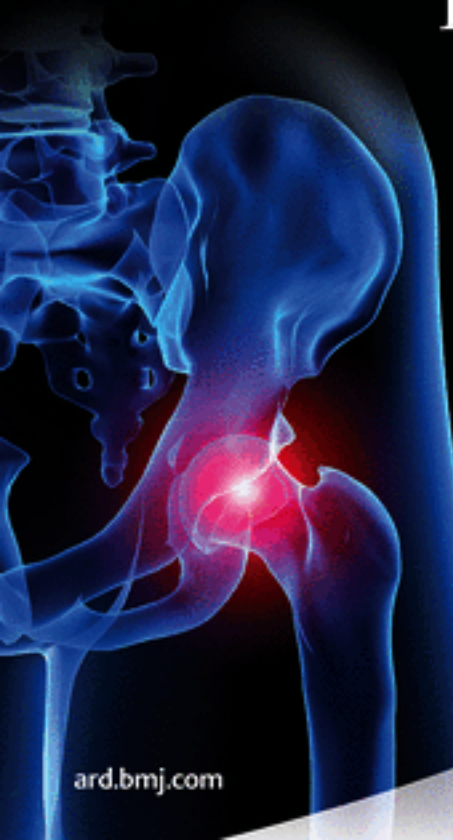
August 2018 Volume 77 Issue 8

Impact Factor 12.350

Annals of the Rheumatic Diseases

The EULAR Journal



ard.bmj.com

eular BMJ

ARD
The EULAR Journal

Guidelines for Authors and Reviewers

Full instructions are available online at http://ard.bmj.com/pages/authors. Articles must be submitted electronically at http://mc.manuscriptcentral.com/ard. Authors retain copyright but are required to grant ARD an exclusive licence to publish. (http://authors.bmj.com/policies/copyright-and-authors-rights).

Annals of the Rheumatic Diseases publishes original work on all aspects of rheumatology and disorders of connective tissue. Laboratory and clinical studies are equally welcome

Contact Details

Editorial Office
Annals of the Rheumatic Diseases
BMJ Journals, BMA House, Tavistock Square
London WCIH 9JR,UK
E: **ard@bmj.com**

Production Editor
Teresa Jobson
E: **production.ard@bmj.com**

EULAR
EULAR Executive Secretariat
Seestrasse 240, 8802 Kilchberg, Switzerland
E: **eular@eular.org**
www.eular.org

Customer support
For general queries and support with existing and new subscriptions:
W: **support.bmj.com**
T: **+44 (0)20 7111 1105**
E: **support@bmj.com**

Self-archiving and permissions
W: **bmj.com/company/products-services/rights-and-licensing/**
E: **bmj.permissions@bmj.com**

Advertising
W: **bmj.com/company/for-advertisers-and-sponsor/**

Display Advertising ROW
Sophie Fitzsimmons
T: **+44 (0)20 3655 5612**
E: **sfitzsimmons@bmj.com**

Online Advertising ROW
Marc Clifford
T: **+44 (0) 20 3655 5610**
E: **mclifford@bmj.com**

Display & Online Advertising Americas
American Medical Communications (AMC)
T: **+1 973 214 4374**
E: **rgordon@americanmedicalcomm.com**

Reprints

Author Reprints
BMJ Reprints Team
E: **admin.reprints@bmj.com**

Commercial Reprints ROW
Nadia Gurney-Randall
M: **+44 07866 262344**
E: **ngurneyrandall@bmj.com**

Commercial Reprints Americas
Ray Thibodeau
T: **+1 267 895 1758**
M: **+1 215 933 8484**
E: **ray.thibodeau@contentednet.com**

For all other journal contacts
ard.bmj.com/contact-us

Subscription Information

ARD is published monthly; subscribers receive all supplements
ISSN 0003-4967 (print); 1468-2060 (online)

Institutional Rates 2018

Print
£986

Online
Site licences are priced on FTE basis and allow access by the whole institution. Details available online at http://journals.bmj.com/content/subscribers or contact the Subscription Manager in the UK (see above right)

Personal Rates 2018

Print (includes online access at no additional cost)
£407

Online only
£173

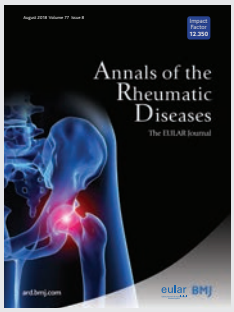
EULAR congress delegates
Delegates receive a Continuous Professional Development package that includes a 12 month complimentary subscription to *ARD* in print and/or online

Personal print or online only and institutional print subscriptions may be purchased online at http://journals.bmj.com/content/subscribers (payment by Visa/Mastercard only)

Residents of some EC countries must pay VAT; for details, call us or visit http://journals.bmj.com/content/subscribers

For more information on subscription rates or to subscribe online please visit ard/bmj.com/pages/contact-us/

ARD
The Eular Journal



Editorial office
Annals of the Rheumatic Diseases
BMJ Publishing Group Ltd
BMA House
Tavistock Square
London WCIH 9JR, UK
T: **+44 (0)20 3655 5889**
E: **ard@bmj.com**
Twitter: **@ARD_BMJ**
ISSN: 0003-4967 (print)
ISSN: 1468-2060 (online)
Impact Factor: 12.350

ARD is published by BMJ Publishing Group Ltd typeset by Exeter Premedia Services Private Ltd, Chennai, India and printed in the UK on acid-free paper.

Annals of the Rheumatic Diseases (ISSN No: 0003-4967) is published monthly by BMJ Publishing Group and distributed in the USA by Air Business Ltd. Periodicals postage paid at Jamaica NY 11431 POSTMASTER: send address changes to *Annals of the Rheumatic Diseases*, Air Business Ltd, c/o Worldnet Shipping Inc., 156-15, 146th Avenue, 2nd Floor, Jamaica, NY 11434, USA.

Editorial

Heroes and pillars of rheumatology

Review

Clinical and epidemiological research

MORE CONTENTS ▶

Basic and translational research

Letters

Electronic pages

Disease activity in ankylosing spondylitis: the global therapeutic target

Daniel Wendling,[1,2] Clément Prati,[1,3] Joachim Sieper[4]

Spondyloarthritis (SpA) is a multifaceted disease with frequent predominant axial involvement.[1] Typical sacroiliac radiographic changes allow to classify the patients as ankylosing spondylitis (AS). Imaging is able to classify patients as AS or non-radiographic axial spondyloarthritis (axSpA) and illustrate and recognise the several steps from inflammation to structural damage, particularly in sacroiliac joints and spine. For decades, these radiographic findings have been the cornerstone for the classification and diagnosis of the disease.[1] Contrary to other chronic rheumatic diseases such as rheumatoid arthritis, radiographic progression over time is only of limited interest as an outcome measure of the disease in the follow-up of patients with AS in current practice. In fact, radiographic progression is slow and does not even occur in all patients, has a low sensitivity to change over time and is associated with unidirectional evolution without regression. The tool used in current research to quantify the structural damage of the spine in AS is the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), which gives higher scores for ossification and bridging of the vertebrae (which may represent a repair mechanism) than for erosive ('inflammatory') changes. Advanced structural changes are associated with functional and spinal mobility impairment.[2]

During the last decade, the use of anti-TNF agents has represented a major breakthrough in the treatment of patients with AS and with SpA in general as well.[3] But, whereas they demonstrated high effectiveness in controlling signs and symptoms of the disease (including extra-articular manifestations, quality of life, productivity), the attempts to illustrate/demonstrate a potential reduction in radiographic progression under TNF inhibition (using mSASSS over a 2-year period and comparison to a historical cohort of patients with AS not treated with TNF blockers) have failed.[4] Several potential risk factors for radiographic progression in AS have been suggested, such as smoking, elevated C reactive protein (CRP) levels, low non steroidal anti inflammatory drug (NSAID) intake, baseline presence of syndesmophytes, high scores for disease activity and various biomarkers (vascular endothelial growth factor (VEGF), calprotectin, adipokines).[3]

In Annals of the Rheumatic Diseases, Molnar *et al*[5] evaluated radiographic progression in AS, using the database of the Swiss cohort patients with AS and spine radiographic follow-up every 2 years, although this analysis was based in about 2/3 of the patients on only one radiographic interval of 2 years. This study included 432 with long-standing, real-life classical AS patients with AS and syndesmophytes, and 616 intervals with two consecutive X-rays and used a statistical model adjusted for the potential factors associated with radiographic progression of the spine and a model adjusted for ASDAS (Ankylosing Spondylitis Disease Activity Score) value before start of anti-TNF agents. In multivariable analysis, prior anti-TNF treatment was associated with a reduction by 50% of the odds for radiographic progression (defined as an increase of at least 2 units of the mSASSS or appearance of at least one new syndesmophyte in 2 years) during the next 2-year interval. Their results suggest that a longer duration of anti-TNF exposition is associated with a stronger protective effect. Moreover, using the above-mentioned model, they found that this effect seems to be mediated through the control of disease activity; patients with an ASDAS less or equal 1.3 (inactive disease) under anti-TNF treatment did not show radiographic progression at all.

Several aspects from this study should be discussed.

First, this study shows an effect of TNF-blocker therapy on structural damage in the spine with a fair level of evidence. Previous reports suggested a potential relationship, using retrospective analysis over a long period,[6–9] but in the absence of a controlled study (that would probably never been performed), confirmation applying sophisticated statistical models is of value.

This kind of study with results drawn from retrospective data analysis demonstrates the usefulness of well-built cohorts; several are available, and some focused on early stages of the disease,[10 11] with promising forthcoming results. Moreover, this study gives the opportunity for validation of a definition of radiographic progression (at least 2 mSASSS units over 2 years) and validation of an operational ASDAS cut-off (less or equal 1.3) for remission or inactive disease in real life.[12 13] These are useful tools for further studies and in clinical settings as well in case of the ASDAS.

Second, the results of this study underline the importance of controlling disease activity, thus confirming previous studies suggesting such a relationship between disease activity, measured by CRP[14] or ASDAS,[15 16] and radiographic progression on a cross-sectional level. Regarding the association disease activity-radiographic progression, the observation of non-progression after reaching remission may represent an argument of a link of causality between these two, also suggested by the association between dose tapering of TNF inhibitors and more rapid progression in AS patients with syndesmophytes.[17] However, suppression of clinical disease activity by long-term TNF-blockers might be more relevant than radiographic progression for clinical outcome parameters such as function and spinal mobility.[18]

The results of the study by Molnar *et al*[5] raise the question: can the equation/association 'induced remission leading to absence of radiographic evolution' be extrapolated to other treatments with different mode of action, such as NSAIDs or other biologics and to other subsets of SpA? This needs to be demonstrated.[19 20]

Finally, these data give sense to a treat-to-target (T2T) strategy[21] with benefit for signs and symptoms and for structural damage as well and, as a consequence of this, on function, even in more advanced diseases. Clinical remission reached by an effective anti-inflammatory treatment such as TNF-blockers may lead to non-progression of structural damage, particularly in case

[1]Department of Rheumatology, CHRU de Besançon, University Teaching Hospital, Besancon, France
[2]EA4266 EPILAB, Université Bourgogne Franche-Comté, Besançon, France
[3]FHU Increase, Université Bourgogne Franche-Comté, Besançon, France
[4]Department of Rheumatology, Charité- Campus Benjamin Franklin, Berlin, Germany

Correspondence to Dr Daniel Wendling, Department of Rheumatology, CHRU de Besançon, University Teaching Hospital, Besancon 25030, France; dwendling@chu-besancon.fr

of early initiation,[22] since bone formation seems secondary to local inflammation.[23]

In conclusion, the results of this study represent a plea for a tight control of disease activity in AS and potentially in SpA in general as well, assessed by the ASDAS. There is now more and more evidence that remission/inactive disease defined by an ASDAS <1.3 is a worthwhile treatment ('T2T') aim with long-term consequences. ASDAS is easy to evaluate and to use in current practice compared with radiographic scoring. This defines a clear target for the therapeutic strategies in axial SpA.

Contributors All authors contributed significantly to this Editorial.

Competing interests DW received speaking fees from Abbvie, BMS, MSD, Pfizer, RochebChugai, Amgen, SOBI, Novartis, Janssen, Hospira, Lilly, Sandoz, UCB, and advisory board for Novartis, Sandoz, Celgene. CP received speaking fees from UCB, Pfizer, Roche Chugai, Lilly, Novartis, MSD, Abbvie, BMS, and advisory board for Novartis. JS received speaking fees from Abbvie, BMS, MSD, Pfizer, UCB, Novartis, Janssen and Lilly

Provenance and peer review Commissioned; externally peer reviewed.

To cite Wendling D, Prati C, Sieper J. *Ann Rheum Dis* 2018;**77**:1095–1096.

► http://dx.doi.org/10.1136/annrheumdis-2017-211544

Ann Rheum Dis 2018;**77**:1095–1096. doi:10.1136/annrheumdis-2017-212363

REFERENCES

1 Sieper J, Poddubnyy D. Axial spondyloarthritis. *Lancet* 2017;390:73–84.
2 Wanders A, Landewé R, Dougados M, *et al*. Association between radiographic damage of the spine and spinal mobility for individual patients with ankylosing spondylitis: can assessment of spinal mobility be a proxy for radiographic evaluation? *Ann Rheum Dis* 2005;64:988–94.
3 Prati C, Claudepierre P, Goupille P, *et al*. TNFα antagonist therapy in axial spondyloarthritis: can we do better? *Joint Bone Spine* 2016;83:247–9.
4 van der Heijde D, Landewé R, Baraliakos X, *et al*. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. *Arthritis Rheum* 2008;58:3063–70.
5 Molnar C, Scherer A, Baraliakos X, *et al*. Rheumatologists of the Swiss Clinical Quality Management Program. TNF blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss Clinical Quality Management cohort. *Ann Rheum Dis* 2018;77:63–9.
6 Haroon N, Inman RD, Learch TJ, *et al*. The impact of tumor necrosis factor α inhibitors on radiographic progression in ankylosing spondylitis. *Arthritis Rheum* 2013;65:2645–54.
7 Baraliakos X, Haibel H, Listing J, *et al*. Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. *Ann Rheum Dis* 2014;73:710–5.
8 Maas F, Arends S, Wink FR, *et al*. Ankylosing spondylitis patients at risk of poor radiographic outcome show diminishing spinal radiographic progression during long-term treatment with TNF-α inhibitors. *PLoS One* 2017;12:e0177231.
9 Maas F, Arends S, Brouwer E, *et al*. Reduction in spinal radiographic progression in ankylosing spondylitis patients receiving prolonged treatment with tumor necrosis factor inhibitors. *Arthritis Care Res* 2017;69:1011–9.
10 van den Berg R, de Hooge M, Rudwaleit M, *et al*. ASAS modification of the Berlin algorithm for diagnosing axial spondyloarthritis: results from the SPondyloArthritis Caught Early (SPACE)-cohort and from the Assessment of SpondyloArthritis international Society (ASAS)-cohort. *Ann Rheum Dis* 2013;72:1646–53.
11 Dougados M, Etcheto A, Molto A, *et al*. Clinical presentation of patients suffering from recent onset chronic inflammatory back pain suggestive of spondyloarthritis: the DESIR cohort. *Joint Bone Spine* 2015;82:345–51.
12 Wendling D, Prati C. Remission in axial spondyloarthritis: the ultimate treatment goal? *Joint Bone Spine* 2016;83:117–9.
13 Wendling D, Guillot X, Gossec L, *et al*. Remission is related to CRP and smoking in early axial spondyloarthritis. The DESIR cohort. *Joint Bone Spine* 2017;84:473–6.
14 Poddubnyy D, Haibel H, Listing J, *et al*. Baseline radiographic damage, elevated acute-phase reactant levels, and cigarette smoking status predict spinal radiographic progression in early axial spondylarthritis. *Arthritis Rheum* 2012;64:1388–98.
15 Ramiro S, van der Heijde D, van Tubergen A, *et al*. Higher disease activity leads to more structural damage in the spine in ankylosing spondylitis: 12-year longitudinal data from the OASIS cohort. *Ann Rheum Dis* 2014;73:1455–61.
16 Poddubnyy D, Protopopov M, Haibel H, *et al*. High disease activity according to the Ankylosing Spondylitis Disease Activity Score is associated with accelerated radiographic spinal progression in patients with early axial spondyloarthritis: results from the GErman SPondyloarthritis Inception Cohort. *Ann Rheum Dis* 2016;75:2114–8.
17 Park JW, Kwon HM, Park JK, *et al*. Impact of dose tapering of tumor necrosis factor inhibitor on radiographic progression in ankylosing spondylitis. *PLoS One* 2016;11:e0168958.
18 Poddubnyy D, Fedorova A, Listing J, *et al*. Physical function and spinal mobility remain stable despite radiographic spinal progression in patients with ankylosing spondylitis treated with TNF-α Inhibitors for Up to 10 Years. *J Rheumatol* 2016;43:2142–8.
19 Proft F, Muche B, Listing J, *et al*. Study protocol: COmparison of the effect of treatment with Nonsteroidal anti-inflammatory drugs added to anti-tumour necrosis factor a therapy versus anti-tumour necrosis factor a therapy alone on progression of StrUctural damage in the spine over two years in patients with ankyLosing spondylitis (CONSUL) - an open-label randomized controlled multicenter trial. *BMJ Open* 2017;7:e014591.
20 Sieper J, Listing J, Poddubnyy D, *et al*. Effect of continuous versus on-demand treatment of ankylosing spondylitis with diclofenac over 2 years on radiographic progression of the spine: results from a randomised multicentre trial (ENRADAS). *Ann Rheum Dis* 2016;75:1438–43.
21 Smolen JS, Schöls M, Braun J, *et al*. Treating axial spondyloarthritis and peripheral spondyloarthritis, especially psoriatic arthritis, to target: 2017 update of recommendations by an international task force. *Ann Rheum Dis* 2018;77:3–17.
22 Zhang JR, Liu XJ, Xu WD, *et al*. Effects of tumor necrosis factor-α inhibitors on new bone formation in ankylosing spondylitis. *Joint Bone Spine* 2016;83:257–64.
23 Tseng HW, Pitt ME, Glant TT, *et al*. Inflammation-driven bone formation in a mouse model of ankylosing spondylitis: sequential not parallel processes. *Arthritis Res Ther* 2016;18:35.

Jacques FORESTIER, a visionary of the clinical epidemiology in rheumatology

Maxime Dougados

Handling editor Josef S Smolen

Correspondence to
Professor Maxime Dougados, Department of Rheumatology, Hopital Cochin, Université Paris Descartes, Paris 75014, France; maxime.dougados@aphp.fr

Received 25 April 2018
Revised 3 May 2018
Accepted 5 May 2018
Published Online First 31 May 2018

Starting my personal research work on spondyloarthritis in the 1980s, I was surprised that my mentor Bernard AMOR proposed me to read the book written in the 1950s by Jacques FORESTIER.[1] In fact, I have been really impressed by the content of this book in terms of extreme detailed clinical data (eg, the description of the four different patterns of hip involvement: (a) arthritis a minima, (b) sclerotic pattern, (c) crenellated shape and (d) ankylosing pattern; the latter being probably in relation with periarticular enthesitis) and in terms of the quality of the provided statistics (prevalence and incidence of the different clinical presentations of spondyloarthritis).

Thanks to a specific book dedicated to the personal and professional life of Jacques FORESTIER written by Professor Jacques ARLET,[2] I am now even more impressed by this 'colleague' (who was one of the first doctors opening an outpatient clinic dedicated to rheumatic patients in Cochin hospital) for several reasons:

This French doctor did not hesitate to cross the ocean in order to present his data in different departments in the USA. At this time (in the 1920s), Jacques FORESTIER started medicine as a neurologist and discovered the advantages of the use of lipiodol in the diagnostic approach of neurological syndromes (he was known in the USA as 'Doctor lipiodol'[3]).

More importantly (at least in my opinion) and thanks to the benefit of the visits he did in different departments in the USA and in particular at the MAYO Clinic, he perfectly implemented the concept of standardised outcome measures. He was working 6 months per year in a SpA resort in Aix les Bains where the patients were spending 3–4 weeks per year. For each specific disease, he created a specific file with the following information: demographics, socioprofessional status, impact of the disease in terms of quality of life, clinical findings, laboratory and radiological findings. This procedure allowed him to provide some interesting and detailed statistics in terms of prevalence and incidence over time since he had the privilege to yearly monitor the majority of the patients.

Moreover, apart from these standardised operating procedures, and thanks to his endless curiosity, he was able to distinguish some diseases and also to recognise the benefit of some therapeutic modalities. Here, we will focus on the recognition of two diseases and the description of the treatment with gold salts despite the fact that Jacques FORESTIER did a lot of work in different areas.

In the book on ankylosing spondylitis,[1] there is a specific section where he is mentioning the difficulties of the diagnosis at an advanced stage of the disease. For this purpose, he described nine typical cases of what we call today the Forestier's disease.[4] He described this disease as 'senile ankylosing spondylitis of the spine'. Most of the rheumatologists are currently still referring to the name of Forestier's disease as diffuse idiopathic skeletal hyperostosis.[5]

Concerning polymyalgia rheumatica,[6] there is still a debate concerning who, between J FORESTIER and GD KERSLEY, was the first to recognise the disease. Whatever the discussion at this time (in the 1950s), Dr KERSLEY did not hesitate to write the obituary of Jacques FORESTIER.[7]

In the SpA in Aix les Bains, patients with tuberculosis were receiving gold salts. Jacques FORESTIER noticed that patients suffering from rheumatoid arthritis took a better benefit of the treatment than patients with tuberculosis.[8] Thereafter, and based on his own personal experience, Jacques FORESTIER proposed the optimal dose regimen which was still on place in France in the 2000s. At this time (eg, a decade of use of methotrexate and the beginning of use of biologics), there was a debate concerning the benefit to continue to use gold salts in the treatment of rheumatoid arthritis[9] with data suggesting that gold salts might be more efficient than methotrexate[10] and/or efficient in case of methotrexate inadequate responders.[11] Even its toxicity has been a

To cite: Dougados M. *Ann Rheum Dis* 2018;**77**:1097–1098.

source of debate since on the one hand all the data suggested a high rate of treatment discontinuation because of toxicity[10] and on the other hand the fact the toxicity was mainly due to reversible harmless skin or mucose membrane reactions.[9] Whatever the final result (no more use of gold salts in many countries), one should recognise that thanks to the findings of Jacques Forestier, a lot of patients have seen their quality of life dramatically improved by this therapy.

Obviously, Jacques FORESTIER was recognised as an excellent clinician and an excellent teacher. Thanks to his capacity to communicate in English, the visit of the SpA in Aix les Bains was recognised all around the world, in particular the annual meeting 'Week of rheumatology'.[12] He became the president of European League Against Rheumatism. However, in France, he was invited in 1976 by the President of the French Republic Giscard d'Estaing not as a rheumatologist but as a rugby player who won the silver medal during the Olympic games in 1920. Another reason to be impressed by this 'colleague' and to try to follow in his footsteps.

Funding The author has not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

1 Forestier J, Jacqueline F, Rotes-querol J. La spondyloarthrite ankylosante. *Masson*, 1951:330.
2 ARLET J. *Jacques Forestier : des stades aux thermes : vie d'un grand rhumatologue*: Privat, 1988:130.
3 Forestier J. The X-Ray examination for respiratory cavities with iodized oil (lipiodol. *Annals of clinical medicine* 1926;4:n0–11.
4 Forestier J, Rotes-querol J. Senile ankylosing hyperostosis of the spine. *Ann Rheum Dis* 1950;9:321–30.
5 Mader R, Verlaan JJ, Eshed I, *et al*. Diffuse idiopathic skeletal hyperostosis (DISH): where we are now and where to go next. *RMD Open* 2017;3:e000472.
6 Forestier J, Certonciny A. Pseudo polyarthrite rhizomélique. *Revue Rheum* 1953;12:854–62.
7 Kersley GD. Obituary, Dr. Jacques Forestier. *Ann Rheum Dis* 1978;37:388.
8 Forestier J. The treatment of rheumatoid arthritis with gold salt injections. *The Lancet* 1932;219:441–4.
9 Rau R. Have traditional DMARDs had their day? Effectiveness of parenteral gold compared to biologic agents. *Clin Rheumatol* 2005;24.
10 Hamilton J, McInnes IB, Thomson EA, *et al*. Comparative study of intramuscular gold and methotrexate in a rheumatoid arthritis population from a socially deprived area. *Ann Rheum Dis* 2001;60:566–72.
11 Lehman AJ, Esdaile JM, Klinkhoff AV, *et al*. A 48-week, randomized, double-blind, double-observer, placebo-controlled multicenter trial of combination methotrexate and intramuscular gold therapy in rheumatoid arthritis: results of the METGO study. *Arthritis Rheum* 2005;52:1360–70.
12 Rakic M. In memorian: Dr Jacques Forestier. *Arthritis and rheumatism* 1978;21:989–90.

Ability of disease-modifying antirheumatic drugs to prevent or delay rheumatoid arthritis onset: a systematic literature review and meta-analysis

Stéphane Hilliquin,[1,2] Benjamin Hugues,[1,2] Stéphane Mitrovic,[1,2] Laure Gossec,[1,2] Bruno Fautrel[1,2]

Handling editor Josef S Smolen

[1]Rheumatology, UPMC, Institut Pierre Louis d'épidémiologie et Santé publique, GRC 08, Paris, France
[2]Department of Rhumatologie, AP-HP, GH Pitié Salpêtrière, Paris, France

Correspondence to
Prof Bruno Fautrel, UPMC, Institut Pierre Louis d'épidémiologie et Santé publique, GRC 08, Paris 75646, France; bruno.fautrel@aphp.fr

SH and BH contributed equally.

Received 28 October 2017
Revised 18 May 2018
Accepted 19 May 2018
Published Online First 8 June 2018

ABSTRACT

Background Recent advances in knowledge of the pathogenesis of rheumatoid arthritis (RA) has led to promoting very early intervention.

Objectives To assess the efficacy of therapeutic interventions in preventing or delaying RA onset with a systematic literature review (SLR) and meta-analysis (MA).

Methods The SLR aimed to include all reports of randomised controlled trials of disease-modifying antirheumatic drugs or glucocorticoids used in patients presenting genetic and/or environmental risk factors for RA and/or systemic autoimmunity associated with RA, and/or symptoms without clinical arthritis and/or unclassified arthritis and in patients with RA. We searched PubMed, EMBASE and Cochrane databases for English articles published from 2006 to 2016 using the keywords 'undifferentiated arthritis' or 'very early rheumatoid arthritis' with 'therapy' or 'treatment'. Main outcome was RA occurrence, defined as fulfilment of the 1987 ACR criteria. The MA was performed with RevMan with the Mantel-Haenszel method.

Results Among 595 abstracts screened, 10 reports of trials were selected. The studies included 1156 patients, with mean symptom duration 16.2±12.6 weeks. The occurrence of RA was available for nine studies, assessing methylprednisolone, methotrexate, a tumour necrosis factor blocker, abatacept or rituximab. In the group arthralgia without arthritis (people at risk of RA), the MA of the two available studies did not show significant reduction in RA occurrence at week 52 or more (pooled OR 0.74, 95% CI 0.37 to 1.49). For people with undifferentiated arthritis, the MA of the seven available studies revealed significant risk reduction with OR 0.73(95% CI 0.56 to 0.97).

Conclusions This MA demonstrates that early therapeutic intervention may significantly reduce the risk of RA onset in this very first phase of the disease.

INTRODUCTION

During the last decades, substantial knowledge has accumulated on the very early stages of rheumatoid arthritis (RA), notably the very early immunological pathogenic mechanisms leading to RA.[1–4] This knowledge has deeply altered the nosological RA concept and its diagnosis.

For a long time, RA diagnosis required a quite complete and comprehensive clinical presentation, including bilateral symmetrical polyarthritis, involving the hands, eventually associated with serum rheumatoid factor (RF), nodules or radiographic joint erosions, as included in the 1987 American College of Rheumatology (ACR) classification criteria.[5] This 'full presentation' does not fit with RA early stages, and the 1987 ACR criteria were found to adequately classify patients as having RA only 2 years after disease onset.[6] In 2010, the joint effort of the ACR and European League Against Rheumatism (EULAR) widened the spectrum of early RA by reducing the minimal synovitis number to 1 and including serum anticitrullinated peptide antibody (ACPA) positivity in addition to RF as immunological biomarkers.[7] The new classification showed higher sensitivity to detect patients with early RA[8] and affected the diagnostic concept of unclassified arthritis (UA) as well as very early RA (VeRA).[9 10]

The combination of advances in RA pathogenesis and progress in RA diagnosis has contributed to redefining the RA early stages as a continuum spreading over several years[4 11] starting from (1) a first autoimmune phenomenon related to a host–environment interaction (eg, interaction between smoking and the presence of the shared epitope leading to ACPA production); (2) preclinical RA (pre-RA), in which levels of autoimmunity biomarkers increase and mature, potentially associated with mild inflammatory features (eg, arthralgia without arthritis/synovitis); (3) UA with at least one synovitis present, without satisfaction of the 1987 ACR criteria (but potentially satisfying the 2010 ACR/EULAR criteria); and finally (4) defined RA, with 'full-picture' RA and satisfaction of 1987 ACR classification criteria.[10 11] These concepts have been retained in recent EULAR recommendations for research of individuals at risk of RA.[12]

Besides diagnosis, the therapeutic issue is also important. Early therapeutic interventions, within the first months after RA onset, were clearly found to be associated with better RA outcomes,[13–15] thereby validating the concept of a 'window of opportunity'. In addition, the PROMPT trial demonstrated the ability of early methotrexate initiation to prevent onset of RA in patients with UA.[16 17] This situation raised the question of delaying or preventing RA if RA treatments are started at preclinical or in the very early clinical stages of the disease.[11]

Although international clinical practice guidelines focus on methotrexate or other conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) in early RA,[18] numerous other therapeutic options are available, and several, including glucocorticoids or biologic DMARDs (bDMARDs), have been tested in individuals at risk of RA. A

To cite: Hilliquin S, Hugues B, Mitrovic S, *et al*. *Ann Rheum Dis* 2018;**77**:1099–1106.

recent meta-analysis (MA) of studies of experimental animal models suggested that DMARDs are not equally efficacious in the prevention or treatment of the early arthritis animal model.[19]

Thus, we conducted a systematic literature review (SLR) and MA of randomised controlled trials (RCTs) of patients at risk of RA to assess the efficacy of glucocorticoids, csDMARDs or bDMARDs for preventing or delaying RA development and/or blocking structural damage. The notion of prevention of RA refers to the ability of a treatment to block the pathogenic process and prevent more established forms of RA. Thus, the target population for such an action is people at risk of RA (family history and presence of (high titre) autoantibodies, or with arthralgia and autoantibodies, or people with UA).

METHODS

Search strategy

We performed a systematic literature search of PubMed, Medline, EMBASE and the Cochrane Controlled Trials Register up to June 2017 for articles published from 2006 to 2016 and EULAR and ACR scientific meeting abstracts from the last 2 years (2015 and 2016). We used the following key words: 'Arthritis, Rheumatoid'(MeSH) AND 'very early' AND 'treatments'(all fields) OR 'therapy'(all field), or 'undifferentiated arthritis'(All Fields) AND 'therapy'(all fields) or 'treatments'(all fields). We limited our search to English-language reports of RCTs of adults ≥18 years old. In addition, we hand-searched reference lists of papers initially detected to identify additional relevant reports. The reports of clinical trials were initially selected on the basis of the title and abstract, then the full text. Duplicate references were removed.

Study selection criteria

To be selected, reports had to satisfy the following:

1. The study design should be an RCT.
2. The enrolled patient diagnosis should be one of (A) patients presenting genetic and/or environmental risk factors for RA and/or systemic autoimmunity associated with RA, and/or symptoms without clinical arthritis and/or UA[7 12 20]; (B) patients with clinical arthritis evolving for <16 weeks and fulfilling the 2010 ACR/EULAR criteria but not the 1987 ACR criteria.
3. Study treatment should be glucocorticoids or any other DMARD, either a csDMARD (methotrexate) or bDMARD (tumour necrosis factor (TNF) blocker or other mode of action).
4. Study outcomes should be measured at week 52 or closest time point.

Data extraction

Two independent readers (SH, BH) extracted the following data by using a standardised form: patient characteristics at baseline (ie, demographics and disease characteristics, including classification criteria fulfilment); therapeutic intervention; occurrence of RA at week 52 or closest time point, defined as fulfilment of the 1987 ACR classification criteria[17 21–25] or ACR EULAR 2010 or according to the rheumatologist's opinion[21 26]; clinical remission rates at week 52, defined by validated composite criteria (ie, Disease Activity Score in 28 joints (DAS28),[17 21–25 27] Simple Disease Activity Index (SDAI), Clinical DAI (CDAI) or boolean,[25] with adequate threshold); structural damage progression seen on X-rays at week 52 (table 1) based on the van der Heijde-modified Sharp score or any other validated score[17 21 23–25 27] and safety based on a descriptive analysis.

To define specific phases of RA, patients were classified into groups according to the EULAR recommendations for terminology[12] as follows: (a) genetic risk factors for RA; (b) environmental risk factors for RA; (c): systemic autoimmunity associated with RA; (d) symptoms without clinical arthritis; (e) UAs; (f) RA.

Study quality was assessed by the Jadad scale[28] with two questions (answer Yes/No) for randomisation, two for masking, and one (answer Yes/No) evaluating the reporting of withdrawals and dropouts. A total of 5 points could be awarded, with higher scores indicating higher quality.

Statistical analysis and MA

The MA was performed accordingly to the Cochrane Collaboration guidelines[29] for RA occurrence, defined as a *definite* RA, which is mostly according to the 1987 ACR classification criteria in the literature (or ACR EULAR 2010) at week 52 or closest time point, radiographic progression and clinical remission. Concerning RA occurrence, data at week 52 and beyond this time were pooled to strengthen the results. A sensitivity analysis was conducted to isolate the impact of TNF-blocker treatment. Statistical heterogeneity was tested by the χ^2 Q test[30]; with significant heterogeneity, a random-effects model was used. The MA was performed with RevMan V.5.3 (The Nordic Cochrane Centre, Copenhagen, 2014) with the Mantel-Haenszel method, estimating ORs and 95% CIs. A descriptive analysis was performed for other measures such as the DAS28, Health Assessment Questionnaire (HAQ) and side effects (infectious and intolerance).

RESULTS

Selected studies

The search identified 595 abstracts, with reports of 10 RCTs selected (including 2 congress abstracts) and 10 exploited for analysis (figure 1). The main reasons for exclusion were disease duration at baseline (many studies included patients with RA evolving for >16 weeks), study design (ie, non-RCTs), study endpoints different from the outcomes of interest and incomplete results (ie, missing data for means and/or SD). The mean Jadad score was 5, which indicates high methodological quality of studies.

Seven were related to (e) criteria, two to (d) and one to (f).[12] The therapeutic strategies tested were methylprednisolone at a single dose of 80[21] or 120 mg,[22] intramuscularly in two studies; dexamethasone at the dose of 100 mg IM at week 0 and week 6[31] oral methotrexate up to 30 mg/week for X weeks or months in one study[17]; TNF blockers—infliximab (3 mg/kg at weeks 0-2-4-6−14+/-22)[23 32] or etanercept (50 mg/kg/wk)[25 27] at labelled doses—in four studies, used alone or with methotrexate (up to 30 mg/wk); intravenous abatacept (100 mg/kg every 2 weeks for 1 month, then monthly)[24] at a labelled dose in one study; and finally intravenous rituximab at 1 g once only in one study (table 2).

The SLR and MA included 1239 patients (mean percentage of women 66.0% with weighted mean age 45.8±15.2 years and mean symptom duration 16.2±12.6 weeks.

Data synthesis

Preventing or delaying RA occurrence

RA occurrence, defined as satisfaction of the 1987 ACR classification criteria, was found in 9 of 10 papers assessing methylprednisolone (80 to 120 mg intramuscularly), dexamethasone, methotrexate, TNF blocker (infliximab in the Saleem and Durez trial; etanercept in EMPIRE), abatacept or rituximab.

Table 1 Main outcome results

Trial name reference)	Group	RA occurrence W52 or more		% Clinical remission W52		% No radiographic progression W52 or more	
Bos *et al* 2009[31]	DXM IM Pcb	16,7 22.5	7/42 9/40	n.a.	n.a.	n.a.	n.a.
Gerlag *et al* 2016 (PRAIRI)[26]*	RTX+GC Pcb	34 40	14/41 16/40	n.a.	n.a.	n.a.	n.a.
Verstappen *et al* 2009 (STIVEA)[21]*	MP IM Pcb	48.6 60.4	54/111 67/111	20.7 11.7	23/111 13/111	12.7 14.8	9/71 9/61
Machold *et al* 2009 (SAVE)[22]	MP Pcb	47.6 52.4	69/145 76/145	16.2 17.8	32/198† 33/185	n.a.	n.a.
van Dongen *et al* 2007 (PROMPT)[16]	MTX Pcb	40 53	22/55 29/55	n.a.	n.a.	88 73	48/55 40/55
Saleem *et al* 2008[23]	IFN Pcb	100 71.4	10/10 5/7	20 14.3	2/10 1/7	80.0 71.4	8/10 5/7
Durez 2011[32]	INF Pcb	73.3 66.7	11/15 10/15	50.0 21.4	7.5/15‡ 3.2/15	n.a.	n.a.
Nam *et al* 2013 (EMPIRE)[25]	MTX+ETN MTX+Pcb	61.5 63.5	33/52 35/53	68.8 47.5 62.5 37.0	38/55‡ 26/55§ 34/55‡ 20/55§	93.1 (87.1=M18) 95.5 (80.0=M18)	51.2/55 52.5/55
Emery *et al* 2009 (ADJUST)[24]	ABA Pcb	46.2 66.7	12/26 16/24	47.4 38.5	9/19‡ 5/13	n.a.	n.a.
Emery *et al* 2011 (COMET) post hoc[27]	ETN+MTX MTX	n.a.	n.a.	69.8 24.1 34.7 13.6	44/63‡ 15.2/63§ 17/49‡ 6.7/49§	80.6 73.9	50.8/63 36.2/49

*Diagnosis of RA relied on the rheumatologist's opinion.
†No SJ and ≤2TJ +2/3 of following: normal CRP level, visual analogue scale score for pain or activity <10/100+ no past or current treatment with DMARDs or glucocorticoids except study drug.
‡DAS28 <2.6.
§Simple DAI ≤ 3.3.
ABA, abatacept; ADJUST, Abatacept study to Determine the effectiveness in preventing the development of rheumatoid arthritis in patients with Undifferentiated inflammatory arthritis and to evaluate Safety and Tolerability; COMET, COmbination of Methotrexate and Etanercept in early rheumatoid arthriTis; CRP, C reactive protein; DAI, Disease Activity Index; DAS28, Disease Activity Score in 28 joints; DMARD, disease-modifying antirheumatic drug; DXM, dexamethasone; EMPIRE, Etanercept and Methotrexate to Induce Remission in Early Inflammatory Arthritis; ETN, etanercept; GC, glucocorticoids; INF, infliximab; MP, methylprednisolone; MTX, methotrexate; n.a., not available; Pcb, placebo; PRAIRI, Prevention of RA by Rituximab; PROMPT, Probable Rheumatoid Arthritis Methotrexate Versus Placebo Trial; RA, rheumatoid arthritis; RTX, rituximab; SAVE, Stop Arthritis Very Early ; STIVEA, Steroids in very early arthritis; SJ, soft joint; TJ, tender joint; W52, week 52.

Two studies are related to arthralgia without arthritis (d)[26 31] evaluating dexamethasone and rituximab. seven are related to UA (e).[21–25 32 33]

In the group arthralgia without arthritis (d), the MA of the two available studies did not show significant reduction in RA occurrence at week 52 or more (pooled OR 0.74, 95% CI 0.37 to 1,49) (figure 2A).

For people with undifferentiated arthritis (e), the MA of the seven available studies revealed significant risk reduction with OR 0.73 (95% CI 0.56 to 0.97). All drugs tended to reduce the risk of RA occurrence, except TNF blockers (figure 2A).

As a sensitivity analysis, the MA was performed without the 2 TNF-blocker studies, which resulted in a more significant pooled OR 0.68 (95% CI 0.50 to 0.92) (figure 2B).

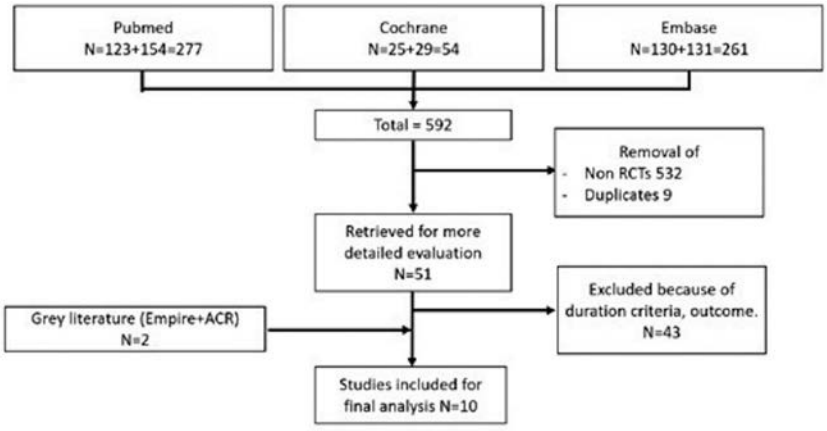


Figure 1 Flow of studies in the review. ACR, American College of Rheumatology; RCT, randomised controlled trial.

Table 2 Study characteristics

Study or subgroup (reference)	Inclusion	Terminology (REF)	N	Intervention	Outcome	Evaluation Time of outcome
Bos *et al* 2009[31]	Arthralgia without synovitis	(d)	83	Dexamethasone 100 mg IM, W0 and W6	► RA occurrence	► W52 and more (mean duration of follow-up: 52.5 months)
Gerlag *et al* 2016 (PRAIRI)[26]	Arthralgia without synovitis	(d)	81	RTX 1000 mg J0	► RA occurrence	► 29 months
Verstappen *et al* 2009 (STIVEA)[21]	UA ACPA/RF+naive of treatment	(e)	224	80 mg MP W0-1-2	► DAS ► HAQ ► Radiographic score ► RA occurrence*	► Baseline, W24, 52 ► Baseline, W52 ► W52 ► W52
Machold *et al* 2009 (SAVE)[22]	UA ACPA/RF + Naive	(e)	303	120 mg MP J0	► DAS ► RA occurrence	► Baseline, W12, W52 ► 12 months
van Dongen *et al* 2007 (PROMPT)[16]	UA ACPA/RF + GC allowed	(e)	55	MTX until 30 mg/wk	► DAS ► Radiographic score ► RA occurrence	► Baseline, W12, W52 ► Baseline, M18 ► 30 months, 60 months
Saleem *et al* 2008[23]	UA ACPA/RF + GC allowed	(e)	17	INF 3 mg/kg W0-2-4-6-14	► DAS ► HAQ ► Radiographic score ► RA occurrence	► Baseline, W12, 24 ► Baseline, W12, 24, ► W52 ► W52
Durez 2011[32]	UA ACPA +	(e)	30	INF 3 mg/kg W0, 2, 6, 14, 22	► RA occurrence ► DAS28 ► ACR 20 –50–70	► 12 months ► W52 ► W14
Nam *et al* 2013 (EMPIRE)[25]	UA ACPA/RF + GC allowed	(e)	82	ETN50 mg/wk+MTX	► DAS ► HAQ ► Radiographic score ► RA occurrence	► Baseline, W12, W52, M18 ► Baseline, W52, M18 ► Baseline, W52, M18 ► 12 months
Emery *et al* 2009 (ADJUST)[24]	UA ACPA/RF+or VERA GC allowed (<10 mg/day)	(e) or (f)	11	ABA 100 mg/kg Day: 1-15-29-57-85-113-141-169	► DAS ► Radiographic score ► HAQ ► RA occurrence	► Baseline, W24, 52 ► Baseline, W52 ► Baseline ► 6 months
Emery *et al* 2011 (COMET) post hoc[27]	VERA	(f)	112	MTX vs MTX+ETN 50 mg/wk	► DAS28 ► Radiographic score	► W52 ► Baseline, W52

RA occurrence: according to ACR 1987 for all studies except for PRAIRI and STIVEA which correspond to the rheumatologist's opinion.
(*a) genetic risk factor of RA; (b) environmental risk factor of RA; (c) systemic auto-immunity associated with RA; (d) symptoms without clinical arthritis; (e) UA; (f) RA (according EULAR 2012 recommendations for terminology[12]).
ABA, abatacept; ACPA, anticitrullinated protein antibody; ACR, DAS, disease activity score; ETN, etanercept; GC, glucocorticoids; HAQ, health assessment questionnaire; INF, infliximab; MP, methylprednisolone; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor; RTX, rituximab; UA, unclassified arthritis (ie, patients presenting arthritis and ultrasound-detected synovitis, without ACPA or RF positivity); VeRA (ie, patients with clinical arthritis evolving for <16 weeks and fulfilling the 2010 ACR/EULAR criteria but not the 1987 ACR criteria); W, week.

Clinical remission

Clinical remission at week 52, according to the DAS28, SDAI, CDAI or boolean definitions, was available for five studies of glucocorticoids and TNF blockers (etanercept or infliximab) or abatacept. The Saleem and Durez study used another criteria (no swollen joint and C reactive protein level <10 mg/L). Only the COMET trial[27] demonstrated a significant effect of etanercept on remission. The MA revealed that early intervention increased the odds of achieving remission (pooled OR 1.84, 95% CI 1.08 to 3.16) (figure 3).

Radiographic progression

Data on radiographic progression were available for five studies, evaluating methylprednisone, methotrexate or a TNF blocker (etanercept and infliximab). The outcomes were the Sharp score (modified or not) and Larsen score. No significant risk reduction was revealed for radiographic progression (figure 4). The MA yielded a pooled OR of 1.36 (95% CI 0.82 to 2.27). We found no difference between treatments for radiographic progression. The analysis without TNF blockers did not alter the results.

Other outcomes and side effects

With the descriptive analysis, similar side effects were observed between placebo and methylprednisone without notable difference.[21 22] The observed side effects were the expected ones: hypertension, lower limb oedema,[22] anaphylactic reaction and mood swings.[21] The PROMPT study found no significant safety difference for methotrexate versus placebo (26/55 with methotrexate and 18/55 with placebo, p=0.17). Side effects described were benign gastrointestinal events, elevated serum liver enzyme levels and dermal/mucosal events with methotrexate. bDMARDs were associated with respiratory and urinary tract infections, with two severe cases.[25] No malignancy was identified. There were 10 safety events with abatacept versus 11 with placebo.[24] The most frequently reported events were nasopharyngitis, urinary tract infection and gastroenteritis. The abstract for the PRAIRI study (rituximab) did not specify side effects.[26]

DISCUSSION

The present SLR and MA provide information that a very early therapeutic intervention may significantly reduce the risk of RA onset with patients at risk of RA and significantly increase the rate of clinical remission. Although the notion of a window of opportunity is well accepted in people with already diagnosed RA,[15 34] our work reveals that an even earlier therapeutic intervention could prevent RA or delayed its onset. This conclusion seems to be available in patients with arthritis. In symptomatic patients without

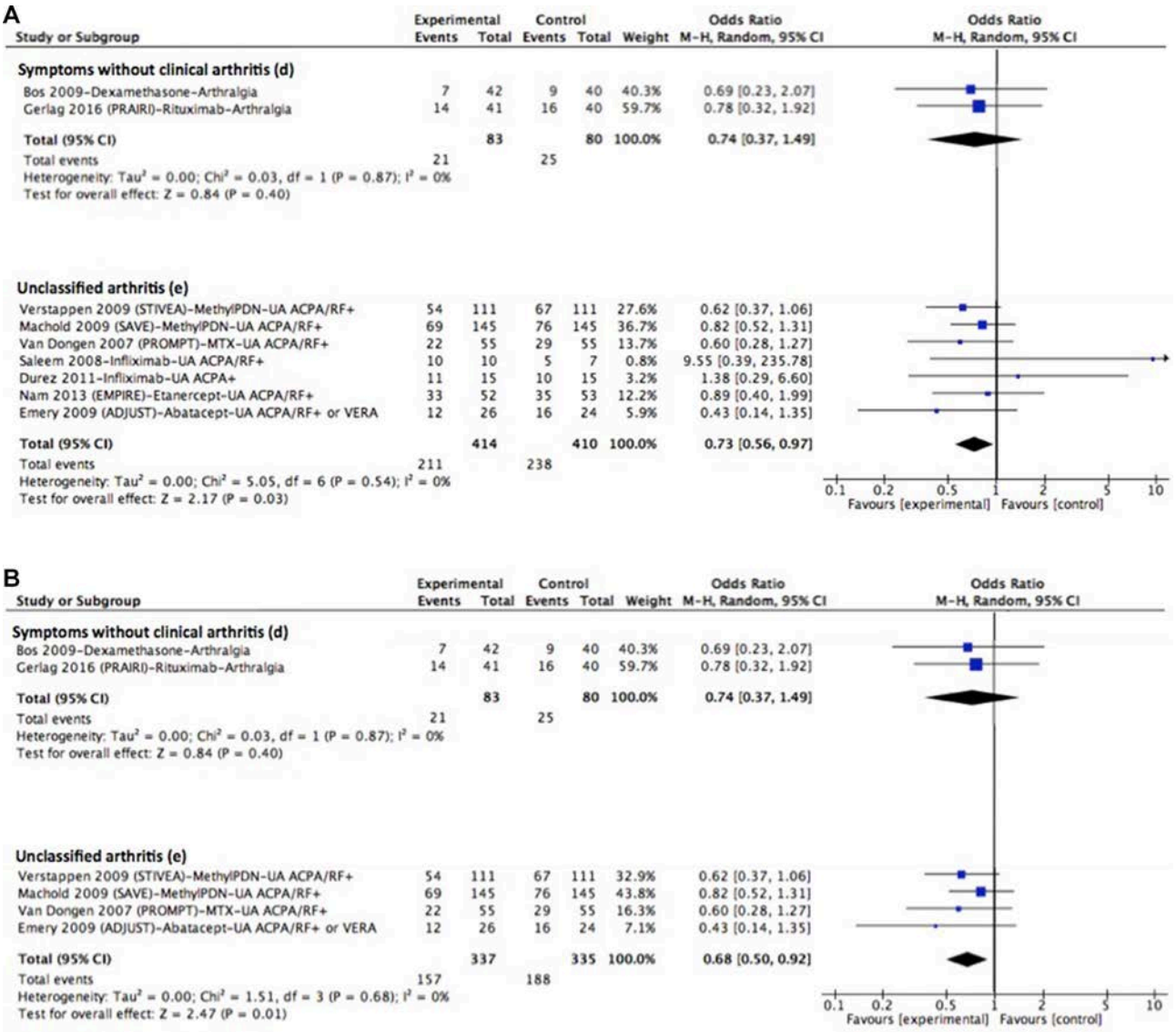


Figure 2 (A) RA diagnosis at week 52 or more including tumour necrosis factor (TNF) blockers. (B) RA diagnosis at week 52 or more not including TNF blockers. ACPA, anti-citrullinated protein antibody; MethylPDN, methylprednisolone; MTX, methotrexate; PDN, prednisolone; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor; UA, undifferentiated arthritis; VeRA, very early rheumatoid arthritis.

arthritis, no significant reduction was observed, potentially partly due to a lack of power with the only two available studies.

This work also suggests that the beneficial effect of very early treatment in RA could differ between csDMARDs or bDMARDs. Although a reduced risk of RA occurrence was observed with glucocorticoids, methotrexate, abatacept or rituximab, the trend seemed not confirmed for TNF blockers.[23 25] This finding is likely to be a class effect rather than a single molecule effect because it was observed with two different agents, one soluble receptor (etanercept) and one monoclonal antibody (infliximab). They

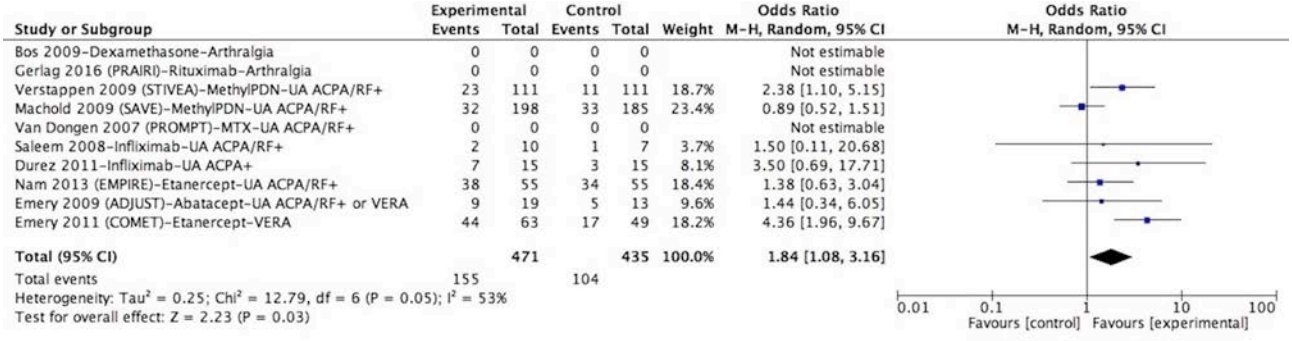


Figure 3 Clinical remission at week 52. ACPA, anti-citrullinated protein antibody; MethylPDN, methylprednisolone; MTX, methotrexate; PDN, prednisolone; RA, rheumatoid arthritis; RF, rheumatoid factor; UA, undifferentiated arthritis; VeRA, very early rheumatoid arthritis.

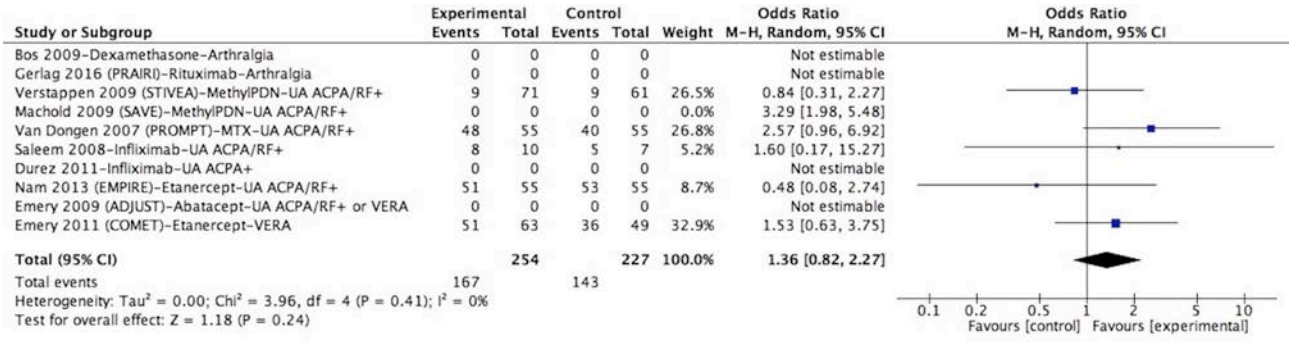


Figure 4 Absence of radiographic progression at week 52. ACPA, anti-citrullinated protein antibody; MethylPDN, methylprednisolone; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor; UA, undifferentiated arthritis; VeRA, very early rheumatoid arthritis.

may be not as important in the very early steps of the disease in which autoimmune phenomenon are present but not joint or systemic inflammation.[35] TNF blockers have been mainly tested, and are currently recommended, in established active RA and eventually in early RA with pejorative prognostic factors such as high swollen joint count, increased acute-phase reactant levels or joint erosions; this is in line with the association between TNF and detectable inflammation.[36–40] Pharmacological agents such as glucocorticoids, methotrexate, rituximab or abatacept may have a broader effect and act higher in the pathogenic cascade, including antigen presentation and early steps of the autoimmune reaction; they could thus prevent the immune system activation, whereas TNF blockers could only reduce already existing inflammation. We have no data available for interleukin 6 (IL-6) blocking agents, although IL-6 seems to be involved in the very early steps of RA pathogenesis.[41]

Several strengths of the study must be underlined. The study applied the methodological standards recommended by the Cochrane collaboration for an SLR and MA, including double data extraction and entry.[29] Although people in very early phases of the disease constitute a challenging population for clinical research, nine reports of RCTs were identified and the data could be integrated in the MA. These trials cover almost all possible modes of action for RA, except IL-6 blockers and JAK inhibitors.

However, our work has some limitations. Although data about about RA diagnosis according to 1987 ACR classification criteria were available in most of the studies (seven of nine), only a few reported data on clinical remission (five of nine). Structural damage information was assessable in five studies; however, the progression was small in RA, and we could not identify any significant benefit of early therapeutic intervention for this outcome. In addition, we found substantial heterogeneity in the outcome measures used in the selected trials: DAS28, SDAI, CDAI or Boolean definitions for remission and Larsen or van der Heijde-modified Sharp score for radiographic progression. For feasibility reasons, we pooled the remission rates or percentages for patients without structural damage progression, regardless of the tool used. This move could have biased our results in part.

An important concern comes with the distinction of the very early steps of RA.[12 35] We chose the cut-off of 4 months of disease duration to select the studies for our MA, which was based on data from a few studies[25 27] using the cut-off of 3 to 4 months to define the very early RA phase. This choice is of course partly arbitrary and reveals the complexity to define the initial RA phases, which constitute a continuum[1] rather than a succession of clearly different health states.[35 42] The 2010 ACR/EULAR criteria[7] allowed for identifying patients with RA at an earlier stage than did the 1987 criteria.[8] However, the criteria are operational only for patients with significant RA symptoms and do not cover the whole spectrum of people with less specific symptoms such as those with arthralgia or limited arthritis, with or without family history of RA, and/or with or without serum ACPA positivity. The development of clinical practice guidelines for people at risk of RA was an important step forward[12] but did not completely fix the overlap of the existing definitions of the RA early phases.[35 42]

Despite these difficulties, our results reinforce the view of 'the sooner the better' in terms of therapeutic decision-making within the pathogenic RA continuum. This paradigm raises an important additional question related to the duration of such a very early therapeutic intervention aiming to prevent RA onset or completely abate the disease. Whatever the RA stage, the risk of relapse seems substantial when treatments are not maintained.[17 21–26] However, there is potential for 'immunological remission' in some patients with RA (ie, resolution of any sign of joint or systemic inflammation with disappearance of serum autoantibodies (RF or ACPA)).[43] Early or very early intervention may favour such immunological remission and could correspond to some kind of resetting of the immune system with complete resolution of any autoimmune phenomenon. The optimal strategy for such patients could then be an induction therapeutic sequence to prevent RA or achieve immunological remission, then a drug tapering or discontinuation sequence to reach sustained and stable drug-free and disease-free states.[44–47] This move would probably require as an intermediary step a better assessment and quantification of the risk of developing RA for a given patient to facilitate the implementation of more personalised therapeutic schemes. A risk stratification score, based on family history as well as patient clinical and biological features, has been proposed in the context of the Leiden Early Arthritis Clinic.[47] Finally, this notion of prevention in patients at risk of developing RA needs to be handled with caution for two reasons. First, in all the trial, the treatment of patients with arthralgia and autoantibodies or with UA have mainly shown their capacity to delay or postpone RA onset, but only a minority of them will durably remain asymptomatic if the tested DMARD is discontinued.[16 17 26] Second, it must be kept in mind that these patients may correspond to patients achieving spontaneous remission with no or only little role for the DMARD. In a recent work conducted in the ESPOIR and the Leiden early arthritis cohorts, such an evolution—that is, DMARD-free sustained remission—could be observed in 5.4% (29/533 in ESPOIR) to 11.5% (85/738 in LEAC).[15] It is important to note that delay is not prevention. It remains to date unknown whether a minority will remain asymptomatic after DMARD is discontinued.

This question deserves to be further studied, why not by creating a two arms study with early intervention in case of arthralgia and autoimmunity comparing intervention only when clinical arthritis develops.

In conclusion, these SLR and MA clearly demonstrate the potential benefits of very early therapeutic intervention for people who start RA and specifically its ability to prevent established RA. Our results fit perfectly with the 2017 EULAR campaign on early actions in rheumatic disorders: 'Don't delay, Connect today' (https://www.eular.org/what_we_do_dont_delay_connect_today.cfm).

Contributors BF had the initiative for drafting this article. The literature search and writing of the article were performed equally by BH and SH. The data layout, meta-analysis and rereading was carried out by SM. The overall proofreading was achieved by both BF and LG.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Not required.

Ethics approval Ethics Committee/Institutional Review Board approval obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Klareskog L, Alfredsson L, Rantapää-Dahlqvist S, *et al*. What precedes development of rheumatoid arthritis? *Ann Rheum Dis* 2004;63(Suppl 2):ii28–31.
2 Klareskog L, Padyukov L, Lorentzen J, *et al*. Mechanisms of disease: genetic susceptibility and environmental triggers in the development of rheumatoid arthritis. *Nat Clin Pract Rheumatol* 2006;2:425–33.
3 Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. *Lancet* 2009;373:659–72.
4 Klareskog L, Catrina AI. Autoimmunity: lungs and citrullination. *Nat Rev Rheumatol* 2015;11:261–2.
5 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
6 Saraux A, Berthelot JM, Chalès G, *et al*. Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. *Arthritis Rheum* 2001;44:2485–91.
7 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
8 Fautrel B, Combe B, Rincheval N, *et al*. Level of agreement of the 1987 ACR and 2010 ACR/EULAR rheumatoid arthritis classification criteria: an analysis based on ESPOIR cohort data. *Ann Rheum Dis* 2012;71:386–9.
9 Krabben A, Huizinga TW, van der Helm-van Mil AH. Undifferentiated arthritis characteristics and outcomes when applying the 2010 and 1987 criteria for rheumatoid arthritis. *Ann Rheum Dis* 2012;71:238–41.
10 van der Helm-van Mil AH, Huizinga TW. The 2010 ACR/EULAR criteria for rheumatoid arthritis: do they affect the classification or diagnosis of rheumatoid arthritis? *Ann Rheum Dis* 2012;71:1596–8.
11 Deane KD. Can rheumatoid arthritis be prevented? *Best Pract Res Clin Rheumatol* 2013;27:467–85.
12 Gerlag DM, Raza K, van Baarsen LG, *et al*. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann Rheum Dis* 2012;71:638–41.
13 Anderson JJ, Wells G, Verhoeven AC, *et al*. Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. *Arthritis Rheum* 2000;43:22–9.
14 Lard LR, Visser H, Speyer I, *et al*. Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies. *Am J Med* 2001;111:446–51.
15 van Nies JA, Tsonaka R, Gaujoux-Viala C, *et al*. Evaluating relationships between symptom duration and persistence of rheumatoid arthritis: does a window of opportunity exist? Results on the Leiden early arthritis clinic and ESPOIR cohorts. *Ann Rheum Dis* 2015;74:806–12.
16 van Dongen H, van Aken J, Lard LR, *et al*. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 2007;56:1424–32.
17 van Aken J, Heimans L, Gillet-van Dongen H, *et al*. Five-year outcomes of probable rheumatoid arthritis treated with methotrexate or placebo during the first year (the PROMPT study). *Ann Rheum Dis* 2014;73:396–400.
18 Combe B, Landewe R, Daien CI, *et al*. 2016 update of the EULAR recommendations for the management of early arthritis. *Ann Rheum Dis* 2017;76:948–59.
19 Dekkers JS, Schoones JW, Huizinga TW, *et al*. Possibilities for preventive treatment in rheumatoid arthritis? Lessons from experimental animal models of arthritis: a systematic literature review and meta-analysis. *Ann Rheum Dis* 2017;76:458–67.
20 Hua C, Daien CI, Combe B, *et al*. Diagnosis, prognosis and classification of early arthritis: results of a systematic review informing the 2016 update of the EULAR recommendations for the management of early arthritis. *RMD Open* 2017;3:e000406.
21 Verstappen SM, McCoy MJ, Roberts C, *et al*. Beneficial effects of a 3-week course of intramuscular glucocorticoid injections in patients with very early inflammatory polyarthritis: results of the STIVEA trial. *Ann Rheum Dis* 2010;69:503–9.
22 Machold KP, Landewé R, Smolen JS, *et al*. The Stop Arthritis Very Early (SAVE) trial, an international multicentre, randomised, double-blind, placebo-controlled trial on glucocorticoids in very early arthritis. *Ann Rheum Dis* 2010;69:495–502.
23 Saleem B, Mackie S, Quinn M, *et al*. Does the use of tumour necrosis factor antagonist therapy in poor prognosis, undifferentiated arthritis prevent progression to rheumatoid arthritis? *Ann Rheum Dis* 2008;67:1178–80.
24 Emery P, Durez P, Dougados M, *et al*. Impact of T-cell costimulation modulation in patients with undifferentiated inflammatory arthritis or very early rheumatoid arthritis: a clinical and imaging study of abatacept (the ADJUST trial). *Ann Rheum Dis* 2010;69:510–6.
25 Nam JL, Villeneuve E, Hensor EM, *et al*. A randomised controlled trial of etanercept and methotrexate to induce remission in early inflammatory arthritis: the EMPIRE trial. *Ann Rheum Dis* 2014;73:1027–36.
26 Gerlag DM, Safy M, Maijer K, *et al*. A Single Infusion of Rituximab Delays the Onset of Arthritis in Subjects at High Risk of Developing RA. *ACR Meet. Abstr* 2016.
27 Emery P, Kvien TK, Combe B, *et al*. Combination etanercept and methotrexate provides better disease control in very early (<=4 months) versus early rheumatoid arthritis (>4 months and <2 years): post hoc analyses from the COMET study. *Ann Rheum Dis* 2012;71:989–92.
28 Jadad AR, Moore RA, Carroll D, *et al*. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;17:1–12.
29 The Cochrane Collaboration. Cochrane handbook for systematic reviews of interventions. http://handbook.cochrane.org/ (accessed 14 Jul 2016).
30 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21:1539–58.
31 Bos WH, Dijkmans BA, Boers M, *et al*. Effect of dexamethasone on autoantibody levels and arthritis development in patients with arthralgia: a randomised trial. *Ann Rheum Dis* 2010;69:571–4.
32 Durez P. Talk: Infliximab Versus Placebo in Adult Patients with ACPA Positive Undifferentiated Arthritis (2011 ACR/ARHP Annual Scientific Meeting). https://acr.confex.com/acr/2011/webprogram/Paper21665.html (accessed 23 Sep 2017).
33 van der Helm-van Mil AH, le Cessie S, van Dongen H, *et al*. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007;56:433–40.
34 Quinn MA, Emery P. Window of opportunity in early rheumatoid arthritis: possibility of altering the disease process with early intervention. *Clin Exp Rheumatol* 2003;21:S154–7.
35 Deane KD, Striebich CC, Holers VM. Editorial: prevention of rheumatoid arthritis: now is the time, but how to proceed? *Arthritis Rheumatol* 2017;69:873–7.
36 Smolen JS, Landewé R, Breedveld FC, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;73:492–509.
37 Singh JA, Hossain A, Tanjong Ghogomu E, *et al*. Biologics or tofacitinib for rheumatoid arthritis in incomplete responders to methotrexate or other traditional disease-modifying anti-rheumatic drugs: a systematic review and network meta-analysis. *Cochrane Database Syst Rev* 2016:CD012183.
38 PubMed J. Biologic or tofacitinib monotherapy for rheumatoid arthritis in people with traditional disease-modifying anti-rheumatic drug (DMARD) Failure: A Cochrane Systematic Review and Network Meta-Analysis (NMA). https://ncbi.nlm.nih.gov/labs/articles/27855242/ (accessed 31 Aug 2017).
39 Singh JA, Hossain A, Mudano AS, *et al*. Biologics or tofacitinib for people with rheumatoid arthritis naive to methotrexate: a systematic review and network meta-analysis. *Cochrane Database Syst Rev* 2017;5:CD012657.
40 Vanier A, Smolen J, Allaart C, *et al*. OP0247 An improved matrix to predict rapid radiographic progression of early rheumatoid arthritis patients: pooled analyses from several databases. *Ann Rheum Dis* 2017;76:158.
41 Gottenberg JE, Dayer JM, Lukas C, *et al*. Serum IL-6 and IL-21 are associated with markers of B cell activation and structural progression in early rheumatoid arthritis: results from the ESPOIR cohort. *Ann Rheum Dis* 2012;71:1243–8.
42 Raza K, Saber TP, Kvien TK, *et al*. Timing the therapeutic window of opportunity in early rheumatoid arthritis: proposal for definitions of disease duration in clinical trials. *Ann Rheum Dis* 2012;71:1921–3.
43 Schett G, Emery P, Tanaka Y, *et al*. Tapering biologic and conventional DMARD therapy in rheumatoid arthritis: current evidence and future directions. *Ann Rheum Dis* 2016;75:1428–37.

44 Wevers-de Boer K, Visser K, Heimans L, *et al*. Remission induction therapy with methotrexate and prednisone in patients with early rheumatoid and undifferentiated arthritis (the IMPROVED study). *Ann Rheum Dis* 2012;71:1472–7.
45 Heimans L, Wevers-de Boer KV, Visser K, *et al*. A two-step treatment strategy trial in patients with early arthritis aimed at achieving remission: the IMPROVED study. *Ann Rheum Dis* 2014;73:1356–61.
46 Espinoza F, Fabre S, Pers YM. Remission-induction therapies for early rheumatoid arthritis: evidence to date and clinical implications. *Ther Adv Musculoskelet Dis* 2016;8:107–18.
47 Nagy G, van Vollenhoven RF. Sustained biologic-free and drug-free remission in rheumatoid arthritis, where are we now? *Arthritis Res Ther* 2015;17:181.

EXTENDED REPORT

Consensus-based recommendations for the management of uveitis associated with juvenile idiopathic arthritis: the SHARE initiative

Tamas Constantin,[1] Ivan Foeldvari,[2] Jordi Anton,[3] Joke de Boer,[4] Severine Czitrom-Guillaume,[5] Clive Edelsten,[6] Raz Gepstein,[7] Arnd Heiligenhaus,[8,9] Clarissa A Pilkington,[10] Gabriele Simonini,[11] Yosef Uziel,[12] Sebastian J Vastert,[13] Nico M Wulffraat,[13] Anne-Mieke Haasnoot,[4] Karoline Walscheid,[8] Annamária Pálinkás,[1] Reshma Pattani,[6] Zoltán Györgyi,[1] Richárd Kozma,[1] Victor Boom,[14] Andrea Ponyi,[1] Angelo Ravelli,[15] Athimalaipet V Ramanan[16]

Handling editor Josef S Smolen

For numbered affiliations see end of article.

Correspondence to
Prof Athimalaipet V Ramanan, University Hospitals Bristol NHS Foundation Trust & Bristol Medical School, University of Bristol, Bristol BS8 1TH, UK; avramanan@hotmail.com

Received 28 January 2018
Revised 7 March 2018
Accepted 11 March 2018
Published Online First 28 March 2018

ABSTRACT

Background In 2012, a European initiative called *S*ingle Hub and Access point for pediatric Rheumatology in Europe (SHARE) was launched to optimise and disseminate diagnostic and management regimens in Europe for children and young adults with rheumatic diseases. Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children and uveitis is possibly its most devastating extra-articular manifestation. Evidence-based guidelines are sparse and management is mostly based on physicians' experience. Consequently, treatment practices differ widely, within and between nations.

Objectives To provide recommendations for the diagnosis and treatment of JIA-associated uveitis.

Methods Recommendations were developed by an evidence-informed consensus process using the European League Against Rheumatism standard operating procedures. A committee was constituted, consisting of nine experienced paediatric rheumatologists and three experts in ophthalmology from Europe. Recommendations derived from a validated systematic literature review were evaluated by an Expert Committee and subsequently discussed at two consensus meetings using nominal group techniques. Recommendations were accepted if >80% agreement was reached (including all three ophthalmologists).

Results In total, 22 recommendations were accepted (with >80% agreement among experts): 3 on diagnosis, 5 on disease activity measurements, 12 on treatment and 2 on future recommendations.

Conclusions The SHARE initiative aims to identify best practices for treatment of patients suffering from JIA-associated uveitis. Within this remit, recommendations for the diagnosis and treatment of JIA-associated uveitis have been formulated by an evidence-informed consensus process to suggest a standard of care for JIA-associated uveitis patients throughout Europe.

To cite: Constantin T, Foeldvari I, Anton J, *et al*. *Ann Rheum Dis* 2018;**77**:1107–1117.

INTRODUCTION

In 2012, Single Hub and Access point for pediatric Rheumatology in Europe (SHARE) was launched with the aim of optimising and disseminating diagnostic and management regimens for children and adolescents with rheumatic diseases. The European League against Rheumatism (EULAR) has produced a number of recommendations in the area of paediatric rheumatology such as juvenile dermatomyositis[1] using a standardised procedure[2] and referring to a generalised instrument for guideline assessment: the Appraisal of Guidelines for Research & Evaluation.[3]

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children, and uveitis is the most frequent and potentially most devastating extra-articular manifestation. There are no international consensus statements specifically relating to the diagnosis and treatment of JIA-associated uveitis although there are national guidelines from Germany and Spain.[4 5] Management is therefore based on physicians' personal experience; and considerable variation in clinical practice, both in terms of investigation and management of JIA-associated uveitis, was found in an extensive survey of uveitis experts.[6]

With the rapid development of novel therapies for JIA, clear recommendations based on available best evidence and expert opinion (when trial evidence is lacking) will help physicians in the care of patients with JIA-associated uveitis. The uveitis seen with JIA is usually chronic anterior uveitis which is asymptomatic, but acute anterior uveitis can also be seen in the enthesitis-related arthritis subtype.[7 8] There is a clear need to regularly update those managing patients with JIA uveitis through expert opinion. While the majority of treatments available for the treatment of arthritis have a solid evidence base from clinical trials, corresponding data for patients with uveitis may not be known or the level of evidence may not be as strong at the time of their introduction into clinical practice.

The primary aims of the recommendations forwarded by the Expert Group were to develop agreed strategies to

- prevent or reduce the likelihood of JIA-associated uveitis from occurring and minimise the damage at the time of diagnosis
- recommend treatments and management strategies that would reduce inflammation and prevent the development of those ocular complications most likely to cause irreversible visual loss.

METHODS

A committee of 12 experts (AH, BV, CP, CE, SC-G, IF, JdB, JA, KW, RG, YU, NW) in paediatric rheumatology (n=9) or ophthalmology (n=3) was established to develop recommendations for JIA-associated uveitis based on consensus, but evidence informed, using EULAR standard operating procedures for developing best practice.[2 3]

Systematic literature search

The electronic databases PubMed/MEDLINE, Embase and Cochrane were searched independently by two researchers for eligible articles in February 2015. All synonyms of JIA were searched in Medical Subject Headings/Emtree terms, titles and abstracts (figure 1). Reference tracking was performed in all included studies (full search strategy is shown in figure 1). Experts (TC, AP, VB) selected papers relevant to uveitis associated with JIA investigations and/or treatment to be taken forward for validity assessment (inclusion and exclusion criteria shown in figure 1) by members of the expert committee.

Validity assessment

The selected articles were randomly allocated to the expert group, and two members per paper independently assessed the methodological quality of those papers meeting the inclusion

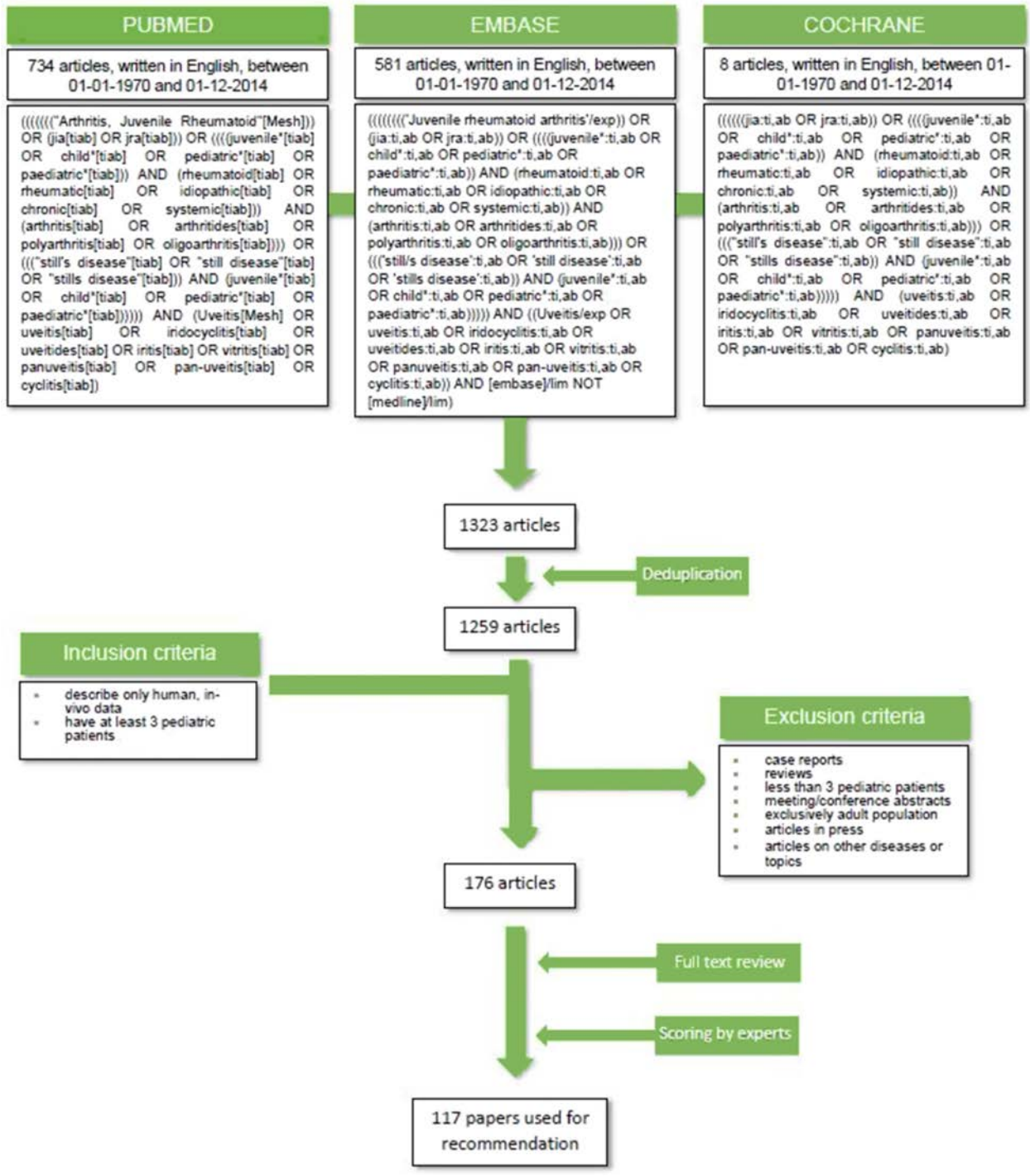


Figure 1 Summary of search strategy for identification of key articles.

Table 1 Recommendations for diagnosis and screening in juvenile idiopathic arthritis (JIA)-related uveitis

Recommendation	L	S	Agreement (%)	References
1. All patients in whom a diagnosis of JIA is being considered should be screened for uveitis according to a contemporary and audited protocol. Formal screening protocol should be administered in all centres, where patients with JIA are seen.	2A	B	100	13–32
2. Frequency of ophthalmological follow-up visits must be based on disease severity and needs to be decided in conjunction with an expert ophthalmologist.	4	D	100	13–27 33–66
3. Patients with JIA stopping any systemic immunosuppressant are at risk of developing new onset uveitis or recurrence of uveitis after a prolonged remission. After stopping systemic immunosuppression, it is recommended that all patients with JIA are screened by an ophthalmologist at least every three months for at least 1 year.	2B	B	100	67–70

Agreement indicates the % of experts that agreed on the recommendation during the final voting round of the consensus meeting.
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; 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. L, level of evidence; S, strength of evidence.

criteria (figure 1). Data were extracted using predefined scoring forms for diagnostic and therapeutic studies. Disagreements were resolved by discussion or by the opinion of a third expert. Adapted classification tables for diagnostic, therapeutic and epidemiological studies were used to determine the level of evidence and strength of each recommendation.[9–11]

Establishment of recommendations

As part of the EULAR standard operating procedure, experts described the main results and conclusions of each paper, along with their validity and level of evidence. These descriptions were collated by five experts (TC, GS, YU, RG, JdB) and used to formulate provisional recommendations which were reviewed by a panel of three experts (IF, NW, JdB). A summary of the evidence was presented along with each provisional recommendation to the expert committee. The recommendations were revised and discussed at a face-to-face meeting in March 2014 (Genova: 12 participants), using a Nominal Group Technique to reach a consensus.[12] A non-voting expert (AR) facilitated the process. Recommendations were accepted when ≥80% (10 of 12) of the experts agreed (including all three ophthalmologists).

RESULTS

Literature review

The literature search yielded a total of 1323 papers which, after deduplication, left 1259 unique articles. Evaluation of inclusion/exclusion criteria in titles and/or abstracts resulted in a core reference database of 176 articles for which full-text copies were obtained for quality screening by the expert committee. Of these, 117 were selected to support the development of consensus-based recommendations by the expert group (figure 1).

Recommendations

The sections that follow report the recommendations of the expert committee based on the supporting literature.[13–128] Tables 1–4 summarise the recommendations, the level of evidence that they provide and the strength of the recommendation, and the percentage of experts who agreed with these assessments.

Background

JIA is the most common chronic rheumatic disease in children with an incidence of 8.2 (7.5–9.0)/100 000 of the population under 16 and an annual prevalence of approximately 70.2 [16 – 140]/100 000.[129 130] The wide prevalence range has been attributed to the different study designs employed, but the incidence is thought to vary little worldwide.[130] The incidence of JIA-associated uveitis is thought to be approximately 1/10 000 and there is some evidence that it is less frequent in oriental populations with JIA.[7 131]

Structural complications, some of them leading to irreversible visual loss, include cataracts, glaucoma, band keratopathy, macular oedema, retinal detachment and sequelae associated with chronic hypotony. The uveitis usually has an insidious time course and can be chronic or recurrent but, most frequently, JIA-associated uveitis is a chronic relapsing condition lasting several years. It almost universally starts as an anterior uveitis, but in rare instances can become a panuveitis. JIA-associated uveitis is usually asymptomatic in the age group in which it

Table 2 Recommendations for disease activity measurement in juvenile idiopathic arthritis (JIA)-related uveitis

	L	S	Agreement (%)	References
4. There should be good communication between the ophthalmologist and the paediatric rheumatologist concerning changes in disease activity treatment changes and responsibility for treatment monitoring.	3	C	100	71
5. There is a need to develop shared outcome measures to help guide decisions on systemic treatment.	4	D	100	
6. At present, there is no validated biomarker to follow the activity of uveitis.	2A	B	100	4 21 29 31 32 37 41 46 72–76
7. At present, no widely accepted definition of inactive disease for JIA-related uveitis is available. The goal of treating JIA-associated uveitis should be no cells in the anterior chamber. The presence of macular and/or disk oedema, ocular hypotony and rubeosis iridis may require anti-inflammatory treatment even in the absence of AC cells.	2B	B	100	4 69 78
8. We recommend 2 years of inactive disease off topical steroids before reducing systemic immunosuppression (both DMARDs and biological therapies).	3	C	92	67

Agreement indicates the % of experts that agreed on the recommendation during the final voting round of the consensus meeting.
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; 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. DMARD, disease-modifying anti rheumatic drugs; L, level of evidence; S, strength of evidence.

Table 3 Recommendations for treatment in juvenile idiopathic arthritis (JIA)-related uveitis

	L	S	Agreement (%)	References
9. Active uveitis in JIA usually requires immediate treatment.	2B	B	100	69 71 78–80
10. Topical corticosteroids (preferably prednisolone acetate or dexamethasone) are the first-line treatment of anterior uveitis.	4	D	100	81
11. Topical and systemic NSAIDs have no demonstrable effect as monotherapy, but may be used as additional therapy.	3	C	92	79 81 82
12. Systemic immunosuppression in active uveitis is recommended if poor prognostic factors are present at the first visit. Poor prognostic factors including lack of remission later on during the disease course require systemic immunosuppression.	2A		100	4 19 22 29 55 56 65 78 83 84
13. Systemic immunosuppression is recommended if inactivity could not be reached within 3 months or inflammation is reactivating during steroid dose reduction.	2B	B	100	55 59 68 69 78 80 85–87
14. Methotrexate is the first choice as systemic immunosuppression.	4	D	100	68 84 88–95
15. In case of methotrexate inefficacy or intolerance, adding or switching to biological treatment is recommended.	3	C	92	91–104
16. The use of anti-TNF treatment strategies *(adalimumab>infliximab>golimumab)* is recommended in patients with uveitis refractory/resistant to DMARD therapy, principally methotrexate.	3	C	100	86 100 101 104–117 120–124 126 127
17. Based on the current evidence, etanercept should not be considered for JIA-associated uveitis.	1B	A	100	87 100 109 117–121
18. Switching between different anti-TNF treatments might be valuable if uveitis is refractory to the first anti-TNF, even though the present evidence comes from small case series or inception cohorts.	3	C	100	87 113 116 122
19. In case of lack of efficacy, consider testing for antidrug antibodies and drug trough level. If the patient has no antibodies but has low trough levels, consider increasing the dose or shortening the interval.	4	D	100	
20. Tocilizumab, rituximab and abatacept might be potential options for cases refractory to previous anti-TNF therapy.	3	C	100	123–125

Agreement indicates the % of experts that agreed on the recommendation during the final voting round of the consensus meeting.
1A, meta-analysis of cohort studies; 1B, meta-analysis of case–control studies; 2A, controlled study without randomisation; 2B, quasi-experimental study; 3, descriptive study; 4, expert opinion; 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.; DMARD, disease-modifying antirheumatic drugs; L, level of evidence; NSAIDs, non-steroidal anti-inflammatory drugs; S, strength of evidence; TNF, tumour necrosis factor.

most commonly develops (age 3–7 years), but severe inflammation may cause symptomatic pain and redness and these symptoms and signs, as well as pupil distortion, may be noticed by carers and lead to early referral. Reduced visual function is an uncommon cause of presentation in small children unless it is severe and usually secondary to irreversible structural damage. The presence of antinuclear antibodies (ANA), oligoarthritis and early onset of arthritis are predominant risk factors for chronic anterior uveitis in those with JIA, and improved identification of children at risk is a key priority for targeted screening programmes. This exercise only looked at chronic anterior uveitis associated with JIA and not at acute anterior uveitis seen in human leukocyte antigen (HLA) B27-positive children with enthesitis-related arthritis.

Table 4 Recommendations for future plans in juvenile idiopathic arthritis (JIA)-related uveitis

	L	S	Agreement (%)	References
21. Validated outcome measures for JIA-associated uveitis are needed	3	C	100	73 126–128
22. Controlled clinical trials are needed for JIA-associated uveitis	1B	A	100	119

Agreement indicates the % of experts that agreed on the recommendation during the final voting round of the consensus meeting.
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 (diagnostic studies); 1A, meta-analysis of randomised controlled trials; 1B, randomised controlled study; 2A, controlled study without randomisation; 2B, quasi-experimental study; 3, descriptive study; 4, expert opinion (therapeutic studies); 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. , level of evidence; S, strength of evidence .

Timely and aggressive treatment is clearly needed where there is significant damage at the time of diagnosis. However, the variable course of the disease means that some significant events may occur years after therapy commences and there remains considerable disagreement among expert practitioners about the timing and indications for treatment escalation.[132 133]

Recommendations for diagnosis and screening in JIA-related uveitis

Screening for JIA-associated uveitis

All patients with JIA should be screened for uveitis according to contemporary and audited screening protocols which should be implemented in all centres in which patients with JIA are being managed.[42 43] There is no necessity for the screening process to take place in the same institution as the rheumatological care. It is especially important, however, that all children in whom the diagnosis of JIA is being considered should have a timely check by a local ophthalmologist rather than wait for confirmation of the diagnosis from a paediatric rheumatology referral centre. It is the responsibility of paediatric rheumatologist to ensure children with JIA are referred for screening. Screening starts with all individuals with a 'suspected' diagnosis of JIA and the clinical responsibility for organising this service needs to be clear. This is independent of whether it is the remit of the ophthalmologist not specialised in uveitis, paediatrician or rheumatologist (table 1). The evidence published to suggest that the risk factors originally proposed for the development of chronic uveitis in the JIA population (early-onset arthritis, ANA positivity and oligoarticular subtype) is suboptimal: the expert group noted that despite the fact that a number of screening protocols have been published there is no evidence to suggest that any one of them is superior.[5 23 26 61 74]

Advances in genetics have not found any more specific diagnostic markers than the HLA types originally reported as being associated with both oligoarticular disease and uveitis.[14 15 30 32 38 48 50 56] There is no evidence, at present, that genotyping adds specificity to the established clinical risk factors on which contemporary screening programmes are based.

Despite the advances in subtyping ANAs in other rheumatic disorders, there has been little advance in our understanding of the association of ANAs with the risk of JIA-associated uveitis[28 34 35 41 60 67] although antibodies to nuclear structures such as histones and chromatin[16 21 41 52–54 64 77] and ocular antigens have been reported.[13 33 51 75] Two studies found that children with higher inflammatory activity (as determined by higher erythrocyte sedimentation rate (ESR) values) when oligoarthritis or polyarthritis was diagnosed had an increased risk of developing uveitis.[27 31]

There is a clear unmet need to adapt screening policies to the contemporary usage of early systemic immunosuppressant treatment of arthritis[57 61 62 64] and novel biomarkers and future genotyping may improve targeting of the population being screened.

Monitoring during follow-up

The risk of visual symptoms and potential for relapse in patients initially responding to treatment highlights the necessity for maintained regular close ophthalmological scrutiny. The expert group recommended that the frequency of ophthalmological follow-up should be based on ocular disease severity and decided upon in conjunction with an expert ophthalmologist.

Screening after stopping treatment of uveitis

Methotrexate (MTX) is the immunosuppressive therapy of choice in patients with JIA-related uveitis (see recommendation 14, table 3). Once long-lasting remission of uveitis is achieved, MTX is usually stopped, but the optimal period of disease control prior to withdrawal of both topical and systemic treatment remains unclear. Patients stopping MTX are at risk of developing new onset uveitis or recurrence of uveitis after prolonged remission in the first year. Indeed, the majority of patients in a recently reported series relapsed within 24 months of stopping therapy.[67–70] Consequently, after stopping immunosuppression with MTX (prescribed for arthritis or uveitis) it is recommended that all patients with JIA are screened by an ophthalmologist at least every three months for a minimum of 1 year.

It also remains unclear for those patients on multiple treatments, in which order treatments are best withdrawn. Relapse of uveitis after withdrawal of MTX appears to be delayed in older patients, those who have been on treatment for longer duration.[67] The authors recommended that the period of uveitis inactivity should be >2 years before MTX is withdrawn.[67] The expert group also recommend that patients should have 2 years of inactive disease while not using topical steroids before reducing systemic immunosuppression.

There is a clear need for continuing monitoring in the early period of remission after medications are stopped, especially in patients on long-term therapy, topical or systemic, which had maintained disease control. The expert group recommends that monitoring of disease in remission by an ophthalmologist should be at least every three months and should continue for at least 3 years off all forms of treatment. The length of remission best predicting lifetime remission remains unknown. More robust data on effective screening strategies are required.

Recommendations for disease activity measurement in JIA-associated uveitis

A pivotal goal in the management of JIA-associated uveitis is to minimise loss of vision through the early diagnosis of ocular morbidity. Early referral and appropriate treatment to eliminate ocular inflammation are seen to be crucial in preserving visual acuity. Visual loss is mainly caused by glaucoma, macular damage from inflammatory oedema, hypotony or amblyopia: structural damage can occur prior to diagnosis and also arise following years of poorly controlled inflammation.

Multiple studies have found delayed presentation with damage at diagnosis, surgery and length of follow-up to be the major risks for lifelong visual loss.[4 68 78] Male gender and non-Caucasian race may be additional risk factors for some complications.[18 19 37 56 65 71] Immunosuppressive treatment is therefore aimed at reducing agreed measures of active intraocular inflammation which include the level of cellular infiltrate, breakdown of vascular barriers (eg, flare) and macular oedema: few of these measures have been validated and different markers may be appropriate in eyes with different levels of structural damage or at different stages of disease.[134]

In one study, an increase in anterior chamber cell grade was associated with elevated rates of visual loss in a dose-dependent fashion, whereas immunotherapy was associated with a reduced risk of visual loss, particularly for the 20/50 or worse outcome (HR 0.40; P<0.01).[78] Others have found anterior chamber flare a better predictor of visual loss.[63]

There are no established biomarkers currently available for predicting disease activity or guiding treatment in JIA-associated uveitis and research efforts in this area need to be intensified (table 2).[24 32 34 35 40 44 49 75–80]

In a small group of children with JIA-related anterior uveitis, serum interleukin-2R levels were significantly increased,[72] while in a larger cohort of children there was a significant correlation between the presence of anterior uveitis and aqueous humour levels of transthyretin.[73] Until reliable biomarkers are found, management relies on frequent ophthalmic examination. Future opportunities might include gene expression and proteomic profiling of the serum, peripheral blood leucocytes and aqueous humour; measurement of acute-phase reactants; HLA typing and determination of ANAs.[74] Evidence-based guidelines by the German Ophthalmological Society, the Society for Childhood and Adolescent Rheumatology and the German Society for Rheumatology noted that macular oedema, ocular hypotony and rubeosis iridis are often associated with chronic inflammation and anti-inflammatory treatment should be initiated (or intensified) even in the absence of cells in the anterior chamber.[4] The expert group recommended that the goal of treating JIA-associated uveitis should be no cells in the anterior chamber although this may not be practically possible (table 2). With reference to the Standardization of Uveitis Nomenclature (SUN) Working Group, a multinational interdisciplinary group (MIWGUC) developed a set of core outcome measures for uveitis that may provide a framework for evaluating disease severity and its course, risk for structural impairment, levels of impairment in visual function and responses to treatment.[74] These would be invaluable in clinical studies involving patients with JIA-associated uveitis. However, these proposed outcome measures remain to be validated in children.[135] To best guide treatment decisions, there should be good communication between the ophthalmologist and the paediatric rheumatologist concerning changes in disease activity treatment changes and responsibility for treatment monitoring. Recently published guidance for management

of non-infectious uveitis in adults has some important principles for the management of panuveitis in all age groups.[136]

Recommendations for treatment in JIA-associated uveitis

Active uveitis in JIA usually requires immediate treatment. In a comparison of two cohorts of patients with new-onset JIA, separated by a 10-year interval, the more recently treated patients received early intensive treatment and close monitoring, and reported fewer complications and milder uveitis with visual loss avoided in most cases.[80] Treatment factors reported to be associated with improved outcomes included introduction of immunosuppressive therapy earlier in the course of the disease or at a younger age[69] and treatment with immunosuppressants generally.[71 78]

Based on past usage, the expert group recommend that topical corticosteroids (preferably prednisolone or dexamethasone) are the first-line treatments of choice for both acute and chronic anterior uveitis.[4 5 81 129] Children with JIA-related uveitis are frequently treated with topical corticosteroids over extended periods, and this increases the risk of cataract formation and glaucoma.

One study found that the increased risk of cataract formation with high-dose topical steroids was independent of active uveitis or presence of posterior synechiae.[81] The risk increased as the number of drops of topical corticosteroids instilled increased. The data suggested that patients may be treated with low doses of topical corticosteroids (≤3 drops daily) over moderate periods of follow-up (median 4 years; range 0.5–15 years) with a low risk of developing cataracts.[81] Among eyes receiving ≤2 drops daily, the incidence of cataract was 0/eye year. There appears to be no evidence to suggest that less potent topical steroids reduce adverse effects in this patient population (table 3). Systemic corticosteroids are not usually preferred in children due to risks of growth suppression and osteopenia; however, they are potentially helpful in individual cases for rapid control of severe uveitis or in the presence of macular oedema.

In a retrospective study, the adjunctive use of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of chronic iridocyclitis was evaluated in 14 patients, 8 with JIA and 6 with idiopathic iridocyclitis.[82] In all patients, the activity of the iridocyclitis improved with the addition of NSAIDs to their treatment regimens, permitting reduction in the dose of corticosteroid drugs. These data suggest that NSAID therapy may have an adjunctive role in the treatment of chronic iridocyclitis in childhood, but not as monotherapy.

Systemic immunosuppression for active uveitis is recommended to reduce complications in those cases where topical steroids are insufficient to eliminate ocular inflammation short term, or such high doses are required that treatment risks outweigh the beneficial effects. However, the threshold for introducing systemic treatment is lower in those with multiple risks for visual loss as discussed earlier.[129] Immediate systemic immunosuppression in active uveitis is recommended if poor prognostic factors are present at the first visit. Studies suggest instituting aggressive immunosuppression in high-risk patients even before there is any clear evidence of complications developing.[27 39] Poor prognostic factors include uveitis antedating arthritis[19 22 74]; posterior synechia[55 68 74 75]; male gender[19 22 55]; band keratopathy, glaucoma and cataract[55]; poor initial vision, hypotony, macular oedema and dense vitreous body opacification[55]; and lack of remission later on during the disease course (table 3). Age at the time of uveitis onset does not appear to be a risk factor.[22 56]

Definition of treatment failure

Treatment failure should lead to a change in treatment dose, route or modality taking into consideration the fact that many of the drugs require different times to achieve their optimal effect and there are sometimes only a limited number of drugs available. Inappropriate drug changes may result from difficulties in achieving consensus on the definition of treatment failure. In a retrospective study, the clinical outcome of 23 patients with JIA-associated uveitis unresponsive to corticosteroids was ascertained. Uveitis was controlled using immunosuppressive therapy in all cases.[86] Patients treated within 4–30 months from onset of uveitis achieved better improvement of vision compared with patients who received immunosuppressive therapy after 3 years ($P<0.005$ for right and left eyes pooled; $P=0.0075$ for best eyes; $P=0.0375$ for worst eyes). These data are supported by findings from the SITE study which reported that among patients with JIA-associated uveitis receiving tertiary care use of immunosuppressants reduced the risk of vision loss by about 60%.[78] The expert group recommended that systemic immunosuppression should be used if inactivity cannot be achieved with topical steroids within 3 months or inflammation is reactivated during steroid dose reduction. The benefits of immunosuppressive therapy are now well recognised, and, in general, patients in remission received this treatment earlier in the course of disease compared with patients who relapsed.[55 59 68 78–87]

Based on findings of efficacy and tolerability/safety from a number of studies, the expert group recommended that MTX is the immunosuppressive therapy of choice in patients with JIA-related uveitis.[68 84 88–90] Other forms of immunosuppressive therapy such as azathioprine, sulfasalazine, mycophenolate mofetil, cyclosporine and leflunomide have been assessed in patients with uveitis, often in MTX-resistant cases.[91 95] The studies have generally included a small numbers of patients, and in the case of cyclosporine, the clinical efficacy was poor and the authors noted that it has limited value in this indication.[94] The results with other forms of immunosuppressive therapy were more encouraging, but were in fewer patients.[91–93 95] At this stage they may prove to be useful treatment options in patients not responding to, or who cannot tolerate, MTX and they also have a place accompanying biological treatment in those who are MTX-intolerant. There is also evidence of the greater effectiveness of MTX in controlling arthritis in JIA compared with other conventional immunosuppressants. It is not uncommon for treatment to fail when treatments other than MTX are used because of recurrent arthritis rather than failure to control the uveitis.

As noted above, the evidence with other forms of immunosuppressive therapy in patients with JIA-related uveitis is low,[91–95] whereas many conventional immunosuppressants are used in adult onset uveitis with no specific agent favoured as the first choice. In both adult and childhood patients who fail to respond to conventional immunosuppressants, expert groups recommend adding or switching to biological treatments. There is a growing number of studies supporting the use of biological therapy in MTX refractory uveitis.[93–108] A wide range of biological therapies have been investigated in JIA-related uveitis refractory to conventional therapy, with adalimumab being the most widely studied; other agents investigated include infliximab, daclizumab, etanercept, golimumab, abatacept, tocilizumab and rituximab.[96–108 123–125]

All of the biological therapies investigated produced some benefit in patients with uveitis refractory to conventional therapy.[86 100–127] There are very few comparative studies. One

study found infliximab to be significantly superior to etanercept in children with refractory JIA-associated chronic uveitis.[100] In another small study, adalimumab was more efficacious than infliximab when used as first-line anti-TNF treatment.[106] Adalimumab also produced higher remission rates versus infliximab in the medium-term treatment (at least 12 months) of patients with JIA-related uveitis[107] and in children with uveitis it was significantly superior to infliximab in maintaining remission over a 3-year treatment period.[102] The expert group recommends treatment with anti-tumour necrosis factor (TNF) agents (adalimumab>infliximab>golimumab) in patients with uveitis refractory/resistant to disease-modifying antirheumatic drug (DMARD) therapy, principally methotrexate.[86 100–127]

The use of TNF inhibitors in uveitis was hypothesised based on their proven efficacy in a range of systemic inflammatory disorders including JIA, rheumatoid arthritis and Crohn's disease.[109] Etanercept is a recombinant DNA dimeric fusion protein that antagonises TNF-α, and it has proven to be effective in children with polyarticular JIA.[118] Evidence for the clinical benefit of etanercept in uveitis has generally been disappointing; it was associated with a high relapse rate and a high risk for developing uveitis flares.[87 100 109 117–121] Based on these findings, the expert group recommend that etanercept should not be considered for JIA-associated uveitis.

Findings from studies including small numbers of patients provide evidence that if treatment with one anti-TNF agent becomes ineffective switching to a different anti-TNF agent could prove to be clinically beneficial.[87 113 116 122] The efficacy and safety of adalimumab was evaluated in in 26 children with JIA resistant to current therapy (disease-modifying drugs in 17 cases and anti-TNF agents in 9 cases). Switching to adalimumab had a beneficial impact on disease control in 17 (65.4%) of patients.[113] In total, 17 patients with severe recalcitrant uveitis (resistant to etanercept, infliximab, adalimumab, rituximab or abatacept) were switched to golimumab and 14 achieved a positive response, and in 12 of these the disease was inactive at the final visit (mean duration 22 months; range 6–29 months).[116] Dhingra *et al* reported preliminary evidence that in seven cases of refractory uveitis switching between biological agents (over a period of 5–24 months) helped to control intraocular inflammation.[122]

While the literature search revealed no direct evidence of the effects of low drug trough levels or the development of anti-drug antibodies (ADAs) on the clinical efficacy of biological agents in patients with uveitis, the expert group considered findings from other clinical settings. They concluded that in cases of loss of efficacy over time consideration should be given to testing for ADAs and drug trough levels.[137 138] If the patient has no antibodies, but has low trough levels, increasing the dose or shortening the interval may be an option.[139]

In keeping with an earlier recommendation (18, table 3), there are data from small studies that in patients with JIA-related uveitis

Table 5 Proposed domains and items for outcome measures of juvenile idiopathic arthritis (JIA)-associated uveitis (from the Multinational Working Group in JIA-related uveitis)[74]

Domains	Items
Grade of cells in anterior chamber	Slit-lamp examination (according to SUN criteria)
Grade of flare in anterior chamber*	Slit-lamp examination for routine clinical practice and prospective trials (according to SUN criteria) Laser flare photometry for prospective trials
Number of visits with active uveitis	Records of treating physician ► Duration of activity over a minimum of four visits/year
Visual acuity (appropriate test for age)	Best-corrected visual acuity Thresholds: ≤20/50, ≤20/200 and no light perception Estimate contribution of amblyopia, yes/no
Development of structural complications	Synechiae, yes/no ► Initial and additional Ocular hypotony, yes/no (<5 mm Hg) Ocular hypertension, yes/no (>21 mm Hg) Glaucoma, yes/no Cataract, yes/no Band keratopathy in the central cornea, yes/no Macular oedema by optical coherence tomography, yes/no ► Funduscopy and optical coherence tomography for routine clinical practice (for macula and optic disc) ► Funduscopy and optical coherence tomography for prospective trials Epiretinal membrane formation, yes/no ► Funduscopy for routine clinical practice ► Funduscopy and optical coherence tomography for prospective trials
Quality of life	Childhood Health Assessment Questionnaire Child Health Questionnaire Pediatric Quality of Life Inventory Uveitis-specific quality of life instrument (not yet available for non-English speaking countries)
Overall uveitis-related disability	Assessment by parents, visual analogue scale Assessment by children, visual analogue scale Assessment by treating ophthalmologist, visual analogue scale Assessment by treating paediatric rheumatologist, visual analogue scale
Social outcome	School/kindergarten absence
Anti-inflammatory medication*	Reduction of corticosteroid dose—topical dose—systemic dose
Surgery*	Yes/no
Biomarkers	Research tools (not currently available

*Not an outcome measure, but should be documented.
SUN, Standardization of Uveitis Nomenclature.

refractory to conventional therapy and at least one anti-TNF therapy switching to drugs such as abatacept, rituximab or tocilizumab may be beneficial.[123–125] This included patients in whom the main cause of poor visual acuity was macular oedema.[125] There is now growing evidence for the role of tocilizumab in macular oedema associated with uveitis.[140 141] There is also an ongoing trial of tocilizumab in children with anti-TNF refractory JIA-associated uveitis (http://www.aptitude-trial.org.uk/).

The optimum time for surgery in children with complications from refractory uveitis has not been addressed as a recommendation due to paucity of evidence. Recent literature does demonstrate that a significant number of children with uveitis still require surgery for complications.[142]

Recommendations for future plans in JIA-related uveitis

A MIWGUC identified the need for clinical trials and longitudinal studies to determine the benefits and costs of health interventions in this setting.[74] To achieve this the group proposed a core set of outcomes aimed at ensuring that changes in relevant outcomes were measured, and that standardisation of outcome measures would facilitate data pooling and comparisons between interventions (tables 4 and 5). The outcomes should be agreed on by both researchers and patients, and they will provide a common focus for interventional studies. Disease-specific and universally agreed on validated outcomes are likely to reduce selective reporting and reporting bias.[74] A limitation of this core set of outcomes is that, despite the fact that there was consensus by the Working Group regarding their utility, they still remain unvalidated. Visual impairment has a significant impact on the quality of life (QOL) of patients with JIA-related uveitis and vision-related QOL relates to the degree to which vision impacts the individual's ability to perform activities of daily living as well as social, emotional and economic well-being.[126–128] The Effects of Youngsters' Eyesight QOL is a useful instrument for measuring the effects of uveitis on QOL which is currently being validated.

The expert group indicated a need for more well-controlled clinical trials in children with JIA-related uveitis to provide the scientific best evidence in the areas of diagnosis, screening, disease activity and treatment to enable the optimal care of these patients.

DISCUSSION

Following a systematic review of the literature and Nominal Group Technique methodology, under the auspices of SHARE and EULAR operating procedures, 22 recommendations for the screening, diagnosis, disease activity monitoring, treatment and future plans for children with JIA-associated uveitis were accepted with at least 80% agreement. In a disease setting where the evidence base is limited by small numbers of patients, and which is developing rapidly, these expert recommendations should help specialists with the evidence-based advice to provide optimal care for their patients.

It should be noted that, in general, the level of evidence was quite low with 13 of 22 recommendations being level 3 or 4, seven level 2 and only two level 1. This highlights the need for more research in this clinical setting where a number of new therapies, particularly biological agents, have been introduced in recent years. At the time that the data search for this article took place, the expert group noted a need for more well-controlled clinical trials in children with JIA-related uveitis. The goal being to ensure that scientific best evidence is used to support optimal treatment. In the interim period, but clearly outside the search time frame of this review, a well-designed randomised controlled trial (RCT) has been published which compared adalimumab with placebo in children with JIA-related uveitis who were taking a stable dose of MTX.[135] Active treatment was shown to control inflammation and was associated with a lower rate of treatment failure compared with placebo. These results do not alter the recommendations of the expert group, but reinforce the intent of recommendation 15 in table 3: 'In case of methotrexate inefficacy or intolerance, adding or switching to biological treatment is recommended'. The only difference being that this recommendation is now supported by level 1 evidence, rather than level 3 evidence. More recently, another smaller RCT of adalimumab has also been published showing efficacy of adalimumab in JIA–uveitis although this study used flare as the primary outcome measure.[143] The utility of biological therapies is receiving wider attention, for example, a number of studies have reported the benefits of tocilizumab.[141 144] These findings from outside the systematic data search time period emphasise the need to regularly update the recommendations of the JIA-associated uveitis expert group so as to provide the highest levels of care in this clinical setting.

Author affiliations
[1]2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary
[2]Klinikum Eilbek, Hamburger Zentrum für Kinder- und Jugendrheumatologie, Hamburg, Germany
[3]Department of Pediatric Rheumatology, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain
[4]Department of Ophthalmology, University Hospital Utrecht, Utrecht, The Netherlands
[5]Service de médecine des adolescents, CHU Bicêtre, AP-HP, 78, rue du Général-Leclerc, Paris, France
[6]Department of Ophthalmology, Great Ormond Street Hospital, London, UK
[7]Department of Ophthalmology, Meir Medical Center, Kfar Sava, Israel
[8]Department of Ophthalmology, Uveitis-Center, and Ophtha Lab, at St. Franziskus Hospital, Muenster, Germany
[9]University of Duisburg-Essen, Duisburg, Germany
[10]Department of Rheumatology, Great Ormond Street Hospital, London, UK
[11]Department of Paediatrics, Rheumatology Unit, Anna Meyer Children's Hospital, University of Florence, Florence, Italy
[12]Department of Paediatrics, Meir Medical Center, Sackler School of Medicine, Tel-Aviv University, Tel Aviv, Israel
[13]Department of Pediatric Rheumatology and Immunology, Wilhelmina Children's Hospital, University Medical Center Utrecht and University of Utrecht, Utrecht, The Netherlands
[14]Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, The Netherlands
[15]Università degli Studi di Genova and Istituto Giannina Gaslini, Genoa, Italy
[16]University Hospitals Bristol NHS Foundation Trust & Bristol Medical School, University of Bristol, Bristol, UK

Acknowledgements The SHARE initiative has been endorsed by the executive committee of the Paediatric Rheumatology European Society and the International Society of Systemic Auto-Inflammatory Diseases. Editorial assistance was provided by Steve Clissold PhD (Content Ed Net, Spain).

Contributors AVR is senior author. NMW and SJV designed the SHARE initiative. AP, VB, A-MH, ZG and RK performed the systematic literature review, supervised by SJV, JdB and NMW. Validity assessment of selected papers was done by TC, IF, JA, JdB, SC-G, CE, RG, AH, CAP, GS, YU, NMW, KW and SJV. Recommendations were formulated by TC, GS, YU, RG and JdB. Provisional recommendations were reviewed by a panel of three experts (IF, NMW and JdB). The expert committee consisted of IF, JA, JdB, SC-G, CE, RG, AH, CAP, GS, YU, SJV and NW; they completed the online surveys and/or participated in the subsequent consensus meeting. AR assisted in the preparation of the live consensus meeting and facilitated the consensus procedure using nominal group technique. TC and AVR wrote the manuscript, with contribution and approval of all coauthors.

Funding Literature search in the preparation of the manuscript was supported by an unrestricted grant from Abbvie. Prof Ramanan is Co-Chief Investigator of APTITUDE Study funded by Arthritis Research UK (Grant Ref 20659).

Competing interests AH: research grants from BMBF, Pfizer and Novartis and honoraria from AbbVie, Alimera Sciences, Allergan, MSD Sharp and Dohme, Pfizer,

Santen, and Xoma. JdB: lectures and advisory board sponsored by AbbVie. SC-G: Pfizer, occasional consulting and registration fees to EULAR/PReS congress and Novartis, registration fees for PReS. IF: advisory board: Abbvie, Novartis, Chugai, Lilly, Medac, Genentech, Bayer. CE: Abbvie: receipt of honoraria and travel expenses. JA has received research grants, speakers bureau or has participated in advisory groups for Abbvie, Pfizer, Roche and BMS. CAP, educational sponsorship from Abbvie. AVR, co-chief investigator of Sycamore Study (funded by NIHR and ARUK). Honoraria/speaker fees from Abbvie, Roche, Lily, UCB and SOBI.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Enders FB, Bader-Meunier B, Baildam E, *et al*. Consensus-based recommendations for the management of juvenile dermatomyositis. *Ann Rheum Dis* 2017;76:329–40.
2 van der Heijde D, Aletaha D, Carmona L, *et al*. 2014 Update of the EULAR standardised operating procedures for EULAR-endorsed recommendations. *Ann Rheum Dis* 2015;74:8–13.
3 Brouwers MC, Kho ME, Browman GP, *et al*. AGREE II: advancing guideline development, reporting and evaluation in health care. *Can Med Assoc J* 2010;182:E839–42.
4 Heiligenhaus A, Michels H, Schumacher C, *et al*. Evidence-based, interdisciplinary guidelines for anti-inflammatory treatment of uveitis associated with juvenile idiopathic arthritis. *Rheumatol Int* 2012;32:1121–33.
5 Bou R, Adán A, Borrás F, *et al*. Clinical management algorithm of uveitis associated with juvenile idiopathic arthritis: interdisciplinary panel consensus. *Rheumatol Int* 2015;35:777–85.
6 Zierhut M, Heiligenhaus A, deBoer J, *et al*. Controversies in juvenile idiopathic arthritis-associated uveitis. *Ocul Immunol Inflamm* 2013;21:167–79.
7 Clarke SLN, Sen ES, Ramanan AV. Juvenile idiopathic arthritis-associated uveitis. *Pediatric Rheumatology* 2016;14:27.
8 Sen ES, Dick AD, Ramanan AV. Uveitis associated with juvenile idiopathic arthritis. *Nat Rev Rheumatol* 2015;11:338–48.
9 Zhang W, *et al*. EULAR evidence based recommendations for gout. Part I: diagnosis. Report of a task force of the standing committee for international clinical studies including therapeutics (ESCISIT). *Ann Rheum Dis* 2006;65:1301–11.
10 Zhang W, Doherty M, Bardin T, *et al*. EULAR evidence based recommendations for gout. Part II: Management. Report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). *Ann Rheum Dis* 2006;65:1312–24.
11 Dougados M, Betteridge N, Burmester GR, *et al*. EULAR standardised operating procedures for the elaboration, evaluation, dissemination, and implementation of recommendations endorsed by the EULAR standing committees. *Ann Rheum Dis* 2004;63:1172–6.
12 Van de Ven AH, Delbecq AL. The nominal group as a research instrument for exploratory health studies. *Am J Public Health* 1972;62:337–42.
13 Uchiyama RC, Osborn TG, Moore TL. Antibodies to iris and retina detected in sera from patients with juvenile rheumatoid arthritis with iridocyclitis by indirect immunofluorescence studies on human eye tissue. *J Rheumatol* 1989;16:1074–8.
14 Giannini EH, Malagon CN, Van Kerckhove C, *et al*. Longitudinal analysis of HLA associated risks for iridocyclitis in juvenile rheumatoid arthritis. *J Rheumatol* 1991;18:1394–7.
15 Malagon C, Van Kerckhove C, Giannini EH, *et al*. The iridocyclitis of early onset pauciarticular juvenile rheumatoid arthritis: outcome in immunogenetically characterized patients. *J Rheumatol* 1992;19:160–3.
16 Murray KJ, Szer W, Grom AA, *et al*. Antibodies to the 45 kDa DEK nuclear antigen in pauciarticular onset juvenile rheumatoid arthritis and iridocyclitis: selective association with MHC gene. *J Rheumatol* 1997;24:560–7.
17 Berk AT, Koçak N, Ünsal E. Uveitis in juvenile arthritis. *Ocul Immunol Inflamm* 2001;9:243–51.
18 Zulian F, Martini G, Falcini F, *et al*. Early predictors of severe course of uveitis in oligoarticular juvenile idiopathic arthritis. *J Rheumatol* 2002;29:2446–53.
19 Chia A, Lee V, Graham EM, *et al*. Factors related to severe uveitis at diagnosis in children with juvenile idiopathic arthritis in a screening program. *Am J Ophthalmol* 2003;135:757–62.
20 Chen CS, Roberton D, Hammerton ME. Juvenile arthritis-associated uveitis: visual outcomes and prognosis. *Can J Ophthalmol* 2004;39:614–20.
21 Nordal EB, Songstad NT, Berntson L, *et al*. Biomarkers of chronic uveitis in juvenile idiopathic arthritis: predictive value of antihistone antibodies and antinuclear antibodies. *J Rheumatol* 2009;36:1737–43.
22 Kalinina Ayuso V, Ten Cate HA, van der Does P, *et al*. Male gender as a risk factor for complications in uveitis associated with juvenile idiopathic arthritis. *Am J Ophthalmol* 2010;149:994–9.
23 Saurenmann RK, Levin AV, Feldman BM, *et al*. Risk factors for development of uveitis differ between girls and boys with juvenile idiopathic arthritis. *Arthritis Rheum* 2010;62:1824–8.
24 Angeles-Han ST, Pelajo CF, Vogler LB, *et al*. Risk markers of juvenile idiopathic arthritis-associated uveitis in the Childhood Arthritis and Rheumatology Research Alliance (CARRA) Registry. *J Rheumatol* 2013;40:2088–96.
25 Cole TS, Frankovich J, Iyer S, *et al*. Profiling risk factors for chronic uveitis in juvenile idiopathic arthritis: a new model for EHR-based research. *Pediatr Rheumatol Online J* 2013;11:45.
26 Calandra S, Gallo MC, Consolaro A, *et al*. Female sex and oligoarthritis category are not risk factors for uveitis in Italian children with juvenile idiopathic arthritis. *J Rheumatol* 2014;41:1416–25.
27 Pelegrín L, Casaroli-Marano R, Antón J, *et al*. Predictive value of selected biomarkers, polymorphisms, and clinical features for oligoarticular juvenile idiopathic arthritis-associated uveitis. *Ocul Immunol Inflamm* 2014;22:208–12.
28 Egeskjold EM, Johansen A, Permin H, *et al*. The significance of antinuclear antibodies in juvenile rheumatoid arthritis associated with chronic bilateral iridocyclitis. *Acta Paediatr Scand* 1982;71:615–20.
29 Cabral DA, Petty RE, Malleson PN, *et al*. Visual prognosis in children with chronic anterior uveitis and arthritis. *J Rheumatol* 1994;21:2370–5.
30 Haas JP, Truckenbrodt H, Paul C, *et al*. Subtypes of HLA-DRB1*03, *08, *11, *12, *13 and *14 in early onset pauciarticular juvenile chronic arthritis (EOPA) with and without iridocyclitis. *Clin Exp Rheumatol* 1994;12(Suppl 10):S7–14.
31 Kotaniemi K, Kotaniemi A, Savolainen A. Uveitis as a marker of active arthritis in 372 patients with juvenile idiopathic seronegative oligoarthritis or polyarthritis. *Clin Exp Rheumatol* 2002;20:109–12.
32 Zeggini E, Packham J, Donn R, *et al*. Association of HLA-DRB1*13 with susceptibility to uveitis in juvenile idiopathic arthritis in two independent data sets. *Rheumatology* 2006;45:972–4.
33 Bloom JN, Ni M, Moore TL, *et al*. Serum antiocular antibodies in patients with juvenile rheumatoid arthritis. *J Pediatr Ophthalmol Strabismus* 1993;30:243–8.
34 Campanilho-Marques R, Bogas M, Ramos F, *et al*. Prognostic value of antinuclear antibodies in juvenile idiopathic arthritis and anterior uveitis. Results from a systematic literature review. *Acta Reumatol Port* 2014;39:116–22.
35 de Boer J, Steijaert A, van den Bor R, *et al*. Development of macular edema and impact on visual acuity in uveitis associated with juvenile idiopathic arthritis. *Ocul Immunol Inflamm* 2015;23:67–73.
36 Ducos de Lahitte G, Terrada C, Tran TH, *et al*. Maculopathy in uveitis of juvenile idiopathic arthritis: an optical coherence tomography study. *Br J Ophthalmol* 2008;92:64–9.
37 Edelsten C, Lee V, Bentley CR, *et al*. An evaluation of baseline risk factors predicting severity in juvenile idiopathic arthritis associated uveitis and other chronic anterior uveitis in early childhood. *Br J Ophthalmol* 2002;86:51–6.
38 Glass D, Litvin D, Wallace K, *et al*. Early-onset pauciarticular juvenile rheumatoid arthritis associated with human leukocyte antigen-DRw5, iritis, and antinuclear antibody. *J Clin Invest* 1980;66:426–9.
39 Grassi A, Corona F, Casellato A, *et al*. Prevalence and outcome of juvenile idiopathic arthritis-associated uveitis and relation to articular disease. *J Rheumatol* 2007;34:1139–45.
40 Heiligenhaus A, Niewerth M, Ganser G, *et al*. Prevalence and complications of uveitis in juvenile idiopathic arthritis in a population-based nation-wide study in Germany: suggested modification of the current screening guidelines. *Rheumatology* 2007;46:1015–9.
41 Ingegnoli F, Del Papa N, Comina DP, *et al*. Autoantibodies to chromatin: prevalence and clinical significance in juvenile rheumatoid arthritis. *Clin Exp Rheumatol* 2004;22:499–501.
42 Kanski JJ. Screening for uveitis in juvenile chronic arthritis. *Br J Ophthalmol* 1989;73:225–8.
43 Kodsi SR, Rubin SE, Milojevic D, *et al*. Time of onset of uveitis in children with juvenile rheumatoid arthritis. *J Aapos* 2002;6:373–6.
44 Kotaniemi K, Sihto-Kauppi K. Occurrence and management of ocular hypertension and secondary glaucoma in juvenile idiopathic arthritis-associated uveitis: An observational series of 104 patients. *Clin Ophthalmol* 2007;1:455–9.
45 Marvillet I, Terrada C, Quartier P, *et al*. Ocular threat in juvenile idiopathic arthritis. *Joint Bone Spine* 2009;76:383–8.
46 Mathur G, Biswas J. Systemic associations of anterior uveitis in a tertiary care ophthalmic centre in south India. *Int Ophthalmol* 2012;32:417–21.
47 McGill NW, Gow PJ. Juvenile rheumatoid arthritis in Auckland: a long term follow-up study with particular reference to uveitis. *Aust N Z J Med* 1987;17:305–8.
48 Melin-Aldana H, Giannini EH, Taylor J, *et al*. Human leukocyte antigen-DRB1*1104 in the chronic iridocyclitis of pauciarticular juvenile rheumatoid arthritis. *J Pediatr* 1992;121:56–60.

49 Merayo-Lloves J, Power WJ, Rodriguez A, *et al*. Secondary glaucoma in patients with uveitis. *Ophthalmologica* 1999;213:300–4.
50 Miller ML, Fraser PA, Jackson JM, *et al*. Inherited predisposition to iridocyclitis with juvenile rheumatoid arthritis: selectivity among HLA-DR5 haplotypes. *Proc Natl Acad Sci U S A* 1984;81:3539–42.
51 Murray P. Serum autoantibodies and uveitis. *Br J Ophthalmol* 1986;70:266–8.
52 Neuteboom GH, Hertzberger-ten Cate R, de Jong J, *et al*. Antibodies to a 15 kD nuclear antigen in patients with juvenile chronic arthritis and uveitis. *Invest Ophthalmol Vis Sci* 1992;33:1657–60.
53 Ohno S, Char DH, Kimura SJ, *et al*. HLA antigens and antinuclear antibody titres in juvenile chronic iridocyclitis. *Br J Ophthalmol* 1977;61:59–61.
54 Ostensen M, Fredriksen K, Kass E, *et al*. Identification of antihistone antibodies in subsets of juvenile chronic arthritis. *Ann Rheum Dis* 1989;48:114–7.
55 Paroli MP, Abbouda A, Restivo L, *et al*. Juvenile idiopathic arthritis-associated uveitis at an Italian tertiary referral center: clinical features and complications. *Ocul Immunol Inflamm* 2015;23:74–81.
56 Paroli MP, Speranza S, Marino M, *et al*. Prognosis of juvenile rheumatoid arthritis-associated uveitis. *Eur J Ophthalmol* 2003;13:616–21.
57 Reininga JK, Los LI, Wulffraat NM, *et al*. The evaluation of uveitis in juvenile idiopathic arthritis (JIA) patients: are current ophthalmologic screening guidelines adequate? *Clin Exp Rheumatol* 2008;26:367–72.
58 Sabri K, Saurenmann RK, Silverman ED, *et al*. Course, complications, and outcome of juvenile arthritis-related uveitis. *J Aapos* 2008;12:539–45.
59 Saurenmann RK, Levin AV, Feldman BM, *et al*. Prevalence, risk factors, and outcome of uveitis in juvenile idiopathic arthritis: a long-term followup study. *Arthritis Rheum* 2007;56:647–57.
60 Schaller JG, Johnson GD, Holborow EJ, *et al*. The association of antinuclear antibodies with the chronic iridocyclitis of juvenile rheumatoid arthritis (Still's disease). *Arthritis Rheum* 1974;17:409–16.
61 Sim KT, Venning HE, Barrett S, *et al*. Extended oligoarthritis and other risk factors for developing JIA-associated uveitis under ILAR classification and its implication for current screening guideline. *Ocul Immunol Inflamm* 2006;14:353–7.
62 Southwood TR, Ryder CA. Ophthalmological screening in juvenile arthritis: should the frequency of screening be based on the risk of developing chronic iridocyclitis? *Br J Rheumatol* 1992;31:633–4.
63 Wakefield D, Herbort CP, Tugal-Tutkun I, *et al*. Controversies in ocular inflammation and immunology laser flare photometry. *Ocul Immunol Inflamm* 2010;18:334–40.
64 Wittemann B, Neuer G, Michels H, *et al*. Autoantibodies to nonhistone chromosomal proteins HMG-1 and HMG-2 in sera of patients with juvenile rheumatoid arthritis. *Arthritis Rheum* 1990;33:1378–83.
65 Woreta F, Thorne JE, Jabs DA, *et al*. Risk factors for ocular complications and poor visual acuity at presentation among patients with uveitis associated with juvenile idiopathic arthritis. *Am J Ophthalmol* 2007;143:647–55.
66 Zannin ME, Buscain I, Vittadello F, *et al*. Timing of uveitis onset in oligoarticular juvenile idiopathic arthritis (JIA) is the main predictor of severe course uveitis. *Acta Ophthalmol* 2012;90:91–5.
67 Kalinina Ayuso V, van de Winkel EL, Rothova A, *et al*. Relapse rate of uveitis post-methotrexate treatment in juvenile idiopathic arthritis. *Am J Ophthalmol* 2011;151:217–22.
68 Papadopoulou C, Kostik M, Böhm M, *et al*. Methotrexate therapy may prevent the onset of uveitis in juvenile idiopathic arthritis. *J Pediatr* 2013;163:879–84.
69 Saboo US, Metzinger JL, Radwan A, *et al*. Risk factors associated with the relapse of uveitis in patients with juvenile idiopathic arthritis: a preliminary report. *J Aapos* 2013;17:460–4.
70 Shakoor A, Esterberg E, Acharya NR. Recurrence of uveitis after discontinuation of infliximab. *Ocul Immunol Inflamm* 2014;22:96–101.
71 Dana MR, Merayo-Lloves J, Schaumberg DA, *et al*. Visual outcomes prognosticators in juvenile rheumatoid arthritis-associated uveitis. *Ophthalmology* 1997;104:236–44.
72 Arocker-Mettinger E, Asenbauer T, Ulbrich S, *et al*. Serum interleukin 2-receptor levels in uveitis. *Curr Eye Res* 1990;9(Suppl 1):25–9.
73 Kalinina Ayuso V, de Boer JH, Byers HL, *et al*. Intraocular biomarker identification in uveitis associated with juvenile idiopathic arthritis. *Invest Ophthalmol Vis Sci* 2013;54:3709–20.
74 Heiligenhaus A, Foeldvari I, Edelsten C, *et al*. Proposed outcome measures for prospective clinical trials in juvenile idiopathic arthritis-associated uveitis: a consensus effort from the multinational interdisciplinary working group for uveitis in childhood. *Arthritis Care Res* 2012;64:1365–72.
75 Walscheid K, Hennig M, Heinz C, *et al*. Correlation between disease severity and presence of ocular autoantibodies in juvenile idiopathic arthritis-associated uveitis. *Invest Ophthalmol Vis Sci* 2014;55:3447–53.
76 Manzotti F, Orsoni JG, Zavota L, *et al*. Autoimmune uveitis in children: clinical correlation between antinuclear antibody positivity and ocular recurrences. *Rheumatol Int* 2002;21:127–32.
77 Massa M, De Benedetti F, Pignatti P, *et al*. Lack of temporal association of iridocyclitis with IgG reactivities to core histones and nucleosome subparticles in pauciarticular juvenile chronic arthritis. *Br J Rheumatol* 1995;34:507–11.
78 Gregory AC, Kempen JH, Daniel E, *et al*. Risk factors for loss of visual acuity among patients with uveitis associated with juvenile idiopathic arthritis: the Systemic Immunosuppressive Therapy for Eye Diseases Study. *Ophthalmology* 2013;120:186–92.
79 Yuce BY, Yilmaz SG, Kose S, *et al*. Outcome of pediatric uveitis at an university clinic. *Turk J Ophthalmol* 2013;43:395–401.
80 Kotaniemi K, Sihto-Kauppi K, Salomaa P, *et al*. The frequency and outcome of uveitis in patients with newly diagnosed juvenile idiopathic arthritis in two 4-year cohorts from 1990-1993 and 2000-2003. *Clin Exp Rheumatol* 2014;32:143–7.
81 Thorne JE, Woreta FA, Dunn JP, *et al*. Risk of cataract development among children with juvenile idiopathic arthritis-related uveitis treated with topical corticosteroids. *Ophthalmology* 2010;117:1436–41.
82 Olson NY, Lindsley CB, Godfrey WA. Nonsteroidal anti-inflammatory drug therapy in chronic childhood iridocyclitis. *Am J Dis Child* 1988;142:1289–92.
83 Wolf MD, Lichter PR, Ragsdale CG. Prognostic factors in the uveitis of juvenile rheumatoid arthritis. *Ophthalmology* 1987;94:1242–8.
84 Sijssens KM, Rothova A, Van De Vijver DA, *et al*. Risk factors for the development of cataract requiring surgery in uveitis associated with juvenile idiopathic arthritis. *Am J Ophthalmol* 2007;144:574–9.
85 Foster CS, Barrett F. Cataract development and cataract surgery in patients with juvenile rheumatoid arthritis-associated iridocyclitis. *Ophthalmology* 1993;100:809–17.
86 Yu EN, Meniconi ME, Tufail F, *et al*. Outcomes of treatment with immunomodulatory therapy in patients with corticosteroid-resistant juvenile idiopathic arthritis-associated chronic iridocyclitis. *Ocul Immunol Inflamm* 2005;13:353–60.
87 Foeldvari I, Nielsen S, Kümmerle-Deschner J, *et al*. Tumor necrosis factor-alpha blocker in treatment of juvenile idiopathic arthritis-associated uveitis refractory to second-line agents: results of a multinational survey. *J Rheumatol* 2007;34:1146–50.
88 Shetty AK, Zganjar BE, Ellis GS, *et al*. Low-dose methotrexate in the treatment of severe juvenile rheumatoid arthritis and sarcoid iritis. *J Pediatr Ophthalmol Strabismus* 1999;36:125–8.
89 Foeldvari I, Wierk A. Methotrexate is an effective treatment for chronic uveitis associated with juvenile idiopathic arthritis. *J Rheumatol* 2005;32:362–5.
90 Heiligenhaus A, Mingels A, Heinz C, *et al*. Methotrexate for uveitis associated with juvenile idiopathic arthritis: value and requirement for additional anti-inflammatory medication. *Eur J Ophthalmol* 2007;17:743–8.
91 Goebel JC, Roesel M, Heinz C, *et al*. Azathioprine as a treatment option for uveitis in patients with juvenile idiopathic arthritis. *Br J Ophthalmol* 2011;95:209–13.
92 Huang JL, Hung IJ, Hsieh KH. Sulphasalazine therapy in chronic uveitis of children with chronic arthritis. *Asian Pac J Allergy Immunol* 1997;15:71–5.
93 Sobrin L, Christen W, Foster CS. Mycophenolate mofetil after methotrexate failure or intolerance in the treatment of scleritis and uveitis. *Ophthalmology* 2008;115:1416–21.
94 Tappeiner C, Roesel M, Heinz C, *et al*. Limited value of cyclosporine A for the treatment of patients with uveitis associated with juvenile idiopathic arthritis. *Eye* 2009;23:1192–8.
95 Molina C, Modesto C, Martín-Begué N, *et al*. Leflunomide, a valid and safe drug for the treatment of chronic anterior uveitis associated with juvenile idiopathic arthritis. *Clin Rheumatol* 2013;32:1673–5.
96 Rajaraman RT, Kimura Y, Li S, *et al*. Retrospective case review of pediatric patients with uveitis treated with infliximab. *Ophthalmology* 2006;113:308–14.
97 Vazquez-Cobian LB, Flynn T, Lehman TJA. Adalimumab therapy for childhood uveitis. *J Pediatr* 2006;149:572–5.
98 Biester S, Deuter C, Michels H, *et al*. Adalimumab in the therapy of uveitis in childhood. *Br J Ophthalmol* 2007;91:319–24.
99 Gallagher M, Quinones K, Cervantes-Castaneda RA, *et al*. Biological response modifier therapy for refractory childhood uveitis. *Br J Ophthalmol* 2007;91:1341–4.
100 Tynjala P, Lindahl P, Honkanen V, *et al*. Infliximab and etanercept in the treatment of chronic uveitis associated with refractory juvenile idiopathic arthritis. *Ann Rheum Dis* 2007;66:548–50.
101 Kotaniemi K, Säilä H, Kautiainen H. Long-term efficacy of adalimumab in the treatment of uveitis associated with juvenile idiopathic arthritis. *Clin Ophthalmol* 2011;5:1425–9.
102 García-De-Vicuña C, Díaz-Llopis M, Salom D, *et al*. Usefulness of adalimumab in the treatment of refractory uveitis associated with juvenile idiopathic arthritis. *Mediators Inflamm* 2013;2013:1–6.
103 Magli A, Forte R, Navarro P, *et al*. Adalimumab for juvenile idiopathic arthritis-associated uveitis. *Graefes Arch Clin Exp Ophthalmol* 2013;251:1601–6.
104 Zholobova E, Galstian L, Nikolaeva MN, *et al*. Effectiveness of adalimumab in the treatment of juvenile idiopathic arthritis associated with uveitis. *Pediatric Rheumatology* 2014;12(Suppl 1):O5.
105 Simonini G, Taddio A, Cattalini M, *et al*. Prevention of flare recurrences in childhood-refractory chronic uveitis: an open-label comparative study of adalimumab versus infliximab. *Arthritis Care Res* 2011;63:612–8.
106 Simonini G, Taddio A, Cattalini M, *et al*. Superior efficacy of Adalimumab in treating childhood refractory chronic uveitis when used as first biologic modifier drug:

Adalimumab as starting anti-TNF-α therapy in childhood chronic uveitis. *Pediatr Rheumatol Online J* 2013;11:16.
107 Zannin ME, Birolo C, Gerloni VM, *et al*. Safety and efficacy of infliximab and adalimumab for refractory uveitis in juvenile idiopathic arthritis: 1-year followup data from the Italian Registry. *J Rheumatol* 2013;40:74–9.
108 William M, Faez S, Papaliodis GN, *et al*. Golimumab for the treatment of refractory juvenile idiopathic arthritis-associated uveitis. *J Ophthalmic Inflamm Infect* 2012;2:231–3.
109 Smith JR, Levinson RD, Holland GN, *et al*. Differential efficacy of tumor necrosis factor inhibition in the management of inflammatory eye disease and associated rheumatic disease. *Arthritis Rheum* 2001;45:252–7.
110 Richards JC, Tay-Kearney ML, Murray K, *et al*. Infliximab for juvenile idiopathic arthritis-associated uveitis. *Clin Exp Ophthalmol* 2005;33:461–8.
111 Kahn P, Weiss M, Imundo LF, *et al*. Favorable response to high-dose infliximab for refractory childhood uveitis. *Ophthalmology* 2006;113:860–4.
112 Tynjälä P, Kotaniemi K, Lindahl P, *et al*. Adalimumab in juvenile idiopathic arthritis-associated chronic anterior uveitis. *Rheumatology* 2008;47:339–44.
113 Trachana M, Pratsidou-Gertsi P, Pardalos G, *et al*. Safety and efficacy of adalimumab treatment in Greek children with juvenile idiopathic arthritis. *Scand J Rheumatol* 2011;40:101–7.
114 Díaz-Llopis M, Salom D, Garcia-de-Vicuña C, *et al*. Treatment of refractory uveitis with adalimumab: a prospective multicenter study of 131 patients. *Ophthalmology* 2012;119:1575–81.
115 Lerman MA, Burnham JM, Chang PY, *et al*. Response of pediatric uveitis to tumor necrosis factor-α inhibitors. *J Rheumatol* 2013;40:1394–403.
116 Miserocchi E, Modorati G, Pontikaki I, *et al*. Long-term treatment with golimumab for severe uveitis. *Ocul Immunol Inflamm* 2014;22:90–5.
117 Saeed MU, Raza SH, Goyal S, *et al*. Etanercept in methotrexate-resistant JIA-related uveitis. *Semin Ophthalmol* 2014;29:1–3.
118 Schmeling H, Horneff G. Etanercept and uveitis in patients with juvenile idiopathic arthritis. *Rheumatology* 2005;44:1008–11.
119 Smith JA, Thompson DJ, Whitcup SM, *et al*. A randomized, placebo-controlled, double-masked clinical trial of etanercept for the treatment of uveitis associated with juvenile idiopathic arthritis. *Arthritis Rheum* 2005;53:18–23.
120 Saurenmann RK, Levin AV, Feldman BM, *et al*. Risk of new-onset uveitis in patients with juvenile idiopathic arthritis treated with anti-TNFalpha agents. *J Pediatr* 2006;149:833–6.
121 Kakkassery V, Mergler S, Pleyer U. Anti-TNF-alpha treatment: a possible promoter in endogenous uveitis? observational report on six patients: occurrence of uveitis following etanercept treatment. *Curr Eye Res* 2010;35:751–6.
122 Dhingra N, Morgan J, Dick AD. Switching biologic agents for uveitis. *Eye* 2009;23:1868–70.
123 Zulian F, Balzarin M, Falcini F, *et al*. Abatacept for severe anti-tumor necrosis factor alpha refractory juvenile idiopathic arthritis-related uveitis. *Arthritis Care Res* 2010;62:821–5.
124 Heiligenhaus A, Miserocchi E, Heinz C, *et al*. Treatment of severe uveitis associated with juvenile idiopathic arthritis with anti-CD20 monoclonal antibody (rituximab). *Rheumatology* 2011;50:1390–4.
125 Mesquida M, Molins B, Llorenç V, *et al*. Long-term effects of tocilizumab therapy for refractory uveitis-related macular edema. *Ophthalmology* 2014;121:2380–6.
126 Angeles-Han ST, Griffin KW, Lehman TJ, *et al*. The importance of visual function in the quality of life of children with uveitis. *J Aapos* 2010;14:163–8.
127 Angeles-Han ST, Griffin KW, Harrison MJ, *et al*. Development of a vision-related quality of life instrument for children ages 8-18 years for use in juvenile idiopathic arthritis-associated uveitis. *Arthritis Care Res* 2011;63:1254–61.
128 Ezzahri M, Amine B, Rostom S, *et al*. The uveitis and its relationship with disease activity and quality of life in Moroccan children with juvenile idiopathic arthritis. *Clin Rheumatol* 2013;32:1387–91.
129 Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. *Lancet* 2011;377:2138–49.
130 Thierry S, Fautrel B, Lemelle I, *et al*. Prevalence and incidence of juvenile idiopathic arthritis: a systematic review. *Joint Bone Spine* 2014;81:112–7.
131 Yu HH, Chen PC, Wang LC, *et al*. Juvenile idiopathic arthritis-associated uveitis: a nationwide population-based study in Taiwan. *PLoS One* 2013;8:e70625.
132 Shen CC, Yeh KW, Ou LS, *et al*. Clinical features of children with juvenile idiopathic arthritis using the ILAR classification criteria: a community-based cohort study in Taiwan. *J Microbiol Immunol Infect* 2013;46:288–94.
133 Heiligenhaus A, Heinz C, Edelsten C, *et al*. Review for disease of the year: epidemiology of juvenile idiopathic arthritis and its associated uveitis: the probable risk factors. *Ocul Immunol Inflamm* 2013;21:180–91.
134 Ramanan AV, Guly C. Adalimumab for childhood onset uveitis. *Ann Rheum Dis* 2018;77:961–2.
135 Ramanan AV, Dick AD, Jones AP, *et al*. Adalimumab plus Methotrexate for Uveitis in Juvenile Idiopathic Arthritis. *N Engl J Med* 2017;376:1637–46.
136 Dick AD, Rosenbaum JT, Al-Dhibi HA, *et al*. Guidance on Noncorticosteroid Systemic Immunomodulatory Therapy in Noninfectious Uveitis: Fundamentals Of Care for UveitiS (FOCUS) Initiative. *Ophthalmology* 2018;S0161-6420:32446–6.
137 Leinonen ST, Aalto K, Kotaniemi KM, *et al*. Anti-adalimumab antibodies in juvenile idiopathic arthritis-related uveitis. *Clin Exp Rheumatol* 2017;35:1043–6.
138 Skrabl-Baumgartner A, Erwa W, Muntean W, *et al*. Anti-adalimumab antibodies in juvenile idiopathic arthritis: frequent association with loss of response. *Scand J Rheumatol* 2015;44:359–62.
139 Correll CK, Bullock DR, Cafferty RM, *et al*. Safety of weekly adalimumab in the treatment of juvenile idiopathic arthritis and pediatric chronic uveitis. *Clin Rheumatol* 2018;37:549–53.
140 Mesquida M, Molins B, Llorenç V, *et al*. Twenty-four month follow-up of tocilizumab therapy for refractory uveitis-related macular edema. *Retina* 2017:1.
141 Calvo-Río V, Santos-Gómez M, Calvo I, *et al*. Anti-Interleukin-6 Receptor Tocilizumab for Severe Juvenile Idiopathic Arthritis-Associated Uveitis Refractory to Anti-Tumor Necrosis Factor Therapy: A Multicenter Study of Twenty-Five Patients. *Arthritis Rheumatol* 2017;69:668–75.
142 Ferrara M, Eggenschwiler L, Stephenson A, *et al*. The Challenge of Pediatric Uveitis: Tertiary Referral Center Experience in the United States. *Ocul Immunol Inflamm* 2018;15:1–8.
143 Quartier P, Baptiste A, Despert V, *et al*. ADJUVITE Study Group. ADJUVITE: a double-blind, randomised, placebo-controlled trial of adalimumab in early onset, chronic, juvenile idiopathic arthritis-associated anterior uveitis. *Ann Rheum Dis* 2018;77:1003–11.
144 Tappeiner C, Mesquida M, Adán A, *et al*. Evidence for Tocilizumab as a Treatment Option in Refractory Uveitis Associated with Juvenile Idiopathic Arthritis. *J Rheumatol* 2016;43:2183–8.

EXTENDED REPORT

Determinants of happiness and quality of life in patients with rheumatoid arthritis: a structural equation modelling approach

Eduardo José Ferreira Santos,[1,2,3] Cátia Duarte,[1,4] Ricardo J O Ferreira,[1,3] Ana Margarida Pinto,[1,4] Rinie Geenen,[5] Jose A P da Silva,[1,6] On behalf of the 'Promoting Happiness Through Excellence of Care' Group

Handling editor Josef S Smolen

[1]Department of Rheumatology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[2]Escola Superior de Enfermagem do Porto, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[3]Health Sciences Research Unit: Nursing, Nursing School of Coimbra, Coimbra, Portugal
[4]Clínica Universitária de Reumatologia, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[5]Department of Psychology, Utrecht University, Utrecht, The Netherlands
[6]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine – University of Coimbra, Coimbra, Portugal

Correspondence to
Professor Jose A P da Silva, Department of Rheumatology, Centro Hospitalar e Universitário de Coimbra, Coimbra 3000-076, Portugal; jdasilva@ci.uc.pt

Received 31 December 2017
Revised 15 March 2018
Accepted 22 March 2018
Published Online First 6 April 2018

To cite: Santos EJF, Duarte C, Ferreira RJO, *et al*. *Ann Rheum Dis* 2018;**77**:1118–1124.

ABSTRACT

Objectives Besides increasing longevity, the ultimate goal of medical care is to improve patients' enjoyment of life, a concept akin to happiness. This study examined the determinants of happiness and quality of life (QoL) in patients with rheumatoid arthritis (RA).

Methods In this observational, cross-sectional study, patients were assessed on disease activity, disease impact, personality, QoL and happiness. Structural equation modelling estimation was used to analyse the associations between these dimensions, pursuing three hypotheses: H_1—disease activity and perceived impact of disease are negatively associated with overall QoL and happiness in patients with RA; H_2—'positive' personality traits are related to happiness both directly and indirectly through perceived disease impact; H_3—happiness has a mediating effect in the relation between impact of disease and QoL.

Results Data from 213 patients were analysed. Results supported all driving hypotheses. Happiness was positively related to 'positive' personality and, to a lesser extent, negatively related to impact of disease. Impact of disease, in turn, was positively related to disease activity and mitigated by 'positive' personality traits. Impact of disease had a much stronger relation with QoL than with happiness. Happiness mitigated the negative effect of disease impact on QoL.

Conclusion Optimisation of QoL and happiness of people with RA requires effective control of the disease process and also improvement of the disease impact domains. Personality seems to play a pivotal mediating role in these relations.

INTRODUCTION

The current paradigm for the management of rheumatoid arthritis (RA), in both clinical and research settings, is epitomised by the treat-to-target strategy[1 2] which establishes that the target of remission, or at least low disease activity, should be pursued and achieved as early and consistently as possible. This target is defined essentially by measures designed to gauge the disease process: number of tender and swollen joints and acute phase reactants supplemented by the patient's and physician's global impression of disease activity.[3] The incorporation of patient-reported outcomes (PROs), designed to provide the patient's perspective of the disease[4–9] into clinical practice and research, is widely supported by international organisations and professional groups.[2 4 10]

Many studies have shown that the control of inflammation through immunosuppressive therapy has a markedly positive impact on PROs: controlling the disease process is, undoubtedly, as important to prevent long-term damage as to improve patients' quality of life (QoL).[2 4–6 11 12] Despite this, a sizeable proportion of patients with RA who are in remission still describe a high impact of disease[13 14] and reduced QoL.[15]

Our group has recently highlighted this view by proposing that the management of RA should pursue two different targets: disease process remission and disease impact control.[13 14] Controlling the disease impact, in terms of quality and duration of life, are the final objectives of disease management, while controlling the disease process should be seen as an important means to that end, but not a guarantee.

Within this perspective, the concept of overall subjective well-being, equivalent to 'happiness', emerges as a decisive goal as well ('the ultimate currency').[16–18] All healthcare professionals know patients who lead a reasonably happy and fulfilling life despite aggressive disease, while others seem to succumb to the diagnosis. Understanding the main determinants of happiness in patients with rheumatic diseases and exploring the potential avenues to maximise it is, in this light, an ethical obligation. Curing or controlling disease is, certainly, an essential contribution, but we need to understand how far disease control can go towards happiness and whether health professionals may contribute to that goal beyond disease control.

Happiness includes different aspects of life such as life satisfaction, healthy interpersonal relationships, personal growth and appreciation of nature, beauty and other people, resulting in a global predominance of positive emotions over negative ones.[16 17] QoL is more focused on physical functioning and negative mental aspects, such as depressed mood and anxiety.[18 19] Happiness is, therefore, a broader concept than QoL, as it goes beyond the ability to do things and incorporates the satisfaction of doing them, that is, the enjoyment of life as a whole.[18 19] Personality is recognised as a key factor in predicting happiness,[16 20 21] as it provides the context in which the roots of happiness operate.[22] Although happiness levels may be

negatively influenced by the experience of living with a disease, especially if it has a chronic course and causes a marked impairment in daily functioning, several studies in this area have also demonstrated that happiness may have a positive impact on physical health and longevity. This has been mostly attributed to its effect on the perception of impact disease and on the engagement in health-related behaviours.[18]

Based on the previous literature, this study was designed to address the following hypotheses in patients with RA:

- H_1—Disease activity and perceived impact of disease are negatively associated to overall QoL and happiness;
- H_2—'Positive' personality traits are related with happiness, both directly and indirectly through perceived disease impact;
- H_3—Happiness has a mediating effect in the relation between impact of disease and QoL.

METHODS

Participants and study design

We used data from an observational, cross-sectional study, performed in a single rheumatology outpatient department,[14] that aimed at exploring the determinants of patient global assessment. The study included consecutive adult patients with RA[23 24] who (1) were followed and treated according to standard guidelines, (2) had the ability to read and interpret the questionnaires applied, and (3) agreed to participate. The current analysis included data from patients who answered all measurements required.

All participants provided informed written consent before the start of study procedures, and the ethical approval was granted by the University of Coimbra's Faculty of Medicine Ethics Committee (CEU 037/2015).

Measures/instruments

Data collection included the Rheumatoid Arthritis Impact of Disease score,[25 26] which is composed of seven items rated on a 10-point numeric rating scale. A higher score indicates greater impact of the disease. Happiness was assessed through the Subjective Happiness Scale (SHS),[27] a four-item measure (seven-point Likert scale). A higher mean score indicates more intense perception of a 'happy life'. Personality was assessed by the Ten-Item Personality Inventory (TIPI),[28] a brief measure of the Big-Five personality dimensions, each being scored as the mean of two items (seven-point Likert scale) addressing extraversion, agreeableness, conscientiousness, emotional stability and openness to experience. Higher scores indicate a stronger expression of the respective trait. We designated the latent higher order factor derived from TIPI as 'Positive' personality to represent the predominantly adaptive nature of the represented dimensions. We recognise that the term 'positive' is questionable especially in the extremes of expression of certain traits, such as conscientiousness. Health-related QoL was accessed by the EuroQOL (EQ-5D) questionnaire, which includes five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). Each dimension has three levels: no problems, some problems and extreme problems. The combination of the five scores leads to an index score between −0.59 and 1.00.[29] Higher scores indicate a best perceived health status and QoL.

Disease activity was measured with the Disease Activity Score 28 joints (DAS28), in its three variables (3v) and C reactive protein (CRP) variant—DAS28CRP(3v).[30]

For patient's characterisation, demographic data, disease characteristics, comorbidities and current treatment were collected.

Data analysis

Descriptive and correlational analyses were performed with SPSS V.23 (IBM). Pearson correlation analyses were conducted to examine the associations between disease activity, measures of disease impact, personality traits, QoL and happiness and interpreted as small (0.10 to 0.30), moderate (0.30 to 0.50) or large (>0.50).[31]

Structural equation modelling (SEM, latent variable structural model) was used to estimate the association between the variables under analysis in the theoretical model and performed with AMOS V.24.0 (IBM SPSS, Chicago, Illinois, USA), using a maximum-likelihood estimation. SEM defines latent variables (summary constructs) from one or more observed variables and examines in a structured way models specifying relationships between these latent variables.

Prior to this analysis, the assumptions of normality and multicollinearity were confirmed. Skewness values ranged from −0.93 to 0.98, while values of kurtosis ranged from –1.1 to 1.29, indicating no violation of univariate and multivariate normality.[32] Variance inflation factor values were below 5 for all variables included in the model, excluding multicollinearity as an issue.

As recommended, different goodness-of-fit indices were used to estimate the model fit, namely (1) the χ^2, (2) the Comparative-of-Fit Index (CFI), (3) the Goodness-of-Fit Index (GFI), (4) the Tucker-Lewis Index (TLI) and (5) the root mean square error of approximation (RMSEA). A good fit of the models was assumed when the ratio of χ^2 to its df was less than 3.0 and CFI, GFI and TLI were larger than 0.90[33]; RMSEA values <0.06 were considered ideal and values between 0.08 and 0.10 were considered acceptable.[34]

Four covariances were entered in the measurement model following modification indices examination/analysis.

The examination of the structural model included a test of the overall model fit as well as individual tests of the relationships among latent constructs. Statistically significant effects were assumed for $P < 0.05$. Other paths with theoretical and clinical plausibility were also tested (DAS28CRP3v→happiness; 'positive' personality→QoL). Non-significant paths were excluded, and the initially proposed model was readjusted accordingly. Furthermore, the bootstrap resampling method, with 700 bootstrap samples and 95% bias-corrected CIs around the standardised estimates of total, direct and indirect effects, was used to test the significance of the mediational path.[35]

To address the potential bias due to missing data, we tested a model-based missing data method (full information maximum-likelihood), which did not show significant differences. In the end, we preferred to use only truly obtained data.

RESULTS

Patient characteristics

This study included 213 of the original sample of 309 patients with RA due to missing data. Baseline demographic and clinical characteristics of patients are presented in table 1. Participants were aged between 27 and 88 (M=57.8) years and had a mean disease duration of 11.8 years. Around one-third (n=69, 32.4%) of patients had no identified comorbidities. The mean DAS28CRP3v was 2.48, with 59.6% (n=127) of patients being in remission according to this index.

Table 1 Demographic and clinical characteristics of 213 patients with RA

Variables	Scores
Age, years, mean (SD)	57.8 (13.2)
Female gender, n (%)	172 (80.8)
Disease duration, years, mean (SD)	11.8 (8.9)
Rheumatoid factor positive, n (%)*	154 (72.3)
Anticitrullinated antibody positive, n (%)*	101 (70.6)
Comorbidities, yes, n (%)	
Fibromyalgia*	35 (16.4)
Depression*	38 (17.8)
Low back pain*	40 (18.8)
Osteoporotic fractures*	16 (7.5)
Osteoarthritis*	108 (50.7)
Stroke*	4 (1.9)
Current treatment with biologic agents, n (%)	66 (31)
Tender joint counts using 28 joints (0–28), mean (SD)	1.52 (3.2)
Swollen joint counts using 28 joints (0–28), mean (SD)	1.46 (2.7)
C reactive protein, CRP (mg/dL), mean (SD)	0.81 (1.4)
Disease Activity, DAS28CRP3v (0–9.4), mean (SD)	2.48 (0.93)
Remission, n (%)	127 (59.6)
Low, n (%)	49 (23)
Moderate, n (%)	34 (16)
High, n (%)	3 (1.4)
Physician global assessment (VAS, 0–100), mean (SD)	14.2 (15. 9)
Patient global assessment (VAS, 0–100), mean (SD)	47.5 (28.6)
Rheumatoid Arthritis Impact of Disease (0–10), mean (SD)	
Pain	4.8 (2.5)
Functional disability	4.9 (2.6)
Fatigue	5.1 (2.7)
Emotional well-being	4.6 (2.7)
Sleep	4.4 (2.9)
Coping	4.2 (2.7)
Physical well-being	4.9 (2.5)
EuroQOL five dimensions (–0.59 to 1), mean (SD)	0.43 (0.26)
Subjective Happiness Scale (1–7), mean (SD)	4.8 (1.3)
Ten-Item Personality Inventory (1–7), mean (SD)	
Extraversion	4.1 (1.5)
Agreeableness	5.7 (1.3)
Conscientiousness	5.6 (1.3)
Emotional stability	3.7 (1.5)
Openness to experience	4.4 (1.5)

*Percentages of patients with missing data were <2.8%, except for ACPA (32.8%) and erosions (18.8%), fibromyalgia (7%), depression (7.5%), low back pain (10.3%), osteoporotic fractures (19.7%), osteoarthritis (8.9%) and stroke (8.5%).

Correlation coefficients

Pearson correlation coefficients for the measured variables are presented in table 2.

As expected, QoL was found to be strongly and inversely correlated with impact of disease.

The personality traits extraversion, emotional stability and openness to experience were associated, with low correlations, with QoL and with virtually all aspects of impact of disease. Openness to experience was not associated with sleep. All happiness items except item 4 presented moderate positive correlations, with QoL; low to moderate positive correlations with all personality traits, except for agreeableness (not significant at SHS 1 and 3); and negative correlations, with impact of disease. Finally, DAS28CRP3v showed moderate associations with impact of disease (positive correlation) and QoL (negative correlations), low correlations with happiness and no significant correlations with each personality trait.

The fourth question of SHS (which was a complex item with a negative formulation and reversed scoring) showed a totally discordant profile vis-a-vis the other three (ie, harming internal consistency of the SHS). For this reason, this question was not included in the happiness construct when we performed the structural equations model, as technically recommended.[34]

Structural equation modelling

The overall fit of the final measurement model was good, thus permitting the examination of the structural model ($\chi^2_{(111)}$=154.22, χ^2/df=1.38, P=0.004; CFI=0.98; GFI=0.92; TLI=0.97; RMSEA=0.04, 95% CI 0.02 to 0.05). Although the χ^2 statistic was significant (P<0.05), its ratio regarding the df was within the accepted range (χ^2/df <3).[33]

The direct path coefficients for the model are shown in table 3 and figure 1. The bootstrap indirect effects are shown in table 4.

H_1—*Disease activity and perceived impact of disease are negatively associated to overall QoL and happiness in patients with RA.*

Impact of disease showed a significant negative direct relation with QoL (β=−0.70; P<0.001) and happiness (β=−0.17; P=0.02). Impact of disease was higher with higher disease activity (DAS28CRP3v) (β=0.36; P<0.001) (table 3 and figure 1).

Moreover, disease activity had also a negative indirect effect of −0.26 (P=0.003) on QoL, through the perception of impact of disease (table 4).

H_2—*'Positive' personality traits are related with happiness, both directly and indirectly through perceived disease impact.*

'Positive' personality traits had a total effect of 0.56 on happiness, being a direct effect of β=0.50 (P<0.001) and an indirect effect of β=0.06 (P=0.03) through impact of disease.

'Positive' personality traits showed also a negative direct relation with impact of disease (β=−0.37; P<0.001), and an indirect effect of β=0.33 (P=0.004) on QoL, through the impact of disease (tables 3 and 4 and figure 1).

'Positive' personality and disease activity explained 27% of the variance of impact of disease (R^2=0.27) (figure 1).

H_3—*Happiness has a mediating effect in the relation between impact of disease and QoL.*

Impact of disease had a total effect of 0.72 on QoL, of which β=−0.02 (P=0.04) was an indirect effect through happiness, indicating a mediating influence between this relationship. Furthermore, there was a significant direct association between happiness and QoL (β=0.13; P=0.01) (tables 3 and 4 and figure 1).

Disease activity had a negative indirect effect of β=−0.06 (P=0.04) on happiness, through the perception of impact of disease (table 4).

Altogether, happiness and impact of disease explained 57% of the variance of QoL (R^2=0.57), and 35% of the variance of happiness (R^2=0.35) was explained by impact of disease and personality traits (figure 1).

DISCUSSION

This study provides a comprehensive model that illustrates the relationships between disease activity, impact of disease, personality traits, QoL and happiness in people with RA. Overall, the results show that happiness is related to a 'positive' personality and, to a small extent, to the perception of impact of disease. The latter was, in turn, positively related to disease activity and

Table 2 Pearson correlation coefficients among variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Impact of disease																				
Pain (1)	1.00																			
Functional disability (2)	0.82**	1.00																		
Fatigue (3)	0.76**	0.82**	1.00																	
Sleep (4)	0.66**	0.69**	0.71**	1.00																
Physical well-being (5)	0.75**	0.82**	0.84**	0.73**	1.00															
Emotional well-being (6)	0.71**	0.72**	0.77**	0.75**	0.85**	1.00														
Coping (7)	0.72**	0.74**	0.79**	0.70**	0.81**	0.80**	1.00													
RAID score (8)	0.89**	0.91**	0.91**	0.83**	0.92**	0.89**	0.89**	1.00												
Quality of life (9)	−0.60**	−0.69**	−0.68**	−0.59**	−0.69**	−0.64**	−0.63**	−0.73**	1.00											
Positive personality																				
Extraversion (10)	−0.18**	−0.20**	−0.23**	−0.20**	−0.21**	−0.19**	−0.24**	−0.23**	0.23**	1.00										
Agreeableness (11)	−0.03	−0.01	−0.03	−0.10	−0.03	−0.14*	−0.10	−0.07	0.04	0.04	1.00									
Conscientiousness (12)	−0.01	−0.06	−0.08	−0.15*	−0.07	−0.15*	−0.12	−0.1	0.10	0.28**	0.40**	1.00								
Emotional stability (13)	−0.21**	−0.26**	−0.29**	−0.29**	−0.29**	−0.35**	−0.25**	−0.31**	0.25**	0.32**	0.20**	0.21**	1.00							
Openness to experience (14)	−0.16*	−0.21**	−0.27**	−0.11	−0.21**	−0.18**	−0.24**	−0.23**	0.15*	0.39**	0.18**	0.28**	0.21**	1.00						
Happiness																				
SHS 1 (15)	−0.22**	−0.21**	−0.28**	−0.29**	−0.25**	−0.32**	−0.29**	−30**	0.31**	0.36**	0.12	0.24**	0.32**	0.17*	1.00					
SHS 2 (16)	−0.26**	−0.23**	−0.31**	−0.30**	−0.30**	−0.33**	−0.32**	−0.33**	0.36**	0.33**	0.17*	0.28**	0.31**	0.23**	0.82**	1.00				
SHS 3 (17)	−0.26**	−0.18**	−0.28**	−0.25**	−0.29**	−0.31**	−0.32**	−0.30**	0.33**	0.39**	0.10	0.23**	0.31**	0.25**	0.58**	0.60**	1.00			
SHS 4 (18)	0.04	0.09	0.05	0.04	0.08	0.07	0.09	0.08	−0.07	−0.02	0.03	−0.01	−0.04	−0.05	0.04	0.02	0.09	1.00		
SHS three-item score (19)	−0.28**	−0.24**	−0.33**	−0.32**	−0.33**	−0.37**	−0.36**	−0.35**	0.38**	0.41**	0.15*	0.29**	0.36**	0.25**	0.90**	0.91**	0.84**	0.07	1.00	
DAS28CRP3v (20)	0.34**	0.35**	0.31**	0.32**	0.35**	0.30**	0.32**	0.37**	−0.34**	−0.001	−0.05	0.01	−0.11	0.01	−0.15*	−0.21**	−0.15*	0.09	−0.20**	1.00

DAS28CRP3v, DiseaseActivity Score using 28 joints and C reactive protein and three variables; RAID, Rheumatoid Arthritis Impact of Disease; SHS, Subjective Happiness Scale.
*P<0.05, ** P<0.001.

Table 3 Regression weights between structural parameters

	Unstandardised direct effects	Standardised direct effects	SE	Critical ratio	Significance level
Impact of disease←positive personality	−0.84	−0.37	0.19	−4.30	<0.001
Impact of disease←DAS28CRP3v	0.91	0.36	0.16	5.66	<0.001
Happiness←positive personality	0.59	0.50	0.12	4.81	<0.001
Happiness←impact of disease	−0.09	−0.17	0.03	−2.31	0.02
Coping←impact of disease	1.00	0.87			
Emotional well-being←impact of disease	1.01	0.90	0.05	18.99	<0.001
Physical well-being←impact of disease	1.00	0.94	0.04	21.09	<0.001
Sleep←impact of disease	0.98	0.80	0.06	15.23	<0.001
Fatigue←impact of disease	1.02	0.90	0.05	19.05	<0.001
Function disability←impact of disease	0.98	0.89	0.05	18.51	<0.001
Pain←impact of disease	0.88	0.82	0.05	15.84	<0.001
Extraversion←positive personality	1.00	0.67			
Agreeableness←positive personality	0.38	0.32	0.11	3.20	0.001
Conscientiousness←positive personality	0.55	0.46	0.11	5.02	<0.001
Emotional stability←positive personality	0.76	0.52	0.13	5.57	<0.001
Openness to experience←positive personality	0.77	0.54	0.13	5.68	<0.001
SHS 1←happiness	1.00	0.89			
SHS 2←happiness	1.08	0.92	0.06	15.95	<0.001
SHS 3←happiness	0.88	0.67	0.08	10.95	<0.001
Quality of life←impact of disease	−0.08	−0.70	0.01	−12.20	<0.001
Quality of life←happiness	0.03	0.13	0.01	2.44	0.014

Unstandardised direct effects come directly out of the estimation procedure. Due to the metric differences of the instruments, in this case, standardised direct effects should be preferred to indicate the strength of the associations (magnitude between −1 and +1). Higher absolute values indicate a stronger (positive or negative) association. An absolute critical ratio >1.96 reflects that path coefficients are significant at the 0.05 level.
DAS28CRP3v, Disease Activity Score using 28 joints and C reactive protein and three variables; SHS, Subjective Happiness Scale.

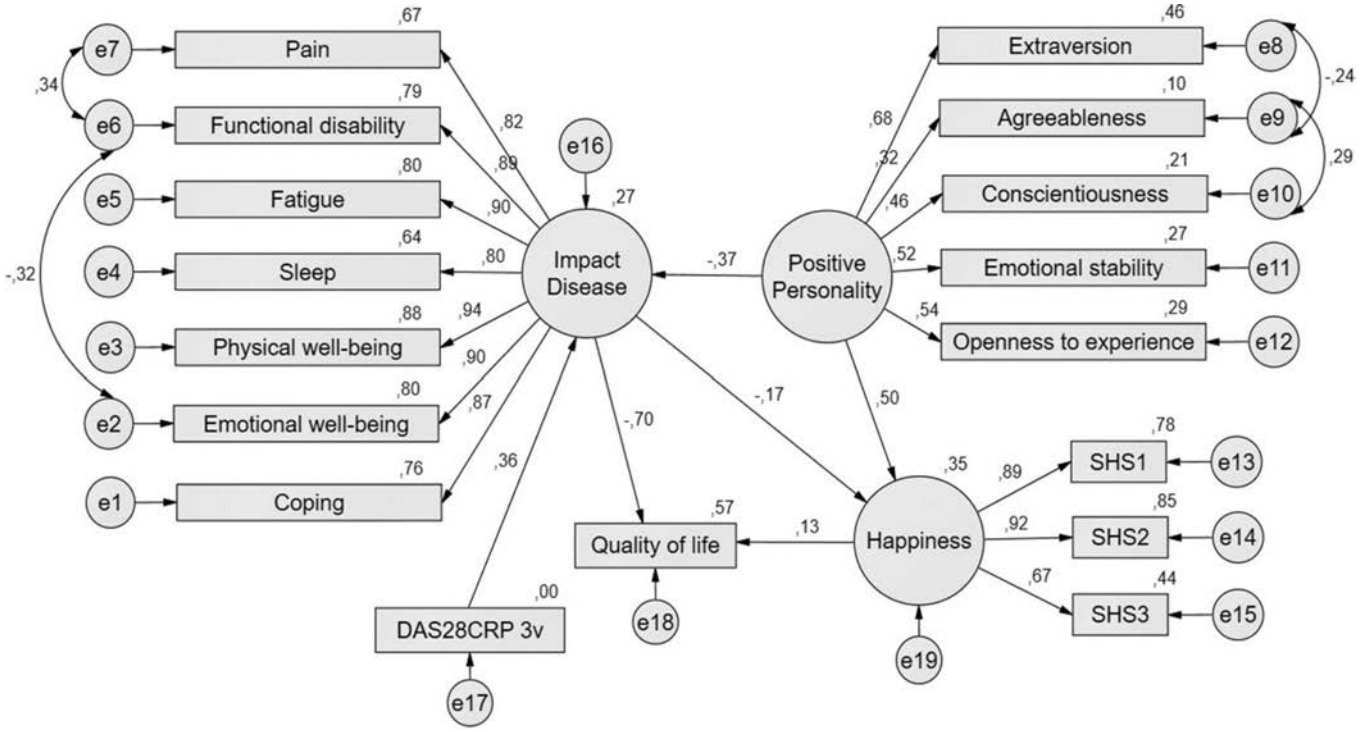


Figure 1 Estimated standardised direct effects for the proposed model. Circles represent latent factors. Squares represent measured variables (the scale scores). Arrows connecting circles and rectangles in one direction show a hypothesized direct relationship between the two variables. Curved lines with an arrow in both directions demonstrate a bi-directional relationship (covariance). Circles with the letter "e" written in it represent the associated error. DAS28CRP3v, Disease Activity Score using 28 joints and C-reactive protein and three variables; SHS, Subjective Happiness Scale.

Table 4 Bootstrap results for indirect effects between structural parameters

	Quality of life		Happiness	
	Estimates, SE	95% CI, significance level	Estimates, SE	95% CI, significance level
DAS28CRP3v	β=−0.26, 0.05	(−0.36 to −0.16), 0.003	β=−0.06, 0.03	(−0.13 to −0.01), 0.04
Positive personality	β=0.33, 0.06	(0.21 to 0.45), 0.004	β=0.06, 0.03	(0.01 to 0.14), 0.03
Impact of disease	β=−0.02, 0.01	(−0.06 to −0.001), 0.04	–	

Standardised indirect effects indicate the strength of the associations (magnitude between −1 and +1). Higher absolute values indicate a stronger (positive or negative) association.
DAS28CRP3v, Disease Activity Score using 28 joints and C reactive protein and three variables.

mitigated by 'positive' personality with very similar weights. Our findings also show that happiness mediates (and mitigates) the association between impact of disease and QoL. Impact of disease has a stronger relation with QoL than with happiness, further supporting the distinct nature of the latter two concepts.

Taken together, these findings imply important clinical implications. Assuming that the perceived impact of disease is, in itself, a valuable treatment target, the model suggests that healthcare professionals should consider personality traits while making the best efforts to control the disease process. In fact, disease activity and personality explained around 27% of the variance in perceived impact, with similar weights for each.

If quality of life is elected as a high-priority treatment objective,[8] the perceived impact of disease should be acknowledged as major determinant,[36 37] but, to a lesser extent, happiness should be considered an ameliorating factor as well. Happiness has been shown to be related to QoL[38 39] and to a variety of better health outcomes, also in a prospective study.[39]

If happiness is taken as the ultimate goal of disease management, the model suggests that personality traits are the most important determinants, with small influences of perceived impact of disease and QoL. The relationship between personality traits, most clearly extraversion, and happiness is well established in the literature.[16 20 21] Our results highlight that this association persists even in the presence of a severely impacting disease, such as RA. Four personality domains seem particularly important in this association: extraversion, emotional stability, conscientiousness and openness to experience. Multiple potential mechanisms may explain these associations: the ability to establish positive personal relationships,[40] to adopt positive attitudes in life's challenging events[41 42] and to accept novel attitudes and unaccustomed values[16] have all been shown to be important ingredients of happiness. It is easy to conceive that they become even more important when facing such a challenging health condition. According to our model, the disease activity control on happiness is indirect, through perceived disease impact, and accounts only for ∼6% of its variance.

Our results should be interpreted while taking into account some limitations. First, although the sample size and the diversity of patients' characteristics were satisfactory, the recruitment was performed in a single centre, which advises caution in results' generalisation. Second, this was a cross-sectional design, not allowing testing causal relationships: longitudinal studies are thus indispensable to further assess the associations suggested here. Third, although we have accessed the presence of some comorbidities, we did not use a validated index for that purpose. This precluded the inclusion of this variable in the statistical analyses, despite its potential confounder effect. Fourth, all variables of this study are also influenced by other factors, such as material wealth, occupation and loneliness, which were not accounted for in the present study, as it was focused on exploring the relevance of disease activity. Finally, the reader should take into account that the concepts of happiness and QoL herein should be interpreted according to the instruments used to define them.

In summary, our results indicate, in line with a substantial literature, that personality traits have a considerable influence on how impactful/disrupting patients perceive their disease to be, with decisive consequences on their QoL, and also on how happy they feel towards life. Taken together, our observations indicate that treatment strategies focused solely on the control of disease activity can be expected to have only a limited impact on QoL and a probably minor effect on happiness. Personality traits represent another realm of potential intervention towards minimising the effects of disease on patients' lives. They seem to be as important as disease control regarding QoL and more important than the disease process if happiness is taken as the ultimate goal. Fully gauging these dimensions would require a more detailed evaluation of patients and a wider scope of interventions than usually done in rheumatology practice.

This can only be attained by multidisciplinary teams working to optimise RA management through tight control of the disease process and also by exploring the full potential of interventions beyond immunosuppression. Within this context, appropriate pain control and non-pharmacological interventions, such as patient education, counselling and support[43 44] and occupational therapy,[45] deserve additional consideration. Interventions in the scope of the positive psychology movement, including 'third wave' cognitive–behavioural therapies designed to boost resilience factors such as acceptance, mindfulness, positive affect and happiness,[46 47] may be of paramount importance for the individual patient's global health and enjoyment of life.

Acknowledgements Special thanks to all participating patients and to their families. To Luís Inês for its critical revision of the article. We are indebted to Ana Maria Abrantes, Catarina Brás and Eliana Maia for valuable secretarial support. To the memory of Christopher Petterson "Because other people matter".

Contributors EJFS performed the statistical analyses (assisted by RJOF and AMP) and wrote the manuscript. CD, RJOF and JAPdS designed the study. AMP and RG revised the final manuscript from their respective specialist perspective. JAPdS supervised and contributed to all steps of the work. All members of the 'Promoting Happiness Through Excellence of Care ' contributed through inspiring discussions on the topic of happiness and medical care, through examining and interviewing patients and revising the manuscript. Co-authors: "Promoting Happiness Through Excellence of Care" is the registered moto of the Rheumatology Department at the Faculty of Medicine and University Hospital of Coimbra. Additional members of this group: Alexandra Daniel, Ana Pinto, Anabela Silva, Andréa Marques, Armando Malcata, Carlos Costa, Cristiana Silva, Diogo Jesus, Flávio Costa, Gisela Eugénio, João Freitas, João Rovisco, Jorge Silva, José Laranjeiro, Luísa Brites, Margarida Coutinho, Maria Salvador, Mariana Luís, Mariana Santiago, Marília Rodrigues, Mary Marques, Pedro Carvalho, Pedro Freitas, Sara Serra, Tânia Santiago.

Funding No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

Competing interests None declared.

Patient consent Not required.

Ethics approval University of Coimbra's Faculty of Medicine Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Smolen JS, Breedveld FC, Burmester GR, *et al*. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann Rheum Dis* 2016;75:3–15.
2 Smolen JS, Landewé R, Breedveld FC, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;73:492–509.
3 Felson DT, Smolen JS, Wells G, *et al*. American College of Rheumatology/European League against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. *Ann Rheum Dis* 2011;70:404–13.
4 Boers M, Kirwan JR, Wells G, *et al*. Developing core outcome measurement sets for clinical trials: OMERACT filter 2.0. *J Clin Epidemiol* 2014;67:745–53.
5 Castrejón I, Pincus T. Patient self-report outcomes to guide a treat-to-target strategy in clinical trials and usual clinical care of rheumatoid arthritis. *Clin Exp Rheumatol* 2012;30(Suppl 73):S50–5.
6 Curtis JR, Shan Y, Harrold L, *et al*. Patient perspectives on achieving treat-to-target goals: a critical examination of patient-reported outcomes. *Arthritis Care Res* 2013;65:1707–12.
7 Desthieux C, Hermet A, Granger B, *et al*. Patient–physician discordance in global assessment in rheumatoid arthritis: a systematic literature review with meta-analysis. *Arthritis Care Res* 2016;68:1767–73.
8 Kilic L, Erden A, Bingham CO, *et al*. The reporting of patient-reported outcomes in studies of patients with rheumatoid arthritis: a systematic review of 250 articles. *J Rheumatol* 2016;43:1300–5.
9 Kirwan JR, Bartlett SJ, Beaton DE, *et al*. Updating the OMERACT filter: implications for patient-reported outcomes. *J Rheumatol* 2014;41:1011–5.
10 Nikiphorou E, Radner H, Chatzidionysiou K, *et al*. Patient global assessment in measuring disease activity in rheumatoid arthritis: a review of the literature. *Arthritis Res Ther* 2016;18:251.
11 Gossec L, Dougados M, Dixon W. Patient-reported outcomes as end points in clinical trials in rheumatoid arthritis. *RMD Open* 2015;1:e000019.
12 Overman CL, Jurgens MS, Bossema ER, *et al*. Change of psychological distress and physical disability in patients with rheumatoid arthritis over the last two decades. *Arthritis Care Res* 2014;66:671–8.
13 Ferreira RJO, Dougados M, Kirwan JR, *et al*. Drivers of patient global assessment in patients with rheumatoid arthritis who are close to remission: an analysis of 1588 patients. *Rheumatology* 2017;56:1573–8.
14 Ferreira RJO, Duarte C, Ndosi M, *et al*. Suppressing inflammation in rheumatoid arthritis: does patient global assessment blur the target? A practice-based call for a paradigm change. *Arthritis Care Res* 2018;70.
15 Matcham F, Scott IC, Rayner L, *et al*. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: a systematic review and meta-analysis. *Semin Arthritis Rheum* 2014;44:123–30.
16 Bakhshipour B, Panahiyan S, Hasanzadeh R, *et al*. Relationship between personality traits and happiness in patients with thalassemia. *Zahedan J Res in Med Scien* 2014;16:28–32.
17 Argyle M. *The Psychology of happiness*. 2nd ed. New York: Routledge, 2001.
18 Angner E, Ray MN, Saag KG, *et al*. Health and happiness among older adults: a community-based study. *J Health Psychol* 2009;14:503–12.
19 Diener E, Pressman SD, Hunter J, *et al*. If, Why, and when subjective well-being influences health, and future needed research. *Appl Psychol Health Well Being* 2017;9:133–67.
20 DeNeve KM, Cooper H. The happy personality: a meta-analysis of 137 personality traits and subjective well-being. *Psychol Bull* 1998;124:197–229.
21 Cheng H, Furnham A. Personality, self-esteem, and demographic predictions of happiness and depression. *Pers Individ Dif* 2003;34:921–42.
22 Pishva N, Ghalehban M, Moradi A, *et al*. Personality and happiness. *Procedia—Social and Behavioral Sciences* 2011;30:429–32.
23 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
24 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
25 Gossec L, Paternotte S, Aanerud GJ, *et al*. Finalisation and validation of the rheumatoid arthritis impact of disease score, a patient-derived composite measure of impact of rheumatoid arthritis: a EULAR initiative. *Ann Rheum Dis* 2011;70:935–42.
26 Ferreira R, Gossec L, Hewlett S, *et al*. FRI0748-HPR Cross-cultural validation of the Portuguese "rheumatoid arthritis impact of disease" score: cross-sectional study. *Ann Rheum Dis* 2017;76(Suppl 2):1501–01.
27 Lyubomirsky S, Lepper HS. A measure of subjective happiness: preliminary reliability and construct validation. *Soc Indic Res* 1999;46:137–55.
28 Gosling SD, Rentfrow PJ, Swann WB. A very brief measure of the Big-Five personality domains. *J Res Pers* 2003;37:504–28.
29 Ferreira LN, Ferreira PL, Pereira LN, *et al*. EQ-5D Portuguese population norms. *Qual Life Res* 2014;23:425–30.
30 van der Heijde DM, van 't Hof M, van Riel PL, *et al*. Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatol* 1993;20:579–81.
31 Cohen J. *Statistical power analysis for the behavioral sciences*. 2nd ed. Hillsdale: Lawrence Earlbaum Associates, 1988.
32 Kline RB. *Principles and practice of structural equation modeling*. New York: The Guilford Press, 2005.
33 Hair J, Black WC, Babin B, *et al*. *Multivariate data analyses*. 6th ed. New York, NY: Prentice-Hall, 2005.
34 Byrne BM. *Structural equation modeling with AMOS: basic concepts, applications, and programming*. Mahwah: NJ: Lawrence Erlbaum Associates, 2000.
35 Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav Res Methods* 2008;40:879–91.
36 Haroon N, Aggarwal A, Lawrence A, *et al*. Impact of rheumatoid arthritis on quality of life. *Mod Rheumatol* 2007;17:290–5.
37 Pollard L, Choy EH, Scott DL. The consequences of rheumatoid arthritis: quality of life measures in the individual patient. *Clin Exp Rheumatol* 2005;23(5 Suppl 39):S43–52.
38 Safdari S, Tarkhan M, Hatami G. Relationship of happiness and quality of life in patients with multiple sclerosis (MS) disorder. *J Appl Environ Biol Sci* 2013;3:35–8.
39 Siahpush M, Spittal M, Singh GK. Happiness and life satisfaction prospectively predict self-rated health, physical health, and the presence of limiting, long-term health conditions. *Am J Health Promot* 2008;23:18–26.
40 Chan R, Joseph S. Dimensions of personality, domains of aspiration, and subjective well-being. *Personality and Individual Differences* 2000;28:347–54.
41 Hayes N, Joseph S. Big 5 correlates of three measures of subjective well-being. *Pers Individ Dif* 2003;34:723–7.
42 Chioqueta AP, Stiles TC. Personality traits and the development of depression, hopelessness, and suicide ideation. *Pers Individ Dif* 2005;38:1283–91.
43 Cramp F, Hewlett S, Almeida C, *et al*. Non-pharmacological interventions for fatigue in rheumatoid arthritis. *Cochrane Database Syst Rev* 2013;8:Cd008322.
44 Vliet Vlieland TP, van den Ende CH. Nonpharmacological treatment of rheumatoid arthritis. *Curr Opin Rheumatol* 2011;23:259–64.
45 Steultjens EM, Dekker J, Bouter LM, *et al*. Occupational therapy for rheumatoid arthritis. *Cochrane Database Syst Rev* 2004;1:Cd003114.
46 Hayes SC, Villatte M, Levin M, *et al*. Open, aware, and active: contextual approaches as an emerging trend in the behavioral and cognitive therapies. *Annu Rev Clin Psychol* 2011;7:141–68.
47 Bolier L, Haverman M, Westerhof GJ, *et al*. Positive psychology interventions: a meta-analysis of randomized controlled studies. *BMC Public Health* 2013;13:119.

CONCISE REPORT

The use of MRI-detected synovitis to determine the number of involved joints for the 2010 ACR/EULAR classification criteria for Rheumatoid Arthritis – is it of additional benefit?

Aleid C Boer,[1] Debbie M Boeters,[1] Annette H M van der Helm-van Mil[1,2]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2018-213143).

[1]Department of Rheumatology, Leids Universitair Medisch Centrum, Leiden, The Netherlands
[2]Department of Rheumatology, Erasmus Medical Centre, Leiden, The Netherlands

Correspondence to
Aleid C Boer, Department of Rheumatology, Leiden University Medical Centre, Leiden 2300 RC, The Netherlands; a.c.boer@lumc.nl

Received 31 January 2018
Revised 27 March 2018
Accepted 14 April 2018
Published Online First 20 April 2018

ABSTRACT

Objective To assess the value of MRI-detected synovitis to determine the number of involved joints on the performance of the 2010-ACR/EULAR classification criteria for rheumatoid arthritis (RA).

Methods 277 patients with a clinical suspicion of RA consecutively included in the Leiden Early Arthritis Clinic (EAC)-cohort underwent 1.5T MRI of MCP-, wrist- and MTP-joints. Test characteristics of the 2010-criteria were calculated when the number of involved joints was determined with and without including MRI-detected synovitis. Two outcomes were studied: disease modifying anti-rheumatic drug (DMARD)-initiation and 1987-criteria fulfilment during the first year.

Results At baseline, 143 patients were classified as RA. When MRI-detected synovitis was considered, 14 patients additionally fulfilled the 2010-criteria. Of these, 64% (9/14) started DMARDs. When MRI-detected synovitis was also used to determine the number of involved joints the sensitivity changed from 62% to 67%, the specificity from 90% to 84% and the AUC from 0.76 to 0.75. The net reclassification index was −2.4%. When fulfilling the 1987-criteria was used as outcome, results were similar.

Conclusion We found no scientific support that the use of MRI-detected synovitis is of additional benefit for the performance of the 2010 classification criteria.

INTRODUCTION

Because early classification is important in rheumatoid arthritis (RA), the 2010 ACR/EULAR classification criteria have been developed.[1] These criteria are more sensitive and slightly less specific than the 1987-criteria.[2] Differences between these criteria are among others a stronger weight of autoantibodies in the 2010-criteria. In addition, the 2010-criteria suggest the use of imaging tools to ascertain synovitis.[1] This addition seems reasonable as studies on MRI have shown that synovitis in early arthritis patients can be present in a substantial amount of joints that were neither swollen nor tender at clinical examination.[3] Moreover, autoantibody-negative patients require the presence of >10 involved joints to fulfil the criteria for RA.[4] The addition of advanced imaging modalities could substantially increase the number of involved joints and may therefore improve the accuracy of the criteria in the autoantibody-negative group in particular. Although the development of the 2010-criteria was primarily data-driven, the suggestion to also use advanced imaging modalities to detect synovitis was included in the criteria based on expert opinion.[5] Thus far there are no studies published in peer-reviewed journals that evaluated the effects of including information of synovitis detected by MRI on the performance of the 2010-criteria. Therefore, this study determined the effects of the inclusion of MRI-detected synovitis in the evaluation of the number of involved joints on the performance of the 2010-criteria.

METHODS

Patients

We studied 277 patients with clinically evident inflammatory arthritis of ≥1 joint that were consecutively included in the Leiden Early Arthritis Clinic (EAC) cohort between 2013 and 2015, who when the results of regular laboratory investigations were known, had the clinical working diagnosis of RA or undifferentiated arthritis (UA) (figure 1). The EAC is a population-based inception cohort of patients with recent-onset arthritis with a symptom duration <2 years that started in 1993 and is described in detail elsewhere.[6] At baseline 66-swollen and 68-tender joint counts (66-SJC and 68-TJC), laboratory investigations (including c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), immunoglobulin M-rheumatoid factor (RF) (positive if ≥3.5 IU/mL) and anti-citrullinated peptide antibody (ACPA, anti-CCP2, Eurodiagnostica, the Netherlands, positive if ≥25 U/mL; from 2009 EliA CCP, Phadia, the Netherlands, positive if ≥7 U/mL)) and an MRI were performed. Follow-up visits with standard clinical assessments were performed 3 months after the first presentation and yearly thereafter. The study was approved by the Ethics Committee. Written informed consent from each patient was obtained.

MRI

From 2010 onwards an MRI was made at baseline and from June 2013 onwards not only the MCP- and wrist-joints, but also the MTP-joints were imaged after gadolinium enhancement. As contrast enhancement is beneficial for the evaluation of synovitis,[7] patients were selected from June 2013 onwards at the time contrast enhancement of the MTP-joints was added to the protocol. Patients studied here were included between June

To cite: Boer AC, Boeters DM, van der Helm-van Mil AHM. *Ann Rheum Dis* 2018;**77**:1125–1129.

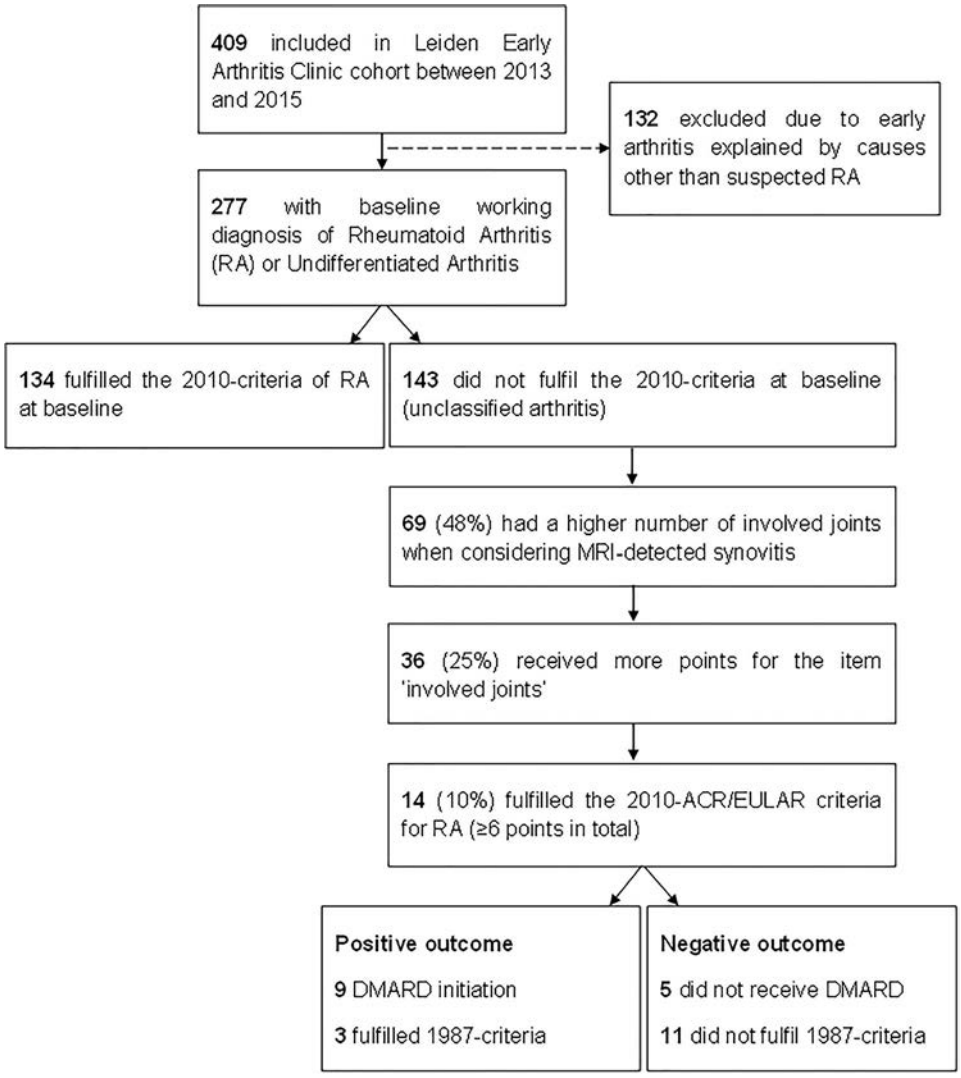


Figure 1 Flowchart of patient selection from the Leiden Early Arthritis Clinic cohort.

2013 and December 2015. A 1.5T MRI was made at the most severely affected symptomatic side or at the dominant side if symptoms were equal at both sides (see online supplementary methods). According to the protocol the MRI was made before disease modifying anti-rheumatic drug (DMARD)-initiation (including glucocorticoids) and patients were asked to stop NSAIDs 24 hours before the scan. The scans were scored according to RA MRI Scoring (RAMRIS) method by two experienced readers (intraclass correlation coefficients (ICC) for synovitis 0.96). More details on the scanning and scoring method are provided supplementary. Mean scores of two readers were calculated and in case of a mean score of ≥1, the MRI was considered positive for MRI-detected inflammation (synovitis, tenosynovitis or bone marrow oedema (BMO)). The MRI reading results were not communicated to the clinicians at any time point.[6 8]

Incorporation of MRI-detected inflammation for the classification of RA-patients

The 2010-criteria were applied to all 277 patients with clinical synovitis of at least one joint that had no alternative explanation for their complaints and were considered as at risk for RA by their treating rheumatologist. Joint counts were performed with and without the addition of MRI-detected inflammation. For example, in case a joint was neither tender nor swollen, but was positive for MRI-detected synovitis (mean score ≥1 per joint by two separate independent readers) it resulted in a positive joint for the calculation of the 2010 ACR/EULAR classification criteria with MRI-detected synovitis. Although the 2010-classification criteria stated that synovitis detected by advanced imaging modalities might be assessed to determine the number of involved joints, MRI also depicts tenosynovitis and BMO. Therefore we also explored if adding information of these features increased the accuracy of the criteria. Patients that fulfilled ≥6 points of the classification criteria were considered 2010-criteria positive RA.[1]

Analyses

After 1 year follow-up patient files were assessed on two outcomes that were used as a proxy of RA. The primary outcome was the initiation of a DMARD (including the start of oral, intra-articular or intramuscular glucocorticoids); this outcome was also used in the data-driven phase of the derivation of the 2010-criteria. The secondary outcome was fulfilment of the 1987-criteria. We calculated test characteristics for the 2010-criteria without and

Table 1 Baseline characteristics of 277 patients studied and for those that did not fulfil the 2010-criteria when MRI results were not considered (Undifferentiated Arthritis, UA)

	All patients (n=277)	UA patients (n=143)
Age, mean (SD)	57 (16)	56 (17)
Female, n (%)	176 (64)	85 (59)
68-Tender joint count, median (IQR)	6 (9)	3 (5)
CRP (mg/L), median (IQR)	7 (18)	5 (11)
Symptom duration in days, median (IQR)	73 (166)	59 (156)
RF positive (≥3.5 IU/mL), n (%)	97 (36)	11 (8)
ACPA positive (≥7 U/mL), n (%)	97 (36)	22 (16)
Either RF or ACPA positive, n (%)	127 (46)	29 (20)

ACPA, anti-citrullinated peptide antibody; CRP, c-reactive protein; IQR, Inter quartile range; RF, rheumatoid factor; SD, standard deviation.

with the addition of MRI-detected synovitis. The net reclassification index was calculated.

RESULTS

At baseline 143 out of the total of 277 patients studied did not fulfil the 2010-criteria when the number of involved joints was determined at clinical evaluation only (figure 1) and 134 did. Table 1 shows the baseline characteristics; in line with previous observations the patients that did not fulfil the criteria were mostly auto-antibody negative. When MRI-detected synovitis was also considered to count the number of involved joints, 69 patients had increased joint counts. Subsequently we determined the number of patients that received more points for the item 'number of involved joints'; this concerned 36 patients. Then we counted the additional number of patients in whom the total points had become six or higher. A total of 14 additional patients now fulfilled the 2010-criteria for RA. Thus, when data on MRI-detected synovitis were included 10% of patients that were formally classified as UA were now additionally classified as having RA.

Then the 1 year follow-up data were studied. When MRI-detected synovitis was not considered, the sensitivity (95% CI) of the 2010-criteria was 62% (55; 69) and the specificity 90% (82; 95) for DMARD initiation as outcome (table 2). Nine of the 14 additionally classified patients (64%) were started on DMARDs and were considered as true positives, whereas the other five patients (36%) were not treated with DMARDs. These five patients developed alternative clinical diagnoses during the first year (gout (n=2), inflammatory osteoarthritis (n=1), paraneoplastic inflammatory arthritis (n=1)) or had spontaneous resolution of arthritis in the first year (n=1). With the addition of MRI-detected synovitis the sensitivity increased to 67% (60; 73) and the specificity decreased to 84% (73; 90). The AUC changed from 0.76 to 0.75. The net reclassification index −2.4% (online supplementary table S1).

Results for the secondary outcome, fulfilment of the 1987-criteria after 1 year, were similar (table 2). The sensitivity changed from 79% (71; 85) to 81% (74; 87) and the specificity from 78% (71; 84) to 71% (63; 78). The net reclassification index was −5.1% (online supplementary table S2).

To investigate whether the additionally classified patients with MRI-detected synovitis could be explained by the definition of MRI-detected synovitis, we also applied a cut-off based on findings from symptom-free volunteers, as previously published,[9] instead of a cut-off of mean ≥1. Then MRI-detected synovitis was considered present in a joint if this was seen in <5% of age matched healthy controls. This caused less UA-patients to fulfil the 2010-criteria and also resulted in both an increase in falsely and correctly additionally classified RA-patients (data not

Table 2 Test characteristics of the 2010 EULAR/ACR criteria for RA without and with considering MRI-detected inflammation for the primary outcome (initiation with DMARDs in the first year) and secondary outcome (fulfilment of the 1987-criteria at year one)

Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
DMARD initiation					
2010-RA without considering MRI					
62 (55; 69)	90 (82; 95)	95 (90; 97)	46 (38; 54)	70 (64; 75)	0.76
2010-RA with considering MRI-detected synovitis					
67 (60; 73)	84 (73; 90)	92 (86; 95)	47 (39; 56)	71 (66; 76)	0.75
2010-RA with considering MRI-detected tenosynovitis					
66 (59; 72)	86 (77; 92)	93 (88; 96)	47 (39; 56)	71 (66; 76)	0.76
2010-RA with considering MRI-detected bone marrow oedema					
64 (57; 70)	86 (77; 92)	93 (87; 96)	46 (38; 54)	70 (64; 75)	0.75
2010-RA with considering any MRI-detected inflammation					
68 (61; 74)	82 (72; 89)	91 (86; 95)	48 (39; 57)	72 (66; 77)	0.75
1987-criteria fulfilment					
2010-RA without considering MRI					
79 (71; 85)	78 (71; 84)	76 (68; 83)	81 (74; 87)	79 (74; 83)	0.79
2010-RA with considering MRI-detected synovitis					
81 (74; 87)	71 (63; 78)	71 (63; 78)	81 (74; 87)	76 (70; 80)	0.76
2010-RA with considering MRI-detected tenosynovitis					
81 (74; 87)	74 (66; 80)	73 (65; 80)	82 (75; 88)	77 (72; 82)	0.78
2010-RA with considering MRI-detected bone marrow oedema					
81 (73; 87)	76 (68; 82)	74 (66; 81)	82 (74; 87)	78 (73; 82)	0.78
2010-RA with considering any MRI-detected inflammation					
82 (75; 88)	69 (61; 76)	70 (62; 76)	82 (74; 87)	75 (70; 80)	0.76

Test characteristics are shown in percentages with a 95% CI except for the AUC, area under the receiver operating characteristic curve. PPV, positive predictive value; NPV, negative predictive value. Any MRI-detected inflammation consists of either synovitis, tenosynovitis or bone marrow oedema.

shown). The area under the receiver operating characteristic curve (AUC) remained 0.76.

Since MRI does not only depict synovitis, but also tenosynovitis and BMO, it was explored if incorporation of these inflammatory findings changed the results. As depicted in table 2, the test characteristics and AUC were almost similar to that of MRI-detected synovitis.

DISCUSSION

This study provided evidence on the value of the inclusion of MRI-detected synovitis in addition to the evaluation of tender and swollen joints for the classification of RA. Our data show that the accuracy as measured by the AUC did not improve. This conclusion is similar to that reported in two abstracts that to our knowledge did not proceed to papers published in peer-reviewed journals.[10 11] We observed that almost 50% of patients had MRI-detected synovitis in joints that were neither swollen nor tender at physical examination. However this resulted in a positive classification for the 2010-criteria in a minority of patients. Furthermore one-third of additionally classified patients did not have RA with DMARD-treatment as reference and could be considered as false-positives.

A meta-analysis on the performance of the 2010-criteria by Radner *et al* reported a sensitivity and specificity for DMARD initiation of 65% and 80% respectively.[2] Our findings are in line with these data.

We also did not identify studies or trials stating that imaging modalities were used for the application of the classification criteria. Hence we are unfamiliar with how often novel imaging modalities are currently used to this end. The value of ultrasound for the classification criteria has been studies previously.[12–15] All studies were differently designed. In two studies the presence of clinically evident inflammatory arthritis was not required for inclusion.[12 13] Another study showed associations between ultrasound-detected synovitis and fulfilment of the 2010-criteria, but test characteristics with and without the use of ultrasound were not provided.[15] One study calculated test characteristics and showed that the use of ultrasound resulted in an increased sensitivity at the cost of specificity, which is in line with our findings.[14] Also these ultrasounds studies showed, similarly to our study, an increase of both correctly and incorrectly classified RA-patients.[15]

The method how MRI-detected synovitis should be incorporated in the 2010-criteria was not thoroughly explained.[1] We used MRI additionally to clinical evaluation of joints. However, the study of Nakagomi *et al* that used ultrasound, included patients without clinical synovitis and determined the number of involved joints solely by imaging.[12] This resulted in patients fulfilling the criteria for RA without any clinically detectable synovitis.

Importantly, concerning the type of inflammation assessed, our main focus was the addition of MRI-detected synovitis, as this was explicitly stated in the table by Aletaha *et al*.[1] To further examine the impact of other types of MRI-detected inflammation, we separately analysed the value of tenosynovitis, BMO and the presence of any type of inflammation as an addition to the criteria. These results were similar to the outcomes of MRI-detected synovitis.

The definition of the presence of synovitis on imaging was not explicated in the 2010-criteria. Several previous studies showed low-grade synovitis in small joints of asymptomatic persons, especially at higher age.[16–18] Although the nature of this phenomenon remains indefinite, not considering this may possibly result in an overestimation of affected joints. Therefore we analysed an alternative definition for synovitis-positivity and investigated the effects if a joint was considered positive when this was present in <5% of age matched healthy controls. This also resulted in an increase in falsely and correctly classified RA-patients. Consequently, we think that the presence of low-grade synovitis in the general population does not explain the lack of increased accuracy when using MRI-detected synovitis in the criteria.

In this study we observed an increased sensitivity at the cost of the specificity. It could be discussed that classification criteria should be sensitive and therefore incorporation of imaging into the 2010-criteria for RA could be considered favourable. At the other hand, here this also resulted in a substantial increase of false positives.

In addition to the outcome studied here, it would also be interesting to evaluate more a long-term outcome like disease persistence. Further, the present findings require external validity in other cohorts of early RA patients to assess if these results are generalizable.

In conclusion, we did not find an increased accuracy of the 2010 ACR/EULAR classification criteria when MRI-detected synovitis was incorporated. Further research on this subject in other longitudinal cohorts is needed. At present there is no scientific proof that MRI-detected synovitis is of additional benefit for classification of RA.

Acknowledgements EC Newsum and WP Nieuwenhuis are acknowledged for scoring MRI-scans.

Contributors ACB, DB and AHMvdHvM made a substantial contribution to the acquisition, analysis and interpretation of the data. All authors made a substantial contribution to the conception and design of the work. ACB and AHMvdHvM drafted the manuscript; DB revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript.

Funding The research leading to these results has received funding from a Vidi-grant of the Netherlands Organization for Health Research and Development and from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Starting grant, agreement No 714312). The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Competing interests None declared.

Patient consent Not required.

Ethics approval 'Commissie Medische Ethiek' of the Leiden University Medical Centre.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
2 Radner H, Neogi T, Smolen JS, *et al*. Performance of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2014;73:114–23.
3 Krabben A, Stomp W, Huizinga TW, *et al*. Concordance between inflammation at physical examination and on MRI in patients with early arthritis. *Ann Rheum Dis* 2015;74:506–12.
4 van der Helm-van Mil AH, Zink A. What is rheumatoid arthritis? Considering consequences of changed classification criteria. *Ann Rheum Dis* 2017;76:315–7.
5 Neogi T, Aletaha D, Silman AJ, *et al*. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis: Phase 2 methodological report. *Arthritis Rheum* 2010;62:2582–91.
6 de Rooy DP, van der Linden MP, Knevel R, *et al*. Predicting arthritis outcomes--what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology* 2011;50:93–100.

7 Stomp W, Krabben A, van der Heijde D, *et al*. Aiming for a shorter rheumatoid arthritis MRI protocol: can contrast-enhanced MRI replace T2 for the detection of bone marrow oedema? *Eur Radiol* 2014;24:2614–22.
8 Nieuwenhuis WP, van Steenbergen HW, Mangnus L, *et al*. Evaluation of the diagnostic accuracy of hand and foot MRI for early Rheumatoid Arthritis. *Rheumatology* 2017;56:1367–77.
9 Boer AC, Burgers LE, Mangnus L, *et al*. Using a reference when defining an abnormal MRI reduces false-positive MRI results-a longitudinal study in two cohorts at risk for rheumatoid arthritis. *Rheumatology* 2017;56:1700–6.
10 Tamai M, Arima K, Uetani M, *et al*. Evaluation of the Patients with Early Arthritis by 2010 RA Criteria in Conjunction with MRI of Wrists and Finger Joints. *Arthritis Rheum* 2011;63:S119.
11 Duer-Jensen A, Horslev-Petersen K, Bak L, *et al*. Using Mri Synovitis to Count Involved Joints in the Acr/Eular 2010 Ra Criteria Increases Their Sensitivity and Specificity. *Ann Rheum Dis* 2012;71:601.
12 Nakagomi D, Ikeda K, Okubo A, *et al*. Ultrasound can improve the accuracy of the 2010 American College of Rheumatology/European League against rheumatism classification criteria for rheumatoid arthritis to predict the requirement for methotrexate treatment. *Arthritis Rheum* 2013;65:890–8.
13 Ji L, Deng X, Geng Y, *et al*. The additional benefit of ultrasonography to 2010 ACR/EULAR classification criteria when diagnosing rheumatoid arthritis in the absence of anti-cyclic citrullinated peptide antibodies. *Clin Rheumatol* 2017;36:261–7.
14 Filer A, de Pablo P, Allen G, *et al*. Utility of ultrasound joint counts in the prediction of rheumatoid arthritis in patients with very early synovitis. *Ann Rheum Dis* 2011;70:500–7.
15 Horton SC, Tan AL, Wakefield RJ, *et al*. Ultrasound-detectable grey scale synovitis predicts future fulfilment of the 2010 ACR/EULAR RA classification criteria in patients with new-onset undifferentiated arthritis. *RMD Open* 2017;3:e000394.
16 Mangnus L, van Steenbergen HW, Reijnierse M, *et al*. Magnetic resonance imaging-detected features of inflammation and Erosions in symptom-free persons from the general population. *Arthritis Rheumatol* 2016;68:2593–602.
17 Zubler V, Agten CA, Pfirrmann CW, *et al*. Frequency of Arthritis-Like MRI findings in the forefeet of healthy volunteers versus patients with symptomatic Rheumatoid Arthritis or Psoriatic Arthritis. *AJR Am J Roentgenol* 2017;208:W45–53.
18 Agten CA, Rosskopf AB, Jonczy M, *et al*. Frequency of inflammatory-like MR imaging findings in asymptomatic fingers of healthy volunteers. *Skeletal Radiol* 2018;47:279–87.

EXTENDED REPORT

ACPA IgG galactosylation associates with disease activity in pregnant patients with rheumatoid arthritis

Albert Bondt,[1,2,3] Lise Hafkenscheid,[3] David Falck,[2] T Martijn Kuijper,[1] Yoann Rombouts,[2,3,4] Johanna M W Hazes,[1] Manfred Wuhrer,[2] Radboud J E M Dolhain[1]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2018-212946).

[1]Department of Rheumatology, Erasmus University Medical Center, Rotterdam, The Netherlands
[2]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands
[3]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[4]Institut de Pharmacologie et Biologie Structurale, IPBS, Université de Toulouse, Toulouse, France

Correspondence to
Dr Radboud J E M Dolhain, Department of Rheumatology, Erasmus University Medical Center, Rotterdam 3015 CE, The Netherlands;
r.dolhain@erasmusmc.nl

Received 2 January 2018
Revised 16 March 2018
Accepted 22 March 2018
Published Online First
3 April 2018

ABSTRACT

Objectives Patients with autoantibody-positive rheumatoid arthritis (RA) are less likely to experience pregnancy-induced improvement of RA disease activity (DAS28-C reactive protein (CRP)) compared with patients with autoantibody-negative RA. Anti-citrullinated protein antibodies (ACPAs) are the most specific autoantibodies for RA. We previously demonstrated that disease improvement is associated with changes in total IgG glycosylation, which regulate antibody effector function. Therefore, we sought to analyse the ACPA-IgG glycosylation profile during pregnancy with the aim to understand the lower change of pregnancy-induced improvement of the disease in patients with autoantibody-positive RA.

Methods ACPA-IgGs were purified from ACPA-positive patient sera (n=112) of the Pregnancy-induced Amelioration of Rheumatoid Arthritis cohort, a prospective study designed to investigate pregnancy-associated improvement of RA. The fragment crystallisable (Fc)glycosylation profile of ACPA-IgGs was characterised by mass spectrometry and compared with that of total IgG derived from the same patients or from ACPA-negative patients.

Results All ACPA-IgG subclasses display significant changes in the level of galactosylation and sialylation during pregnancy, although less pronounced than in total IgG. The pregnancy-induced increase in ACPA-IgG galactosylation, but not sialylation, associates with lower DAS28-CRP. In ACPA-positive patients, no such association was found with changes in the galactosylation of total IgG, whereas in ACPA-negative patients changes in disease activity correlated well with changes in the galactosylation of total IgG.

Conclusions In ACPA-positive RA, the pregnancy-induced change in galactosylation of ACPA-IgG, and not that of total IgG, associates with changes in disease activity. These data may indicate that in ACPA-positive patients the galactosylation of ACPA-IgG is of more pathogenic relevance than that of total IgG.

INTRODUCTION

In rheumatoid arthritis (RA), autoantibodies (AAbs) are thought to be important drivers in the pathogenesis of the disease. A variety of AAbs has been discovered in RA over the years, but rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) are reported to be present in approximately 70% of patients with RA.[1] For both ACPA and RF, the IgM, IgG and IgA isotypes have been characterised.[2 3] An increasing body of evidence suggests that AAb-positive RA is a different disease than AAb-negative RA. For example, it has been shown that AAb-positive RA is more progressive and destructive, in particular when ACPA is present.[3] In addition, while approximately 50% of all patients with RA spontaneously improve with pregnancy, this percentage is lower for AAb-positive disease, and often disease activity in these patients remains moderate to high.[4 5]

IgG is known to bear sugar structures at asparagine 297 residues of the fragment crystallisable (Fc) of the heavy chain, the so-called *N*-glycan. These *N*-glycans on IgG are generally composed of a core structure of seven building blocks (four *N*-acetylglucosamines (GlcNAc) and three mannoses), which can be extended by galactoses, sialic acids, a fucose and a bisecting GlcNAc (figure 1). For ACPA-IgGs, in addition, glycans with only three GlcNAcs in the core structure, called mono-antennary glycans, have been described.[6]

The absence of galactose extensions is known for decades to be associated with higher RA disease activity, whereas the increase in the levels of galactose is associated with the spontaneous improvement of RA during pregnancy.[7 8] The Fc-glycan moiety of IgG regulates antibody effector functions, especially by modulating the binding of IgG to Fc-gamma-receptor or by activating anti-inflammatory pathways via lectins such as Dectin-1.[9 10]

AAbs, in particular ACPA and RF, can be present years before disease onset without clinical phenotype.[11] Their mere presence is not enough to induce arthritis, but shortly before the diagnosis of RA the ACPA-IgG Fc *N*-glycosylation is changed in appearance towards a phenotype associated with increased inflammation.

Since ACPA-IgGs have been described to exhibit a more 'pro-inflammatory' Fc-glycosylation profile compared with total IgGs, and given that patients with AAb-positive RA are less likely to improve during pregnancy, we hypothesised that ACPA-IgGs are more likely to retain their 'pro-inflammatory' glycosylation profile during pregnancy compared with total IgG.[4 5 12]

Therefore, we compared the changes in the glycosylation of ACPA-IgG during pregnancy and after delivery with that of total IgG. In addition, associations thereof with changes in disease activity were investigated, as well as differences between clinical responders and non-responders.

To cite: Bondt A, Hafkenscheid L, Falck D, *et al*. *Ann Rheum Dis* 2018;**77**:1130–1136.

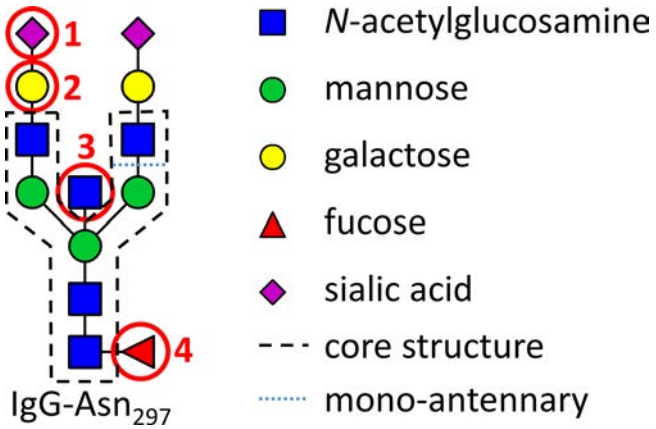


Figure 1 Schematic representation of an *N*-glycan. The stable core is indicated by the dashed line. The dotted blue line indicates the truncation called mono-antennary. The four main glycan characteristics are furthermore shown: (1) sialylation, (2) galactosylation, (3) bisection (for 'bisecting' *N*-acetylglucosamine) and (4) fucosylation.

METHODS

Study population and clinical response

The current study is embedded in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study, a nationwide prospective cohort study on pregnancy and RA for which patients were included between 2002 and 2009. Sera were collected preconception (PC) if possible, at three time points during pregnancy and three time points after delivery.[13] All patients fulfil the 1987 American College of Rheumatology (ACR) criteria for RA. At all time points disease activity was assessed using the disease activity score (DAS28) based on C reactive protein (CRP), and swollen and tender joint count.[14] Responders and non-responders (from first trimester (TM1) to third trimester (TM3)) were categorised based on the EULAR response criteria.[15] The postpartum flare was defined between the TM3 and 12 weeks post partum using the so-called 'reversed EULAR response criteria'.[13] As a reference group 32 healthy pregnant Caucasian volunteers without adverse obstetric history were followed from the TM1 onwards.

Total and ACPA-IgG glycosylation data

ACPAs were captured as described before using CCP2-based affinity chromatography followed by IgG purification on CaptureSelect IgG-Fc (Hu) beads (ThermoFisher).[16] The captured and dried ACPA-IgGs were reconstituted in 50 mM ammonium bicarbonate (Sigma Aldrich, Steinheim, Germany) with 15% acetonitrile. As a next step, the same volume of ultrapure water containing 600 ng of N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Sigma Aldrich) was added before overnight digestion at 37°C. The obtained tryptic digests were sequentially measured by nano-liquid chromatography (LC) coupled online to electrospray ionisation (ESI) mass spectrometry (MS) as described before.[17] Total IgG glycosylation data obtained previously were used to compare with ACPA-IgG glycosylation of the same patients.[8]

Data analysis

Raw LC-MS data were converted to mzXML using MSConvert. LaCyTools was used to align the LC runs, extract and calibrate the mass spectra of each IgG subclass, and integrate the spectra for a selected list of potential analytes.[18] In addition, several quality measures were extracted. After analyte and spectrum curation, several glycosylation traits were calculated in Microsoft Excel as described in the online supplementary material.

Some ACPA-negative sera were used to assess the specificity of the entire procedure. A threshold for inclusion was set at the subclass specific intensity obtained for these negative sera plus two times the SD thereof.

Statistical analysis

Statistical analysis was performed using Stata/SE V.13.1 for Windows (StataCorp). First, all ACPA-IgG and total IgG time lapses of subclass specific glycosylation traits, as well as disease activity, were modelled using multilevel mixed-effects linear regression analysis, similar to what has been described before.[8] In addition, for total IgG separate models were estimated for ACPA-positive and ACPA-negative patients. Within these models the presence of pregnancy-associated changes were studied between PC and TM3, between TM1 and TM3, and between TM3 and the second postpartum time point (12 weeks post partum; PP2). At this postpartum time point, disease activity was highest. After this time point, several patients had already restarted disease-modifying antirheumatic drugs, including methotrexate. Δ values were calculated for the changes in the glycosylation traits and disease activity. It was tested whether the mean ΔACPA-IgG glycosylation was different compared with the total IgG glycosylation changes, and whether Δtotal IgG glycosylation was different between ACPA-positive and ACPA-negative patients. Furthermore, associations between Δglycosylation and ΔDAS28 were explored. Two-group mean-comparison tests of glycosylation traits were performed to identify possible differences between responders and non-responders (during pregnancy), or 'flare' and 'no flare' (after delivery). A false discovery rate of 5% was used in Benjamini-Hochberg correction for multiple testing.

RESULTS

Study population and clinical response

Sera from the 152 ACPA-positive patients included in the PARA cohort (total n=255) with material available from at least three time points were used for this study, and finally resulted in ACPA-IgG glycopeptide spectra for 112 patients: 50 from PC onwards, 48 from the TM1, 9 from the TM2, and 5 from the TM3. Patient characteristics as well as clinical response outcomes are depicted in table 1.

ACPA-glycosylation shows pregnancy-associated changes

First, we analysed the glycosylation changes of ACPA-IgG (with respect to galactosylation, sialylation, incidence of bisecting GlcNAc and fucosylation) during pregnancy and after delivery. All studied glycosylation properties showed differences over time (P<0.001; online supplementary table S1). For example, a typical increase in galactosylation from PC onwards until the TM3 of pregnancy was observed, followed by lower levels of galactosylation after delivery (figure 2; online supplementary tables S1and S2).

An increase of ACPA-IgG galactosylation was observed between TM1 and TM3 for all subclasses (IgG1 +3.40%, P<0.001; IgG2/3 +5.01%, P<0.001; IgG4 +3.99%, P<0.001), followed by a decrease after delivery (IgG1 –4.39%, P<0.001; IgG2/3 –5.89%, P<0.001; IgG4 –5.09%, P<0.001; figure 2, table 2, online supplementary tables S1 and S2).

Furthermore, also ACPA-IgG sialylation showed an increase during and decrease after pregnancy (table 2, online supplementary figure S1, online supplementary table S1 and S2).

Table 1 Cohort characteristics

	ACPA-positive (n=112)	ACPA-negative (n=101)	ACPA-positive vs ACPA-negative*
Age, mean (range)	33.1 (21.9–42.4)	32.3 (24.7–40.5)	ns
RF positive, n (%)	95/105 (90)	28/94 (30)	<0.0001
Erosive disease, n (%)	74/107 (69)	49/99 (49)	0.0037
DAS28-CRP3, mean (SD)			
PC†	3.8 (1.2)	3.6 (1.0)	ns
TM1‡	3.9 (1.2)	3.3 (1.0)	0.0002
TM3§	3.5 (1.0)	3.0 (1.0)	0.0006
Use of sulfasalazine at TM1, n (%)	31/96 (32)	26/89 (29)	ns
Use of prednisone at TM1, n (%)	37/96 (39)	29/89 (33)	ns
Use of hydroxychloroquine at TM1, n (%)	3/96 (3)	2/89 (2)	ns
Use of methotrexate in the past, n (%)	63/112 (56)	53/101 (52)	ns
Use of TNF-blocking agents in the past, n (%)	18/112 (16)	10/101 (10)	ns

*Outcome of the t-test comparing ACPA-positive with ACPA-negative patients.
†ACPA-positive n=46; ACPA-negative n=52.
‡ACPA-positive n=91; ACPA-negative n=85.
§ACPA-positive n=97; ACPA-negative n=96.
ACPA, anti-citrullinated protein antibodies; CRP, C reactive protein; ns, not significant; PC, preconception; PP, post partum; RF, rheumatoid factor; TM, trimester; TNF, tumour necrosis factor.

Levels of bisecting GlcNAc were registered for ACPA-IgG1 and ACPA-IgG2/3 showing a drop between PC and the TM1, followed by a gradual increase during pregnancy which continued post partum. Levels of bisection at TM3 were not different from the PC time point (online supplementary figure S2, supplementary tables S1 and S2).

Afucosylated structures showed a mild decrease after delivery for ACPA-IgG1. No afucosylated species were detected for ACPA-IgG2/3and ACPA-IgG4 (online supplementary figure S3, supplementary tables S1 and S2).

In addition to the previously studied glycosylation traits, we were able to detect glycopeptides with mono-antennary glycans (<1% of all detected ACPA-IgG1 glycopeptides), of which the level decreased during pregnancy (from 0.8% to 0.7%, P<0.001) and increased after delivery (from 0.7% to 0.8%, P=0.001; online supplementary tables S1 and S2).

Pregnancy-associated changes in glycosylation of ACPA-IgG are less pronounced compared with total IgG

Since ACPA-IgG showed the typical pregnancy-associated changes with regards to Fc-glycosylation, we investigated whether ACPA-IgG glycosylation changes were different from total IgG in the same (ACPA-positive) patients. In addition, the changes in glycosylation of total IgG during pregnancy were compared between ACPA-positive and ACPA-negative patients.

The change in galactosylation was on average twofold smaller for ACPA-IgG1 compared with total IgG1 for all studied time spans (P<0.001; figure 3A, table 2). Likewise, the increase of

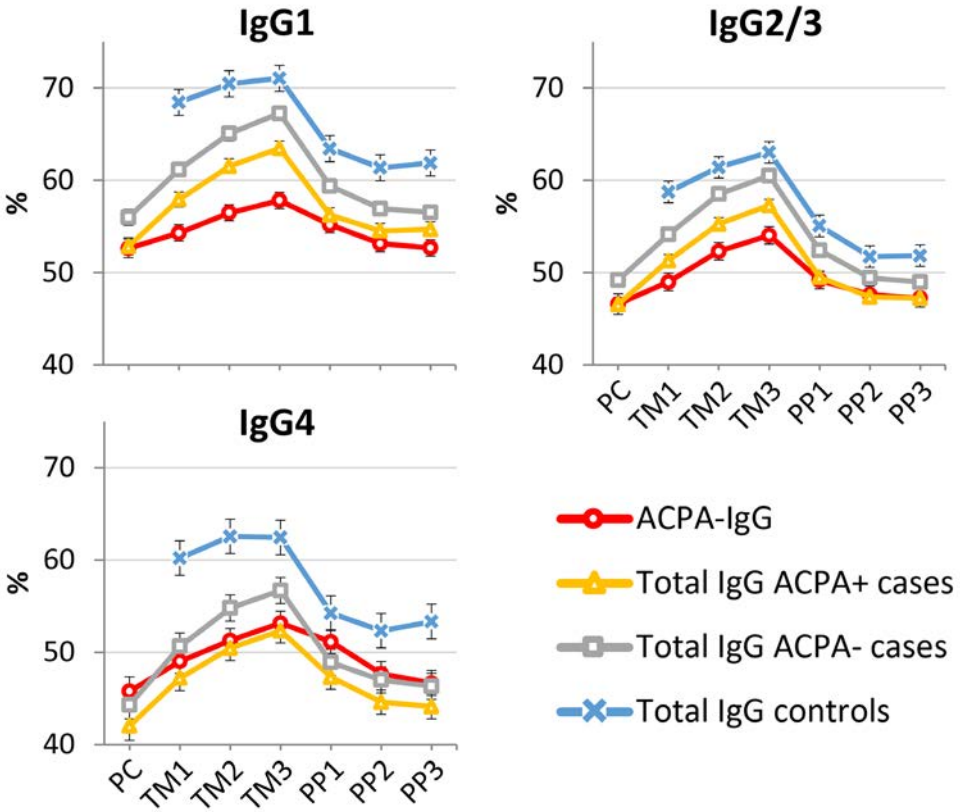


Figure 2 Galactosylation time curves for IgG1, IgG2/3 and IgG4. The anti-citrullinated protein antibodies (ACPAs) galactosylation (red line, circle markers) is different compared with total IgG of ACPA-positive individuals (yellow line, triangle markers). In addition, total IgG galactosylation patterns for ACPA-negative patients (grey line, square markers) and healthy controls (blue line, cross markers) are shown. Error bars represent standard errors. PC, preconception; PP, post partum; TM, trimester.

Table 2 Comparison of the change in glycosylation (%) between anti-citrullinated protein antibodies (ACPAs)-IgG (ACPA) and total IgG (Total) in ACPA-positive patients

	PC-TM3						TM1-TM3						TM3-PP2					
		ACPA		Total				ACPA		Total				ACPA		Total		
	n	Mean	SE	Mean	SE	P values	n	Mean	SE	Mean	SE	P values	n	Mean	SE	Mean	SE	P values
Gal																		
IgG1	25	4.68	1.60	11.15	1.28	**<0.001**	61	3.40	0.55	5.37	0.53	**<0.001**	71	−4.39	0.47	−8.76	0.48	**<0.001**
IgG2/3	13	4.80	1.35	9.91	1.08	**<0.001**	28	5.01	1.52	6.34	0.65	0.266	30	−5.89	0.65	−9.01	0.62	**<0.001**
IgG4	7	8.15	4.10	10.09	3.03	0.550	16	3.99	1.16	6.31	1.40	0.075	20	−5.09	1.44	−7.38	1.33	**0.004**
SA																		
IgG1	25	0.51	0.48	2.48	0.22	**<0.001**	61	0.58	0.13	1.19	0.12	**<0.001**	71	−1.10	0.12	−2.42	0.12	**<0.001**
IgG2/3	13	1.17	0.41	2.76	0.28	**0.003**	28	1.10	0.36	1.74	0.16	**0.019**	30	−1.62	0.20	−2.62	0.16	**<0.001**
IgG4	7	2.42	1.33	3.05	0.71	0.627	16	1.10	0.41	1.81	0.38	0.094	20	−1.82	0.42	−2.60	0.38	**0.014**
Bis																		
IgG1	25	0.43	0.44	−0.80	0.29	**<0.001**	61	1.09	0.18	0.13	0.14	**<0.001**	71	0.63	0.16	1.46	0.12	**<0.001**
IgG2/3	13	−0.10	0.26	0.06	0.28	0.557	28	0.69	0.26	0.58	0.21	0.581	30	1.35	0.17	0.86	0.11	**0.001**
Fuc																		
IgG1	25	−0.08	0.08	−0.47	0.21	0.047	61	−0.10	0.06	−0.35	0.10	**0.019**	71	0.21	0.06	0.50	0.10	**0.005**

P values depicted in bold font were considered statistically significant after Benjamini-Hochberg multiple testing correction under a false discovery rate of 5%.
Bis, bisection; Fuc, fucosylation; Gal, galactosylation; PC, preconception; PP, post partum; SA, sialylation; TM, trimester.

galactosylation during pregnancy and subsequent decrease after delivery was significantly lower for ACPA-IgG2/3 compared with total IgG2/3 (P<0.001). Regarding ACPA-IgG4, only the decrease of galactosylation after delivery showed a difference compared with that of total IgG4 (P=0.004).

A highly comparable set of observations was obtained for sialylation, with a less pronounced increase during pregnancy and postpartum decrease in sialylation of ACPA-IgG1 and ACPA-IgG2/3 compared with their respective total IgG subclasses (figure 3B; table 2). For IgG4 similar trends were observed. Some differential pregnancy-induced changes between ACPA-IgG and total IgG were also observed for the level of bisecting GlcNAc (table 2).

Only minor differences in total IgG glycosylation changes were observed between ACPA-positive and ACPA-negative patients (online supplementary table S3).

Disease activity associates with ACPA-IgG galactosylation in ACPA-positive, and with total IgG galactosylation in ACPA-negative patients

We previously found that the change in total IgG galactosylation is associated with a change in disease activity (DAS28-CRP). Regression analysis was now performed to study this phenomenon for ACPA-IgG. In addition, for total IgG a subgroup analysis for ACPA-positive and ACPA-negative

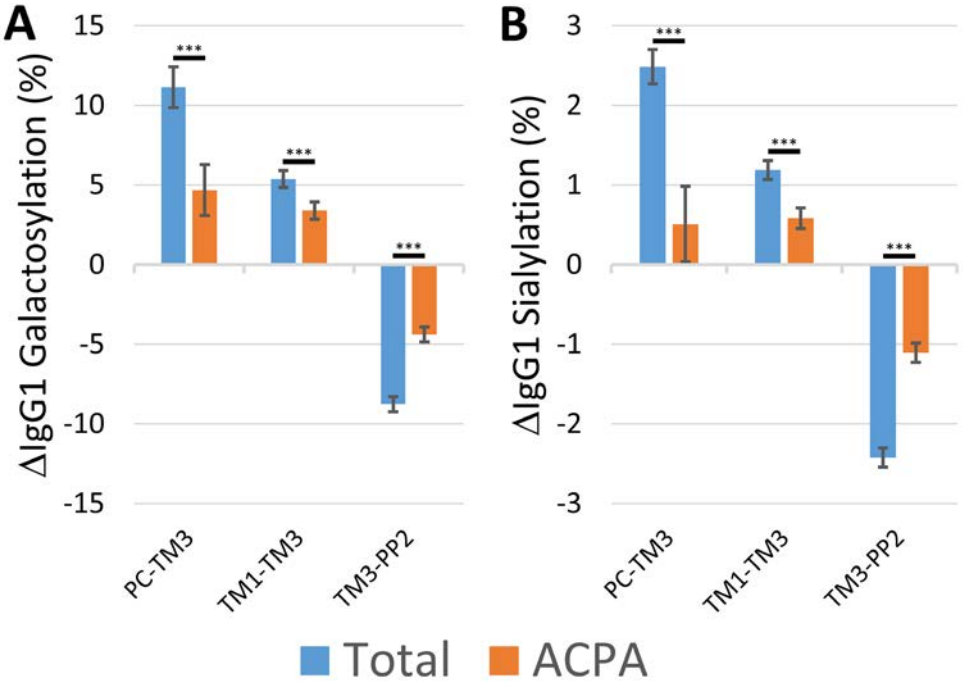


Figure 3 Pregnancy-associated changes in IgG1 galactosylation (A) and sialylation (B) are more pronounced in total IgG compared with anti-citrullinated protein antibodies (ACPAs) in the same patients. Changes from preconception (PC) to the third trimester (TM3), from first trimester (TM1) to TM3, and from TM3 to the second postpartum time point (PP2; 12 weeks post partum) are shown for total IgG in blue and ACPA-IgG in orange. (***P<0.001).

Table 3 Association of the change in galactosylation and sialylation with the change in disease activity during different time spans for anti-citrullinated protein antibodies (ACPAs)-IgG and total IgG in the ACPA-positive patients, and for total IgG in the ACPA-negative patients

	PC-TM3				TM1-TM3				TM3-PP2			
	n	P values	R^2	Beta	n	P values	R^2	Beta	n	P values	R^2	Beta
Galactosylation												
ACPA												
IgG1	25	0.7526	0.004	0.066	59	**0.0058**	0.126	−0.355	69	0.1285	0.034	−0.185
IgG2/3	12	0.6557	0.021	0.144	27	0.1863	0.069	−0.262	30	0.2845	0.041	−0.202
IgG4	12	0.4221	0.066	0.256	33	0.1458	0.067	−0.259	35	0.4659	0.016	−0.127
Total (ACPA-positive)												
IgG1	35	0.9575	0.000	0.009	82	0.0516	0.047	−0.216	94	0.0320	0.049	−0.221
IgG2/3	35	0.9328	0.000	0.015	82	0.0191	0.067	−0.258	94	0.0312	0.050	−0.222
IgG4	11	0.5429	0.043	0.206	30	0.133	0.079	−0.281	34	0.6870	0.025	−0.159
Total (ACPA-negative)												
IgG1	47	0.0542	0.080	−0.283	82	**0.0007**	0.134	−0.366	89	**0.0000**	0.184	−0.429
IgG2/3	47	0.0623	0.075	−0.274	82	**0.0018**	0.116	−0.340	89	**0.0001**	0.159	−0.398
IgG4	15	0.0655	0.237	−0.487	23	0.1559	0.094	−0.306	29	0.0162	0.196	−0.443
Sialylation												
ACPA												
IgG1	25	0.2816	0.050	0.224	59	0.0564	0.062	−0.250	69	0.8384	0.001	−0.025
IgG2/3	12	0.6515	0.021	0.146	27	0.5430	0.015	−0.122	30	0.9885	0.000	0.003
IgG4	12	0.3803	0.078	0.279	33	0.0216	0.159	−0.399	35	0.9037	0.001	0.003
Total (ACPA-positive)												
IgG1	35	0.4646	0.016	−0.128	82	0.1535	0.025	−0.159	94	0.2622	0.014	−0.117
IgG2/3	35	0.5861	0.009	−0.095	82	0.1840	0.022	−0.148	94	0.2877	0.012	−0.111
IgG4	11	0.6651	0.022	0.148	30	0.1847	0.062	−0.249	34	0.6730	0.006	−0.075
Total (ACPA-negative)												
IgG1	47	0.1063	0.057	−0.239	82	0.0154	0.071	−0.267	89	0.0277	0.055	−0.233
IgG2/3	47	0.4120	0.015	−0.123	82	0.1007	0.033	−0.183	89	0.0505	0.043	−0.208
IgG4	15	0.1742	0.137	−0.370	23	0.4198	0.031	−0.177	29	0.2273	0.054	−0.231

P values depicted in bold font were considered statistically significant after Benjamini-Hochberg multiple testing correction under a false discovery rate of 5%.
PC, preconception; PP, post partum; TM, trimester.

patients was performed. A graphical representation of the disease activity time course for these groups is represented in online supplementary figure S4.

A negative association of the change in (Δ)ACPA-IgG1 galactosylation between TM1 and TM3 was seen with changes in disease activity (table 3; P=0.0058, R^2=0.13, β=−0.36), meaning that patients with the most pronounced decrease in disease activity show the highest increase in galactosylation.

Regarding total IgG galactosylation of ACPA-positive patients, no significant association between galactosylation change and the change in disease activity was observed.

In contrast, in ACPA-negative patients the total IgG galactosylation showed negative associations with disease activity for both IgG1 and IgG2/3, during pregnancy (TM1-TM3; IgG1, P=0.001, R^2=0.13; IgG2/3, P=0.002, R^2=0.12) as well as after delivery (TM3-PP2; IgG1, P<0.001, R^2=0.18; IgG2/3, P<0.001, R^2=0.16; table 3).

When focusing on sialylation, we observed no associations of changes in ACPA-IgG sialylation with changes in disease activity (table 3). Also, the changes in total IgG sialylation for ACPA-positive and ACPA-negative patients did not show significant associations with disease activity (table 3).

Change in ACPA-IgG galactosylation in ACPA-positive patients and total IgG galactosylation in ACPA-negative patients differs between responders and non-responders does

Previously we have shown that changes in glycosylation are more pronounced in patients that improve during pregnancy and those that flare after delivery. To determine whether this is related to ACPA status, a subgroup analysis was performed.

Patients were classified as 'responder' and 'non-responder' during pregnancy, or 'flare' and 'no flare' after delivery according to the EULAR response criteria. For ACPA-IgG1, galactosylation was found to increase slightly more in responders compared with non-responders (P=0.044; online supplementary table S4). Total IgG galactosylation in ACPA-positive patients, however, did not show different increases between responders and non-responders during pregnancy, nor a difference in decrease between patients with and without a flare after delivery. Interestingly, a larger increase in total IgG1 galactosylation was observed in the ACPA-negative responders (+8.9%) during pregnancy compared with the non-responders (+4.8%; P=0.005; online supplementary table S4). After delivery the decrease in total IgG1 galactosylation was more pronounced in patients with a flare of disease activity (−13.2% vs −9.3%; P=0.001). Similar findings were observed for total IgG2/3 (online supplementary table S4).

DISCUSSION

In this article, we sought to determine whether the Fc-glycosylation of ACPA-IgG could provide more insights into why patients with RA positive for AAbs are less likely to improve during pregnancy. Previously, we have shown that ACPA titres do not change during pregnancy; therefore, a rise in ACPA titre cannot explain this phenomenon.[4] We therefore tested an alternative hypothesis, namely that ACPA-IgG would retain a more 'pro-inflammatory'

glycan phenotype compared with total IgG, which could explain higher disease activity in the AAb-positive population. In agreement with our hypothesis, our data show that ACPA-IgGs keep a glycan phenotype that is associated with higher inflammation, with lower galactosylation and sialylation as a hallmark. This might be an explanation why patients with AAb-positive RA do not show a similar pregnancy-associated improvement of disease activity as do patients with AAb-negative RA. Changes in the glycosylation of total IgG were comparable between ACPA-positive and ACPA-negative patients.

Notably, in ACPA-negative patients there was a strong association in the change in galactosylation of total IgG with the change of disease activity, whereas in ACPA-positive patients this was found only for ACPA-IgG, but not for total IgG.

The increase in oestrogens during pregnancy is believed to contribute to the pregnancy-induced changes in glycosylation, in particular galactosylation.[19] Overstimulation with oestrogen, however, has been suggested to possess pro-inflammatory effects.[20] An inflammatory environment, like the inflamed joints of patients with RA, with high levels of interleukin-6, is associated with increased levels of oestrogen receptors.[21] The increased expression of oestrogen receptors, together with the increased level of oestrogen during pregnancy, may therefore result in an overstimulated, hence pro-inflammatory, stimulation. It this therefore tempting to speculate that since ACPA-producing plasma cells reside in the joints, this pro-inflammatory stimulation might be an explanation for the aforementioned lower increase in galactosylation of ACPA-IgG compared with total IgG.

An alternative explanation for the less pronounced pregnancy-induced changes in ACPA-IgG glycosylation is that ACPA-producing B cells appear to be generally more matured, with high rates of class-switched memory B cells and plasmablasts.[22] In addition, these cells may persist longer in the synovium.[23] These more matured or 'older' antibody-producing cells may be less sensitive to (hormonal) stimulation which would modify the IgG glycosylation. Such desensitisation has, for example, been reported for matured T cells.[24]

As we have mentioned before, there is no difference in the average increase of total IgG galactosylation between ACPA-positive and ACPA-negative patients. However, only in ACPA-negative patients, but not ACPA-positive patients, an association was observed between the changes in disease activity and the changes in the galactosylation of total IgG. In the ACPA-positive patients, such an association was only found for the ACPA-IgG galactosylation.

These observations indicate that in ACPA-positive patients' disease activity is mainly driven by the glycosylation of ACPA, suggesting that ACPA, as an AAb, could have a pathogenic role in this subgroup of patients. That in AAb-negative patients changes in the glycosylation of total IgG could affect disease activity seems less straightforward. However, it is known that Igs may exert general immunosuppressive properties which are attributed to the degree of galactosylation and/or sialylation.[10] This all is thought to be the underlying mechanism of therapy with intravenous Igs (IVIgs). Several studies on IVIgs have been performed in patients with RA, with proven efficacy also in AAb-negative patients.[25 26]

Antibodies are often called 'pro-inflammatory' or 'anti-inflammatory' when people refer to the glycosylation. The results obtained in the current study appear to support this notion. In ACPA-positive patients, where ACPA is expected to drive pathogenesis, increased ACPA-IgG galactosylation associates with lower disease activity. In contrast, total IgG galactosylation only associates with disease activity in ACPA-negative RA, supposedly due to a different pathogenic mechanism. However, showing a direct link between glycosylation and inflammation is challenging. Recently a murine study on collagen-induced arthritis has provided evidence that antibodies with a more 'anti-inflammatory' glycosylation phenotype do not induce arthritis, whereas a more 'pro-inflammatory' phenotype does.[27 28] For the murine system, the sialic acid is believed to serve as a switch between these phenotypes. Intriguingly, we found in our current study for ACPA-IgG, and previously for total IgG, that galactosylation associates with lower disease activity in RA, and not sialylation.[8] This has also been reported recently for granulomatosis with polyangiitis.[29]

In conclusion, we have shown that during pregnancy ACPA retains a more 'pro-inflammatory' glycosylation profile compared with total IgG. In addition, we found that in ACPA-positive patients' changes in the galactosylation of ACPA-IgG were associated with changes in disease activity during that period, whereas no such association was found for total IgG galactosylation in these patients. Our results support a pathogenic potential of ACPAs and their low levels of galactosylation in RA and might explain why patients with AAb-positive RA improve less during pregnancy.

Acknowledgements The authors thank Agnes Hipgrave Ederveen and Carolien Koeleman for excellent technical support. They also thank Dr Jan Wouter Drijfhout (LUMC, Leiden) for providing the CCP2 peptide. An early version of this manuscript has been published as Chapter 8 of the PhD thesis of AB (https://repub.eur.nl/pub/100423).

Contributors AB, JMWH, MW and RJEMD designed the study. AB, LH, DF and YR were involved in sample preparation and processing. AB, LH, TMK, MW and RJEMD conducted statistical analyses and interpreted the results. AB, LH, JMWH, MW and RJEMD drafted the manuscript. All authors reviewed the manuscript and gave their approval for submission.

Funding AB was funded by the Dutch Arthritis Foundation (NR 10-1-411) and by the European Union's Seventh Framework Program (FP7-Health-F5-2011) under grant agreement no. 278535 (HighGlycan). LH was supported by the Dutch Arthritis Foundation (NR 12-2-403). YR was supported by a Boehringer Ingelheim funded project within BeTheCure and by NWO (435000033).

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was in compliance with the Helsinki Declaration and was approved by the Ethics Review Board at the Erasmus University Medical Center, Rotterdam, The Netherlands.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Ursum J, Bos WH, van de Stadt RJ, *et al*. Different properties of ACPA and IgM-RF derived from a large dataset: further evidence of two distinct autoantibody systems. *Arthritis Res Ther* 2009;11:R75.
2 Lakos G, Soós L, Fekete A, *et al*. Anti-cyclic citrullinated peptide antibody isotypes in rheumatoid arthritis: association with disease duration, rheumatoid factor production and the presence of shared epitope. *Clin Exp Rheumatol* 2008;26:253–60.
3 Rantapää-Dahlqvist S, de Jong BA, Berglin E, *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741–9.
4 de Man YA, Bakker-Jonges LE, Goorbergh CM, *et al*. Women with rheumatoid arthritis negative for anti-cyclic citrullinated peptide and rheumatoid factor are more likely to improve during pregnancy, whereas in autoantibody-positive women autoantibody levels are not influenced by pregnancy. *Ann Rheum Dis* 2010;69:420–3.
5 Ince-Askan H, Hazes JMW, Dolhain R. Identifying clinical factors associated with low disease activity and remission of rheumatoid arthritis during pregnancy. *Arthritis Care Res* 2017;69.
6 Lundström SL, Fernandes-Cerqueira C, Ytterberg AJ, *et al*. IgG antibodies to cyclic citrullinated peptides exhibit profiles specific in terms of IgG subclasses, Fc-glycans and a fab-Peptide sequence. *PLoS One* 2014;9:e113924.

7 Parekh RB, Dwek RA, Sutton BJ, *et al*. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature* 1985;316:452–7.
8 Bondt A, Selman MH, Deelder AM, *et al*. Association between galactosylation of immunoglobulin G and improvement of rheumatoid arthritis during pregnancy is independent of sialylation. *J Proteome Res* 2013;12:4522–31.
9 Subedi GP, Barb AW. The immunoglobulin G1 N-glycan composition affects binding to each low affinity Fc γ receptor. *MAbs* 2016;8:1512–24.
10 Karsten CM, Pandey MK, Figge J, *et al*. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and dectin-1. *Nat Med* 2012;18:1401–6.
11 Nielen MM, van Schaardenburg D, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
12 Scherer HU, van der Woude D, Ioan-Facsinay A, *et al*. Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. *Arthritis Rheum* 2010;62:1620–9.
13 de Man YA, Dolhain RJ, van de Geijn FE, *et al*. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. *Arthritis Rheum* 2008;59:1241–8.
14 de Man YA, Hazes JM, van de Geijn FE, *et al*. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. *Arthritis Rheum* 2007;57:716–22.
15 van Riel P, van Gestel AM, Scott DL, *et al*. *Eular handbook of clinical assessments in rheumatoid arthritis*. Alphen aan den Rijn: Van Zuiden Communications, 2000:39–43.
16 Habets KL, Trouw LA, Levarht EW, *et al*. Anti-citrullinated protein antibodies contribute to platelet activation in rheumatoid arthritis. *Arthritis Res Ther* 2015;17:209.
17 Falck D, Jansen BC, de Haan N, *et al*. High-throughput analysis of IgG Fc glycopeptides by LC-MS. *Methods Mol Biol* 2017;1503:31–47.
18 Jansen BC, Falck D, de Haan N, *et al*. LaCyTools: a targeted liquid chromatography-mass spectrometry data processing package for relative quantitation of glycopeptides. *J Proteome Res* 2016;15:2198–210.
19 Ercan A, Kohrt WM, Cui J, *et al*. Estrogens regulate glycosylation of IgG in women and men. *JCI Insight* 2017;2:e89703.
20 Straub RH, Bijlsma JW, Masi A, *et al*. Role of neuroendocrine and neuroimmune mechanisms in chronic inflammatory rheumatic diseases–the 10-year update. *Semin Arthritis Rheum* 2013;43:392–404.
21 Capellino S, Riepl B, Rauch L, *et al*. Quantitative determination of steroid hormone receptor positive cells in the synovium of patients with rheumatoid arthritis and osteoarthritis: is there a link to inflammation? *Ann Rheum Dis* 2007;66:53–8.
22 Kerkman PF, Fabre E, van der Voort EI, *et al*. Identification and characterisation of citrullinated antigen-specific B cells in peripheral blood of patients with rheumatoid arthritis. *Ann Rheum Dis* 2016;75:1170–6.
23 Kerkman PF, Kempers AC, van der Voort EI, *et al*. Synovial fluid mononuclear cells provide an environment for long-term survival of antibody-secreting cells and promote the spontaneous production of anti-citrullinated protein antibodies. *Ann Rheum Dis* 2016;75:2201–7.
24 Davey GM, Schober SL, Endrizzi BT, *et al*. Preselection thymocytes are more sensitive to T cell receptor stimulation than mature T cells. *J Exp Med* 1998;188:1867–74.
25 Tumiati B, Casoli P, Veneziani M, *et al*. High-dose immunoglobulin therapy as an immunomodulatory treatment of rheumatoid arthritis. *Arthritis Rheum* 1992;35:1126–33.
26 Katz-Agranov N, Khattri S, Zandman-Goddard G. The role of intravenous immunoglobulins in the treatment of rheumatoid arthritis. *Autoimmun Rev* 2015;14:651–8.
27 Pfeifle R, Rothe T, Ipseiz N, *et al*. Regulation of autoantibody activity by the IL-23-T_H17 axis determines the onset of autoimmune disease. *Nat Immunol* 2017;18:104–13.
28 Schwab I, Biburger M, Krönke G, *et al*. IVIg-mediated amelioration of ITP in mice is dependent on sialic acid and SIGNR1. *Eur J Immunol* 2012;42:826–30.
29 Kemna MJ, Plomp R, van Paassen P, *et al*. Galactosylation and sialylation levels of IgG predict relapse in patients with PR3-ANCA associated vasculitis. *EBioMedicine* 2017;17:108–18.

EXTENDED REPORT

Risk of myocardial infarction with use of selected non-steroidal anti-inflammatory drugs in patients with spondyloarthritis and osteoarthritis

Maureen Dubreuil,[1,2] Qiong Louie-Gao,[1] Christine E Peloquin,[1] Hyon K Choi,[3] Yuqing Zhang,[1] Tuhina Neogi[1]

Handling editor Josef S Smolen

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2018-213089).

[1]Boston University School of Medicine, Boston, Massachusetts, USA
[2]VA Boston Healthcare System, Boston, Massachusetts, USA
[3]Massachusetts General Hospital, Boston, Massachusetts, USA

Correspondence to
Dr Maureen Dubreuil, Boston University School of Medicine, Boston, MA 02118, USA; mdubreui@bu.edu

Received 22 January 2018
Revised 28 March 2018
Accepted 5 April 2018
Published Online First 19 April 2018

ABSTRACT

Objectives Spondyloarthritis (SpA) is associated with an increased risk of myocardial infarction (MI) due to underlying inflammation and possibly due to medications such as certain non-steroidal anti-inflammatory drugs (NSAIDs). We sought to describe MI risk among patients with SpA who were prescribed NSAIDs, and to compare the pattern of risk in SpA with that in osteoarthritis (OA).

Methods Nested case-control studies were performed using The Health Improvement Network (THIN). Underlying cohorts included adults with incident SpA or OA who had ≥1 NSAID prescription and no history of MI. Within each cohort, we matched each MI case to four controls without MI. NSAID use was categorised as: (a) current (prescription date 0–180 days prior to index date), (b) recent (181–365 days) or (c) remote (>365 days). We performed conditional logistic regression to compare the odds of current or recent NSAID use relative to remote use of any NSAID, considering diclofenac and naproxen specifically.

Results Within the SpA cohort of 8140 and the OA cohort of 244 339, there were 115 and 6287 MI cases, respectively. After adjustment, current diclofenac use in SpA was associated with an OR of 3.32 (95% CI 1.57 to 7.03) for MI. Naproxen was not associated with any increase (adjusted OR 1.19, 95% CI 0.53 to 2.68). A ratio of ORs for SpA/diclofenac relative to OA/diclofenac was 2.64 (95% CI 1.24 to 5.58).

Conclusions MI risk in SpA is increased among current users of diclofenac, but not naproxen. The MI risk with diclofenac in SpA appears to differ from that in OA.

INTRODUCTION

Myocardial infarction (MI) risk is increased in several systemic rheumatic diseases including rheumatoid arthritis (RA), psoriatic arthritis (PsA) and other forms of spondyloarthritis (SpA).[1–4] Reasons for this increased risk are likely multifactorial, including a greater prevalence of traditional cardiovascular (CV) risk factors, systemic inflammation and use of medications that may predispose to MI.[5–9] While some risk factors cannot be changed, other modifiable risk factors, specifically medication selection, offer an opportunity to prevent morbidity and reduce the premature mortality associated with SpA.

Non-steroidal anti-inflammatory drugs (NSAIDs) are currently first-line therapy for axial SpA and PsA.[10–12] While NSAIDs may relieve pain and stiffness, use may be associated with risk of adverse events such as MI. In particular, several NSAIDs that selectively inhibit cyclooxygenase-2 (COX-2) were withdrawn from the market when their CV risk was publicised. Although drugs with predominantly COX-2 inhibition have been incriminated and limited or removed from the market, NSAIDs with lower COX-2 inhibition ('non-selective NSAIDs') remain available. In fact, the top three NSAIDs, diclofenac, naproxen and ibuprofen are non-selective, and account for >12 million prescriptions annually in the UK.[13]

In people without CV disease, several non-selective NSAIDs have been shown to increase risk of CV events in a dose-dependent fashion. High-dose diclofenac is associated with a 41% increase in risk, and high-dose ibuprofen is also likely associated with an increased risk, although not statistically significant in meta-analysis (rate ratio (RR) 1.44, 95% CI 0.89 to 2.33).[14] Naproxen, on the other hand, did not have an increased risk (RR 0.93, 95% CI 0.69 to 1.27), suggesting drug-specific effects, rather than a class effect. The proposed mechanisms for different effects include the relative degree of COX-2 inhibition compared with COX-1 (rather than the absolute amount of inhibition), drug half-life and platelet inhibition.

Despite the evidence of CV risk in the general population, risk has not been fully studied in systemic rheumatic diseases. We hypothesised that MI risk with specific NSAIDs would follow a similar pattern in patients with SpA as compared with that in the general population, but would be greater in SpA due to systemic inflammation. A competing theory is that NSAID use in inflammatory arthritis may protect against CV events by reducing systemic inflammation, which itself increases risk for MI. For this reason, we examined the risk of MI associated with use of NSAIDs in patients with SpA, and also assessed risk among patients with osteoarthritis (OA), a non-inflammatory form of arthritis.

METHODS

We performed a nested case-control study using 1994–2015 data from The Health Improvement Network (THIN), a database of medical records from over 600 general practitioners in the UK. THIN currently contains data on over 11 million individuals, covering >6% of the UK population.

THIN contains systematically and prospectively recorded data collected by GPs on demographics, diagnoses, consultations, referrals,

To cite: Dubreuil M, Louie-Gao Q, Peloquin CE, *et al*. *Ann Rheum Dis* 2018;**77**:1137–1142.

hospitalisations, testing and prescriptions. Diagnoses are organised according to the Read classification.[15] Prescription data include the dose, strength and formulation of medications, categorised according to the drug dictionary, Multilex. Quality control checks are done regularly, and this database has been validated for several pharmacoepidemiological studies as well as for MI as an outcome.[16]

Underlying cohort establishment

We identified adults, aged 18–89 years in THIN with a diagnosis of ankylosing spondylitis (AS) or PsA, two forms of SpA, after at least 12 months' enrolment without such a diagnosis (incident SpA cohort). Diagnosis was established using Read codes documented by the GP. In previous studies, a Read code alone for PsA had a positive predictive value (PPV) of 85% and the PPV of an AS code was 72%.[17 18] As a control condition, we also identified a cohort of adults with incident OA (any site) documented by the GP. While the PPV of an OA diagnosis has not been assessed in THIN, the high disease prevalence makes it likely PPV will be high. Subjects were excluded if they had any history of MI, to allow identification of incident MIs. Although the NSAIDs of primary interest for this study were diclofenac (which has high COX-2 inhibition) and naproxen (which has low COX-2 inhibition), we required all subjects to have been prescribed at least one NSAID of any type to minimise confounding by indication.

Case and control ascertainment

We identified cases of MI as the first recording of an MI Read code, a definition with a PPV of 95% in a previous THIN study.[19] In SpA and OA separately, each MI case was matched using risk set sampling to 1–4 control subjects without an MI, according to age (within 2 years), date of SpA or OA diagnosis (within 2 years) and sex (figure 1).

Exposure assessment

For each subject, NSAID use was categorised as 'current' if the most recent NSAID prescription was 0–180 days prior to the index date, 'recent' if 181–365 days prior or 'remote' if >365 days. This approach of prescription recency has been used previously in the study of rheumatic diseases.[20 21]

Covariate assessment

Potential confounders included any prior diagnosis of hypertension, hyperlipidaemia, diabetes mellitus, gastrointestinal bleeding, ischaemic heart disease and chronic kidney disease. We assessed use of medications in the year preceding the index date, including aspirin, antihypertensives (beta-blockers and ACE-inhibitors), lipid-lowering agents (statins and fibrates), proton pump inhibitors and disease-modifying antirheumatic drugs (DMARDs) or biologics used in the treatment of SpA. Body mass index (BMI) was classified using the most recent value prior to the index date within 5 years, and smoking status as the most recent value. Missing values for BMI and smoking were imputed using multiple imputation, in which five datasets were generated.[22–25] The imputation model was constructed using all variables used in the analytic model (Statistical Analysis section).

Statistical analysis

We generated descriptive statistics for MI cases and controls, including mean age, sex, prevalence of comorbidities and medication use and BMI and smoking categories.

For the primary analysis, we calculated a crude OR for the odds of current NSAID use relative to remote NSAID use for cases and controls. A conditional logistic regression model was used to adjust for baseline confounders. The SpA and OA cohorts were analysed separately. For each OR, we calculated a 95% CI for current and recent NSAID exposure categories relative to remote use of any NSAID (the referent).

To assess the robustness of the primary analysis findings, we conducted several sensitivity analyses. First, because the mean age in OA was 10 years greater than that in SpA, we performed an analysis restricted to subjects aged 55–70 years to allow a comparison of relative risks among cohorts of comparable ages. Second, we rematched the original SpA cases to controls, using all the original matching factors, and additionally matched on SpA subtype (AS or PsA; within the SpA cohort only) and stratified to assess for effects by SpA subtype. Third, out of concern that aspirin use among subjects may be an indication of pre-existing ischaemic heart disease, we performed an analysis restricted to subjects free of baseline aspirin use.

Finally, to assess for effect modification between arthritis type (SpA vs OA) and the effect of diclofenac, we calculated the ratio of the adjusted ORs with 95% CI.[26] Analyses were performed

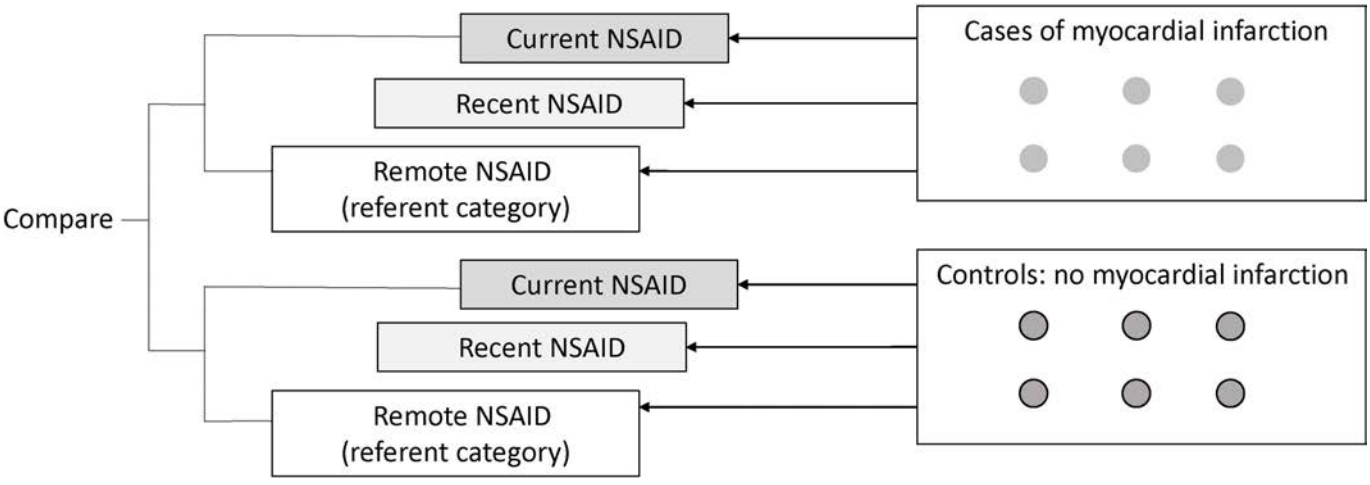


Figure 1 Case-control study design. The study began on the right, with selection of myocardial infarction (MI) cases and matched controls who did not have MI. Subjects' exposure to non-steroidal anti-inflammatory drugs (NSAIDs) was assessed as 'current' (within 180 days), 'recent' (180–365 days) or 'remote' (>365 days). NSAID non-users were excluded. Remote NSAID use was considered the referent category.

Table 1 Characteristics of cases and controls derived from the underlying SpA and OA cohorts

	SpA cohort		OA cohort	
	Cases	Controls	Cases	Controls
Subjects (n)	115	455	6287	25 164
Age, mean±SD	63.0±11.5	62.7±11.4	72.6±10.3	72.5±10.2
Female	35 (30.4%)	138 (30.3%)	2927 (46.6%)	11 716 (46.6%)
Comorbidities*				
Chronic kidney disease	14 (12.2%)	52 (11.4%)	1043 (16.6%)	3248 (12.9%)
Diabetes	23 (20.0%)	52 (11.4%)	1153 (18.3%)	3195 (12.7%)
Gastrointestinal bleeding	5 (4.3%)	17 (3.7%)	339 (5.4%)	1011 (4.0%)
Hyperlipidaemia	22 (19.1%)	58 (12.7%)	1211 (19.3%)	3898 (15.5%)
Hypertension	66 (57.4%)	188 (41.3%)	3626 (57.7%)	12 491 (49.6%)
Ischaemic heart disease	44 (38.3%)	39 (8.6%)	2707 (43.1%)	3048 (12.1%)
Medication use*				
Aspirin	29 (25.2%)	71 (15.6%)	2354 (37.4%)	6400 (25.4%)
ACE-inhibitors	32 (27.8%)	87 (19.1%)	1732 (27.5%)	5568 (22.1%)
Beta-blockers	19 (16.5%)	66 (14.5%)	1687 (26.8%)	4697 (18.7%)
Lipid-lowering drugs	42 (36.5%)	111 (24.4%)	2382 (37.9%)	7956 (31.6%)
Proton pump inhibitors	57 (49.6%)	149 (32.7%)	2520 (40.1%)	7470 (29.7%)
DMARDs	41 (35.7%)	137 (30.1%)	196 (3.1%)	488 (1.9%)
Biologics	0 (0.0%)	1 (0.2%)	0 (0.0%)	0 (0.0%)
BMI*—missing	29 (25.2%)	154 (33.8%)	1580 (25.1%)	7197 (28.6%)
Underweight	5 (4.3%)	9 (2.0%)	168 (2.7%)	546 (2.2%)
Normal	15 (13.0%)	64 (14.1%)	1016 (16.2%)	4181 (16.6%)
Overweight	32 (27.8%)	126 (27.7%)	1929 (30.7%)	7405 (29.4%)
Obese	34 (29.6%)	102 (22.4%)	1594 (25.4%)	5835 (23.2%)
Smoking*—missing	6 (5.2%)	21 (4.6%)	184 (2.9%)	786 (3.1%)
Non-smoker	27 (23.5%)	170 (37.4%)	2364 (37.6%)	11 428 (45.4%)
Ex-smoker	46 (40.0%)	183 (40.2%)	2506 (39.9%)	9894 (39.3%)
Current smoker	36 (31.3%)	81 (17.8%)	1233 (19.6%)	3056 (12.1%)

Values expressed are N (%) unless otherwise noted.
*Assessed prior to index date; comorbidities and any time prior to study, medications within the year prior; most recent BMI and smoking status.
BMI, body mass index; DMARDs, disease-modifying anti-rheumatic drugs; OA, osteoarthritis; SpA, spondyloarthritis.

using SAS V.9.3 or V.9.4 (SAS Institute, Cary, North Carolina, USA).

RESULTS

From an original SpA cohort of 8140, we identified 115 MI cases and 455 matched controls. From the OA cohort of 244 339, we identified 6287 MI cases and 25 164 matched controls. In each cohort, MI cases had a greater prevalence of traditional MI risk factors and greater use of medications for treatment of hypertension and diabetes, including aspirin, ACE-inhibitors, beta-blockers and lipid-lowering agents (table 1). Among subjects with SpA, DMARD use was present in 35% of MI cases and 30% of controls. Biologic use was rare, as expected, occurring in only one control subject with SpA.

NSAID prescriptions

Among those classified as diclofenac users, the majority (92%) were prescribed a daily dosage of 100 mg or more, with 150 mg daily being most common (74%). The daily dosage of diclofenac was 100 mg or more in 92% of subjects with OA, 95% of subjects with AS and 92% of subjects with PsA. For naproxen, the most common daily dosage was 1000 mg (55%) and was 1000 mg or greater in 56% of subjects with OA, 63% of subjects with AS and 72% of subjects with PsA. Among all subjects whose most recent prescription was an NSAID other than diclofenac or naproxen, the most common drug was ibuprofen (55%), followed by celecoxib (11%), meloxicam (10%), rofecoxib (7%), etoricoxib (5%), indomethacin (3%) and etodolac (3%). All other NSAIDs accounted for 2% or less of prescriptions (see online supplementary table 1).

Associations of NSAID use with MI

In the primary analysis, among subjects with SpA, current diclofenac was associated with a greater than twofold increase in the crude risk of MI compared with remote NSAID use (OR 2.23, 95% CI 1.22 to 4.05), which after adjustment for covariates and imputation for missing values of BMI and smoking increased to 3.32 (95% CI 1.57 to 7.03; table 2). With current naproxen use and current other NSAID use, ORs were not significantly increased; the adjusted OR (aOR) for naproxen was 1.19 (95% CI 0.53 to 2.68), current other NSAIDs aOR 1.23 (95% CI 0.61 to 2.46), and recent other NSAID aOR 1.03 (95% CI 0.36 to 2.93).

The OR for risk of MI with current diclofenac use was also increased among subjects with OA; aOR 1.26 (95% CI 1.14 to 1.39). Current naproxen was not associated with an increased aOR (0.98, 95% CI 0.85 to 1.13), but current use of other NSAIDs was (aOR 1.17, 95% CI 1.07 to 1.28) in the OA cohort.

Sensitivity analyses

With restriction to ages 55–70 years, results were not meaningfully changed (table 3); the aOR for current diclofenac in SpA

Table 2 Primary outcome: odds of myocardial infarction with current use of diclofenac, naproxen or other NSAIDs, and recent use of an NSAID, relative to remote use of NSAIDs, among patients with SpA and OA

	SpA				OA			
	Cases (n=115)	Controls (n=455)	Crude OR	aOR*	Cases (n=6287)	Controls (n=25 164)	Crude OR	aOR*
Current† diclofenac	25	62	2.23 (1.22 to 4.05)	3.32 (1.57 to 7.03)	843	2981	1.23 (1.12 to 1.34)	1.26 (1.14 to 1.39)
Current naproxen	14	46	1.60 (0.81 to 3.18)	1.19 (0.53 to 2.68)	339	1365	1.06 (0.93 to 1.20)	0.98 (0.85 to 1.13)
Current other NSAID	29	107	1.48 (0.84 to 2.61)	1.23 (0.61 to 2.46)	1224	4491	1.18 (1.09 to 1.28)	1.17 (1.07 to 1.28)
Recent† NSAID	8	39	1.05 (0.45 to 2.44)	1.03 (0.36 to 2.93)	684	2805	1.05 (0.96 to 1.15)	1.01 (0.91 to 1.12)
Remote† NSAID	39	201	1.0 (ref)	1.0 (ref)	3197	13 522	1.0 (ref)	1.0 (ref)

*Adjusted for potential confounders, using imputed BMI and smoking when missing.
†Current use: prescription date 0–180 days prior to index date; recent use: 180–365 days and remote use: >365 days.
aOR, adjusted OR; BMI, body mass index; NSAID, non-steroidal anti-inflammatory drug; OA, osteoarthritis; SpA, spondyloarthritis (includes ankylosing spondylitis and psoriatic arthritis).

was 3.36 (95% CI 0.88 to 12.79), and for OA 1.30 (95% CI 1.10 to 1.53). When we rematched subjects in the SpA cohort based on SpA subtype, the unadjusted OR for diclofenac use within the whole SpA sample remained similar (OR 2.08, table 4). When stratified by SpA subtype, the unadjusted ORs for current diclofenac were similar; for AS, 2.83 (95% CI 0.92 to 8.68) and for PsA, 1.76 (95% CI 0.85 to 3.64). Interestingly, naproxen did not have an increased OR in AS, but had an increased point estimate in PsA (unadjusted OR 2.09, 95%CI 0.90 to 4.85). SpA subtype stratified results could not be adjusted for confounders due to small event numbers. With restriction to subjects free of aspirin use at baseline, results were unchanged. The unadjusted OR for diclofenac in SpA was 2.31 (95% CI 1.16 to 4.61) and for naproxen was 1.76 (95% CI 0.81 to 3.85). In OA, the crude OR of diclofenac was 1.28 (95% CI 1.15 to 1.42), and for naproxen was 1.06 (95% CI 0.90 to 1.23).

Because current diclofenac was associated with increased MI risk, we also assessed whether recent use (181–365 days from prescription date) conferred risk. In SpA, the unadjusted OR for recent diclofenac was 1.45 (95% CI 0.50 to 4.19) and in OA was 0.94 (95% CI 0.80 to 1.11). Recent naproxen was not associated with an increased or decreased risk in either SpA or OA (results not shown).

Ratio of ratios

Using the results from the primary analysis, the ratio of aORs for current diclofenac (OA as the referent) was 2.64 (95% CI 1.24 to 5.58), suggesting an interaction between the underlying form of arthritis (SpA vs OA) and MI risk with diclofenac use, meaning that MI risk differed between the two groups. When these calculations were repeated using the population from the age-restricted sensitivity analysis, the point estimate was similar but no longer statistically significant (table 5).

DISCUSSION

This nested case-control study, performed using GP electronic medical records, demonstrated that MI risk was increased among patients with SpA using diclofenac, and that risk with diclofenac differed between subjects with SpA and OA. This novel study design, comparing current NSAID users to remote NSAID users, minimised confounding by indication in that all subjects were judged to have an indication for prescription NSAID use by their GP.

While the risk of MI with specific NSAIDs has been studied in the general population, relatively little data exist among patients with inflammatory arthritides. One cohort study, in RA, found that the risk of CV disease (composite outcome) was *lower* in RA

Table 3 Sensitivity analysis: age restricted to 55–70 years. Odds of myocardial infarction with current use of diclofenac, naproxen or other NSAIDs relative to remote use of NSAIDs

	SpA				OA			
	Cases (n=54)	Controls (n=216)	Crude OR	aOR*	Cases (n=2035)	Controls (n=8140)	Crude OR	aOR*
Current† diclofenac	11	29	2.13 (0.88 to 5.14)	3.36 (0.88 to 12.79)	349	1263	1.19 (1.03 to 1.37)	1.30 (1.10 to 1.53)
Current naproxen	8	26	1.64 (0.64 to 4.19)	1.11 (0.27 to 4.50)	144	595	1.02 (0.84 to 1.25)	0.95 (0.75 to 1.19)
Current other NSAID	14	49	1.55 (0.71 to 3.37)	1.60 (0.50 to 5.08)	394	1517	1.12 (0.97 to 1.28)	1.15 (0.98 to 1.34)
Recent† NSAID	3	16	0.95 (0.24 to 3.76)	0.59 (0.08 to 4.20)	250	963	1.11 (0.95 to 1.31)	1.11 (0.92 to 1.33)
Remote† NSAID	18	96	1.0 (ref)	1.0 (ref)	898	3802	1.0 (ref)	1.0 (ref)

*Adjusted for potential confounders.
†Current use: prescription date 0–180 days prior to index date; recent use: 181–365 days and remote use: >365 days.
aOR, adjusted OR; NSAID, non-steroidal anti-inflammatory drug; OA, osteoarthritis; SpA, spondyloarthritis (includes ankylosing spondylitis and psoriatic arthritis).

Table 4 Sensitivity analysis: SpA cases and controls rematched and stratified by SpA subtype. Odds of myocardial infarction with current use of diclofenac, naproxen or other NSAIDs relative to remote use of NSAIDs

	Ankylosing spondylitis			Psoriatic arthritis		
	Cases (n=35)	Controls (n=135)	Crude OR	Cases (n=79)	Controls (n=310)	Crude OR
Current* diclofenac	8	14	2.83 (0.92 to 8.68)	17	49	1.76 (0.85 to 3.64)
Current naproxen	3	14	1.14 (0.26 to 4.94)	11	27	2.09 (0.90 to 4.85)
Current other NSAID	12	38	1.60 (0.62 to 4.14)	17	81	1.05 (0.53 to 2.08)
Recent* NSAID	1	16	0.33 (0.04 to 2.76)	7	22	1.55 (0.60 to 3.97)
Remote* NSAID	11	53	1.0 (ref)	27	131	1.0 (ref)

*Current use: prescription end date 0–180 days prior to index date; recent use: 181–365 days and remote use: >365 days.
NSAID, non-steroidal anti-inflammatory drug; SpA, spondyloarthritis.

than in controls without RA. Specific NSAIDs such as rofecoxib and diclofenac were associated with increased risk, but others were not.[27] A 2014 meta-analysis of NSAIDs in RA and PsA found that COX-2 inhibitors were associated with increased CV risk, possibly due to rofecoxib alone, while non-selective NSAIDs, in combination, were not (RR 1.02, 95% CI 0.94 to 1.24). Notably, only one study of diclofenac met inclusion criteria, finding a significantly increased HR of 1.35.[28] Subsequently, a subgroup analysis of patients with RA from a randomised trial of celecoxib trial (10% of the sample) reported no significant increased risk of the composite CV outcome with celecoxib relative to both naproxen and ibuprofen.[29]

In AS, Essers *et al* performed a cohort study using the British Clinical Practice Research Datalink (CPRD), a database with 60% overlap with THIN. The authors reported that ischaemic heart disease in women was increased (HR 1.88, 95% CI 1.22 to 2.90), but that risk was attenuated after adjustment for NSAID use (HR 1.57, 95% CI 0.99 to 2.48). These results are consistent with our current study findings that specific NSAIDs do increase the risk of ischaemic heart disease.[30]

A cohort study using the Ontario health administrative data assessed the effect of NSAIDs on CV mortality in a subset analysis among patients with AS aged 66 years and older.[4] This older adult subset was selected because prescription data were available only in this group. The authors reported an HR of 0.1 with NSAID use (95% CI 0.01 to 0.61) and broadly stated that 'lack of NSAID exposure' is a risk factor for vascular death. This finding, that NSAID use is associated with 90% reduction in CV mortality in an older adult population lacks face validity. But more importantly, the study design raises concern for prevalent user bias; that persons with AS who survive to late adulthood without a complication from or contraindication to NSAID use reflect the healthiest stratum of patients with AS. The same analysis, demonstrating no increased mortality risk with statin use, hypertension, chronic kidney disease or cancer, illustrates the same bias. In contrast to this study, our present study is not limited to older adults and therefore is less likely to suffer from bias due to prevalent NSAID use. In fact, our analysis demonstrates the findings of the Ontario study should not be assumed to hold true in a younger SpA population.

Table 5 Ratio of ORs for current diclofenac use in SpA relative to OA

	Original SpA cohort		Sensitivity analysis: restricted to subjects aged 55–70 years	
	SpA	OA	SpA	OA
*aOR	3.32	1.26	3.36	1.30
Ratio of ORs (95% CI)	2.64 (1.24 to 5.58)		1.86 (0.40 to 4.33)	

*From the fully adjusted model including imputed values for BMI and smoking when missing.
aOR: adjusted OR; BMI, body mass index; OA, osteoarthritis; SpA, spondyloarthritis.

The present study has several limitations and strengths. Although this study applied validated algorithms for identification of SpA, it was not possible to confirm SpA diagnosis for included subjects. We expect that misclassification of non-diseased persons as having SpA would bias study results towards the null. Second, while prescription data are detailed in THIN, the nature of the data did not allow us to determine if patients adhered to therapy. Some patients may take NSAIDs inconsistently, only on an as-needed basis for pain, and the pattern of use may differ according to the indication for use (SpA vs OA). We provided conservative estimates by using the prescription date to define the exposure window. This may have led to misclassification of some current users as recent or remote users, potentially overestimating MI risk in recent or remote use categories and biassing results for current NSAID users towards the null. Third, confounding by indication still remains a potential concern in that an NSAID prescription may indicate a period of pain or increased disease activity, and it may be that painful condition or disease activity that truly puts a subject at risk. Because it was not possible to assess disease activity within this study, we consider the results of this study to be suggestive of an increased risk of MI with diclofenac and worthy of further exploration. Nonetheless, differential risk of NSAIDs would not be expected if these findings were driven by pain since any type of NSAID may be prescribed for pain. Fourth, while we estimate the ratio of OR to provide some insight into differences in the effect estimates for diclofenac use in SpA relative to OA, we did not perform a formal test for interaction. Nonetheless, it is unlikely that formally accounting for interaction (eg, in the imputation model) would have substantially changed the results of these subanalyses. Finally, the ratio of ORs indicated a difference in the effect of diclofenac in SpA as compared with OA, but failed to reach statistical significance in our sensitivity analysis, and therefore warrants further investigation. Even so, one may speculate that this finding indicates a greater propensity for MI among patients with SpA than patients with OA.

This study has strength in the use of a large, GP-derived database reflecting real-world NSAID use and risk, in contrast to the highly selected populations in trials. Second, the requirement that all subjects had at least one NSAID prescription reduces confounding by indication, and offers an advantage over previous studies that included subjects with SpA who had not received NSAIDs at all. While the primary outcome of MI was established through diagnostic codes, the PPV using this method was high in a previous validation study, and our internal validation study confirmed MI in 89% of cases. Finally, the sensitivity analyses overall agreed with the primary findings of increased risk with diclofenac use in SpA suggesting these results are robust given the assumptions made in our analytical approach.

In conclusion, this study found that current use of diclofenac in SpA was associated with twofold to threefold risk of MI relative to remote use of any NSAID. The risk associated with diclofenac in SpA differed from the risk in OA. Current naproxen use did not increase MI risk in SpA or OA, although effects should be further investigated in SpA subtypes. These results suggest that diclofenac use contributes to risk of MI in patients with SpA, and has the important implication for patients with SpA and clinicians that MIs could be prevented through preferential use of naproxen. If confirmed in other large SpA datasets, these findings may motivate a change in practice guidelines to recommend naproxen as the preferred first-line NSAID in SpA.

Acknowledgements The authors would like to thank Drs David Felson, Michael LaValley and Allan Walkey for their critical reviews.

Contributors Study conception/design: MD, HKC, YZ, TN. Data coding/analysis: QL-G, CEP. Manuscript drafting: MD, CEP, YZ, TN. All authors substantially contributed to the data interpretation, manuscript revising, critical review and final approval.

Funding MD: Arthritis Foundation Clinical to Research Transition Award, NIH/NIAMS K23 AR069127. MD, QL-G, CEP, HKC, YZ and TN: NIH NIAMS P60 AR047785. TN: NIH NIAMS K24 AR070982.

Competing interests None declared.

Patient consent Not required.

Ethics approval This study is not Human Subjects Research and was judged exempt from IRB review.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement THIN is a licensed proprietary database from IMS Health Real World Evidence Solutions.

REFERENCES

1 Lindhardsen J, Ahlehoff O, Gislason GH, *et al*. The risk of myocardial infarction in rheumatoid arthritis and diabetes mellitus: a Danish nationwide cohort study. *Ann Rheum Dis* 2011;70:929–34.
2 Symmons DP, Goodson NJ, Cook MN, *et al*. Men with ankylosing spondyliitis have an increased risk of myocardial infarction. *Arthritis Rheum* 2004;50(Suppl):S477.
3 Solomon DH, Karlson EW, Rimm EB, *et al*. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. *Circulation* 2003;107:1303–7.
4 Haroon NN, Paterson JM, Li P, *et al*. Patients with ankylosing spondylitis have increased cardiovascular and cerebrovascular mortality: a population-based study. *Ann Intern Med* 2015;163:409–16.
5 Radovits BJ, Popa-Diaconu DA, Popa C, *et al*. Disease activity as a risk factor for myocardial infarction in rheumatoid arthritis. *Ann Rheum Dis* 2009;68:1271–6.
6 Dubreuil M, Rho YH, Man A, *et al*. Diabetes incidence in psoriatic arthritis, psoriasis and rheumatoid arthritis: a UK population-based cohort study. *Rheumatology* 2014;53:346–52.
7 Papagoras C, Markatseli TE, Saougou I, *et al*. Cardiovascular risk profile in patients with spondyloarthritis. *Joint Bone Spine* 2014;81:57–63.
8 Ernste FC, Sánchez-Menéndez M, Wilton KM, *et al*. Cardiovascular risk profile at the onset of psoriatic arthritis: a population-based cohort study. *Arthritis Care Res* 2015;67:1015–21.
9 Polachek A, Touma Z, Anderson M, *et al*. Risk of cardiovascular morbidity in patients with psoriatic arthritis: a meta-analysis of observational studies. *Arthritis Care Res* 2017;69:67–74.
10 Braun J, van den Berg R, Baraliakos X, *et al*. 2010 update of the ASAS/EULAR recommendations for the management of ankylosing spondylitis. *Ann Rheum Dis* 2011;70:896–904.
11 Ward MM, Deodhar A, Akl EA, *et al*. American College of Rheumatology/Spondylitis Association of America/Spondyloarthritis Research and Treatment Network 2015 Recommendations for the Treatment of Ankylosing Spondylitis and Nonradiographic Axial Spondyloarthritis. *Arthritis Rheumatol* 2016;68:282–98.
12 Gossec L, Smolen JS, Ramiro S, *et al*. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis* 2016;75:499–510.
13 Centre Prescribing and Medicines Team Health and Social Care Information. *Prescriptions dispensed in the community; England 2004-2014*. UK: National Statistics, 2015.
14 Bhala N, Emberson J, Merhi A, *et al*. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 2013;382:769–79.
15 Chisholm J. The Read clinical classification. *BMJ* 1990;300:1092.
16 Lewis JD, Schinnar R, Bilker WB, *et al*. Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. *Pharmacoepidemiol Drug Saf* 2007;16:393–401.
17 Ogdie A, Alehashemi S, Love TJ, *et al*. Validity of psoriatic arthritis and capture of disease modifying antirheumatic drugs in the health improvement network. *Pharmacoepidemiol Drug Saf* 2014;23:918–22.
18 Dubreuil M, Peloquin C, Zhang Y, *et al*. Validity of ankylosing spondylitis diagnoses in The Health Improvement Network. *Pharmacoepidemiol Drug Saf* 2016;25:399–404.
19 García Rodríguez LA, Tacconelli S, Patrignani P. Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. *J Am Coll Cardiol* 2008;52:1628–36.
20 Graham DJ, Campen D, Hui R, *et al*. Risk of acute myocardial infarction and sudden cardiac death in patients treated with cyclo-oxygenase 2 selective and non-selective non-steroidal anti-inflammatory drugs: nested case-control study. *Lancet* 2005;365:475–81.
21 Lee T, Lu N, Felson DT, *et al*. Use of non-steroidal anti-inflammatory drugs correlates with the risk of venous thromboembolism in knee osteoarthritis patients: a UK population-based case-control study. *Rheumatology* 2016;55:1099–105.
22 Raghunathan TE, Van Hoewyk J, Solenberger P. A multivariate technique for multiply imputing missing values using a sequence of regression models. *Survey Methodology* 2001;27:85–95.
23 Rubin DB. *Introduction in multiple imputation for nonresponse in surveys*. Hoboken, NJ: John Wiley & Sons, Inc, 1987.
24 van Buuren S. *Flexible imputation of missing data*. Boca Raton, FL: Chapman & Hall/CRC, 2012.
25 Carpenter JR, Kenward MG. *Multiple imputation and its application*. Chichester, West Sussex: John Wiley & Sons, 2013.
26 Altman DG, Bland JM. Interaction revisited: the difference between two estimates. *BMJ* 2003;326:219.
27 Lindhardsen J, Gislason GH, Jacobsen S, *et al*. Non-steroidal anti-inflammatory drugs and risk of cardiovascular disease in patients with rheumatoid arthritis: a nationwide cohort study. *Ann Rheum Dis* 2014;73:1515–21.
28 Roubille C, Richer V, Starnino T, *et al*. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis. *Ann Rheum Dis* 2015;74:480–9.
29 Solomon DH, Husni ME, Wolski KE, *et al*. Differences in safety of nonsteroidal antiinflammatory drugs in patients with osteoarthritis and patients with rheumatoid arthritis: a randomized clinical trial. *Arthritis Rheumatol* 2018;70:537-546.

30 Essers I, Stolwijk C, Boonen A, *et al*. Ankylosing spondylitis and risk of ischaemic heart disease: a population-based cohort study. *Ann Rheum Dis* 2016;75:203–9.

EXTENDED REPORT

Comparison of individually tailored versus fixed-schedule rituximab regimen to maintain ANCA-associated vasculitis remission: results of a multicentre, randomised controlled, phase III trial (MAINRITSAN2)

Pierre Charles,[1,2] Benjamin Terrier,[1] Élodie Perrodeau,[3] Pascal Cohen,[1] Stanislas Faguer,[4] Antoine Huart,[4] Mohamed Hamidou,[5] Christian Agard,[5] Bernard Bonnotte,[6] Maxime Samson,[6] Alexandre Karras,[7] Noémie Jourde-Chiche,[8] François Lifermann,[9] Pierre Gobert,[10] Catherine Hanrotel-Saliou,[11] Pascal Godmer,[12] Nicolas Martin-Silva,[13] Grégory Pugnet,[14] Marie Matignon,[15] Olivier Aumaitre,[16] Jean-François Viallard,[17] François Maurier,[18] Nadine Meaux-Ruault,[19] Sophie Rivière,[20] Jean Sibilia,[21] Xavier Puéchal,[1] Philippe Ravaud,[3] Luc Mouthon,[1] Loïc Guillevin,[1] for the French Vasculitis Study Group

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212878).

For numbered affiliations see end of article.

Correspondence to
Professor Loïc Guillevin, Department of Internal Medicine, Hôpital Cochin, Paris 75679, France;
loic.guillevin@orange.fr

Received 19 December 2017
Revised 6 April 2018
Accepted 9 April 2018
Published Online First 25 April 2018

To cite: Charles P, Terrier B, Perrodeau É, *et al*. *Ann Rheum Dis* 2018;**77**:1144–1150.

ABSTRACT

Objective To compare individually tailored, based on trimestrial biological parameter monitoring, to fixed-schedule rituximab reinfusion for remission maintenance of antineutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAVs).

Methods Patients with newly diagnosed or relapsing granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) in complete remission after induction therapy were included in an open-label, multicentre, randomised controlled trial. All tailored-arm patients received a 500 mg rituximab infusion at randomisation, with rituximab reinfusion only when CD19+B lymphocytes or ANCA had reappeared or ANCA titre rose markedly based on trimestrial testing until month 18. Controls received a fixed 500 mg rituximab infusion on days 0 and 14 postrandomisation, then 6, 12 and 18 months after the first infusion. The primary endpoint was the number of relapses (new or reappearing symptom(s) or worsening disease with Birmingham Vasculitis Activity Score (BVAS)>0) at month 28 evaluated by an independent Adjudication Committee blinded to treatment group.

Results Among the 162 patients (mean age: 60 years; 42% women) included, 117 (72.2%) had GPA and 45 (27.8%) had MPA. Preinclusion induction therapy included cyclophosphamide for 100 (61.7%), rituximab for 61 (37.6%) and methotrexate for 1 (0.6%). At month 28, 21 patients had suffered 22 relapses: 14/81 (17.3%) in 13 tailored-infusion recipients and 8/81 (9.9%) in 8 fixed-schedule patients (p=0.22). The tailored-infusion versus fixed-schedule group, respectively, received 248 vs 381 infusions, with medians (IQR) of 3 (2–4) vs 5 (5–5) administrations.

Conclusion AAV relapse rates did not differ significantly between individually tailored and fixed-schedule rituximab regimens. Individually tailored-arm patients received fewer rituximab infusions.

Trial registration number NCT01731561; Results.

INTRODUCTION

Standard induction-remission treatment of anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAVs) combines glucocorticoids and cyclophosphamide or rituximab, a chimeric murine/human monoclonal IgG1 antibody directed against CD20, a specific B-cell antigen.[1] Rituximab was shown to be non-inferior to cyclophosphamide.[2 3] Remission maintenance with immunosuppressants, for example, azathioprine or methotrexate, is the conventional therapeutic approach.[4] MAINRITSAN-trial results demonstrated rituximab superiority (500 mg on days 0 and 14, then at months 6, 12 and 18) to azathioprine to maintain remission.[5] In that trial, at month 28, only 5% of rituximab recipients versus 29% taking azathioprine had experienced a major relapse.

Neither ANCA-positivity nor ANCA-titre change on conventional immunosuppressants is considered a reliable relapse predictor.[6–8] Nevertheless, ANCA reappearance or titre increase, mainly anti-proteinase-3 (PR3), in patients in remission is frequently associated with relapses,[7] especially those given rituximab.[9] ANCA parameters are not recommended for monitoring treatment[1 10] and circulating B-cell detection is not a good predictor of AAV relapse.[11] However, when B cells are undetectable and ANCA remain negative, relapses are rare.[11]

The present trial, MAINRITSAN2, was undertaken to evaluate ANCA and circulating CD19+ B cells as indicators to reinfuse rituximab to maintain remission. To do so, an individually tailored rituximab regimen, adapted to ANCA-positivity or ANCA-titre change and/or circulating CD19+ Bcell repopulation, was compared with fixed-schedule rituximab infusions, previously shown to maintain remission[5] in patients with granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA), who were in complete remission at the time of inclusion.

METHODS

Patients

Eligible patients >18 years old had newly diagnosed or relapsing GPA or MPA, as defined by the Chapel Hill Consensus nomenclature.[12] They had to be in complete remission after induction therapy, combining glucocorticoids and cyclophosphamide, rituximab or methotrexate (as decided by each investigator), in accordance with French and international recommendations. Birmingham Vasculitis Activity Score V.3 (BVAS) of 0 (score range: 0–63, with higher scores indicating more active disease) defined complete remission.[13] The following exclusion criteria were applied: another systemic vasculitis; induction with an agent not recommended; active disease; incapacity or refusal to understand or sign the informed consent form; non-compliance; allergy to the study medication; pregnancy; breastfeeding; human immunodeficiency, hepatitis B or C virus infection; severe infection declared during the 3 months before randomisation; cancer or malignant blood disease diagnosed during the 5 years preceding vasculitis diagnosis; participation in another clinical research protocol during the 4 weeks before inclusion; any clinical or psychiatric disorder that could expose the patient to a greater risk of an adverse event (AE) or could prevent treatment administration and patient follow-up according to the protocol; severe immunosuppression; administration of live vaccine during the 4 weeks before inclusion; severe chronic obstructive pulmonary diseases (maximum expiratory volume <50% or dyspnoea grade III); chronic heart failure (dyspnoea NYHA III or IV); history of recent acute coronary syndrome unrelated to vasculitis; patients not enrolled in the French national health insurance.

All patients provided written informed consent.

Study design

This trial, Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis-2 (MAINRITSAN2), is an open-label, pragmatic, multicentre, randomised controlled trial, with evaluation of the primary outcome by an independent Adjudication Committee, comprising three vasculitis experts (one each specialised in rheumatology (X Puéchal), internal medicine (O Lidove) or both (M Gayraud)), blinded to the treatment arm and circulating CD19+ B cell counts. The two coprincipal investigators (PC, LG) designed the trial and drafted the manuscript, with appropriate input from coauthors and other-site investigators. The trial was funded by the Programme Hospitalier de Recherche Clinique of the French Ministry of Health (PHRC National 2011 AOM11145). The Ethics Committee (Comité de Protection des Personnes Île-de-France 1 (Paris)) approved the study, which received legal, monitoring and administrative management support from the Assistance Publique–Hôpitaux de Paris. Hoffmann-La Roche provided rituximab for the study but was not involved in or consulted about the study design and did not have access to the data.

Patients were randomised at a 1:1 ratio to receive maintenance therapy with either an 'individually tailored' (according to laboratory findings every 3 months) or 'fixed-schedule' (control) rituximab regimen within 1 month after completing induction treatment, if they had received cyclophosphamide or methotrexate, or 4–6 months after the last rituximab infusion, if it had been used to obtain remission. An independent statistician provided the computer-generated randomisation sequence, stratified by newly diagnosed or relapsing AAV. Randomisation was centralised through electronic case-report forms (eCRF) to assure allocation concealment.

Treatment allocation was known by patients and clinicians. The Adjudication Committee that evaluated the primary endpoint was blinded to the treatment arm and circulating CD19+ B cell count.

Tailored-infusion-arm patients always received 500 mg of rituximab at randomisation; then ANCA and CD19+ B lymphocytes were assessed every 3 months. Another 500 mg were infused when ANCA status differed from the previous control (ie, reappearance after being negative, indirect immunofluorescence-determined ≥2-dilution–titre increase and/or at least doubled ELISA PR3 or myeloperoxidase (MPO) arbitrary units) or CD19+ B cell counts exceeded 0/mm^3. That algorithm, implemented in the eCRF, specified rituximab reinfusion when the CD19+ B cell count and/or ANCA changes were documented. The last rituximab infusion could be given at month 18.

The control group received the MAINRITSAN trial regimen[5]: 500 mg rituximab infusion on days 0 and 14 postrandomisation and at months 6, 12, 18 after the first infusion.

Premedication before all rituximab infusions comprised intravenous methylprednisolone (100 mg), dexchlorpheniramine (5 mg) and acetaminophen (1000 mg).

Most patients were still taking low-dose prednisone at randomisation that was tapered and stopped or could be maintained at 5 mg/day at the discretion of each site investigator.

All patients received *Pneumocystis jiroveci* pneumonia prophylaxis (daily sulfamethoxazole (400 mg)–trimethoprim (80 mg) or pentamidine aerosolisations for patients allergic to sulfa drugs).

Assessments

Study visits were scheduled at enrolment, then every 3 months until the endpoint, 28 months postrandomisation. At each visit, BVAS was calculated and blood samples were drawn from every patient. Patients were asked to record their study medication(s) weekly in a specifically designated diary. It was mandatory that all ANCA-testing and CD19+ B cell counts for a given patient be determined in the same laboratory.

The primary endpoint at month 28 was the number of relapses, defined as reappearance or worsening of AAV symptoms, that is, BVAS>0. Secondary endpoints included the number of major relapses, defined as life-threatening or involving at least one major organ; number of minor relapses; potential association of ANCA evolution and CD19+ B cell counts with relapses; glucocorticoid duration and cumulated dose; Vasculitis Damage Index (VDI) evaluated damage severity and number for each group and mortality. We also recorded all AEs, treatment-related or not and AAV evolution. The independent Adjudication Committee assessed all relapses.

Statistical analyses

The trial was designed to detect a 20% absolute between-arm difference for relapses, with a 5% alpha risk and 80% power in a two-sided test, with 35% relapses in the control group and 10% lost-to-follow-up for both groups.

The statistical analyses were conducted according to the intention-to-treat principle, including all randomised patients in their assigned group. For descriptive analyses, qualitative variables are expressed as numbers (%) or mean±SD, as appropriate and quantitative variables as median (IQR).

A generalised estimating equation model with Poisson distribution, adjusted for AAV type (newly diagnosed or relapsing) considered for randomisation and supposing a within-centre correlation (exchangeable correlation structure), compared the means of the numbers of relapses per patient per group at month

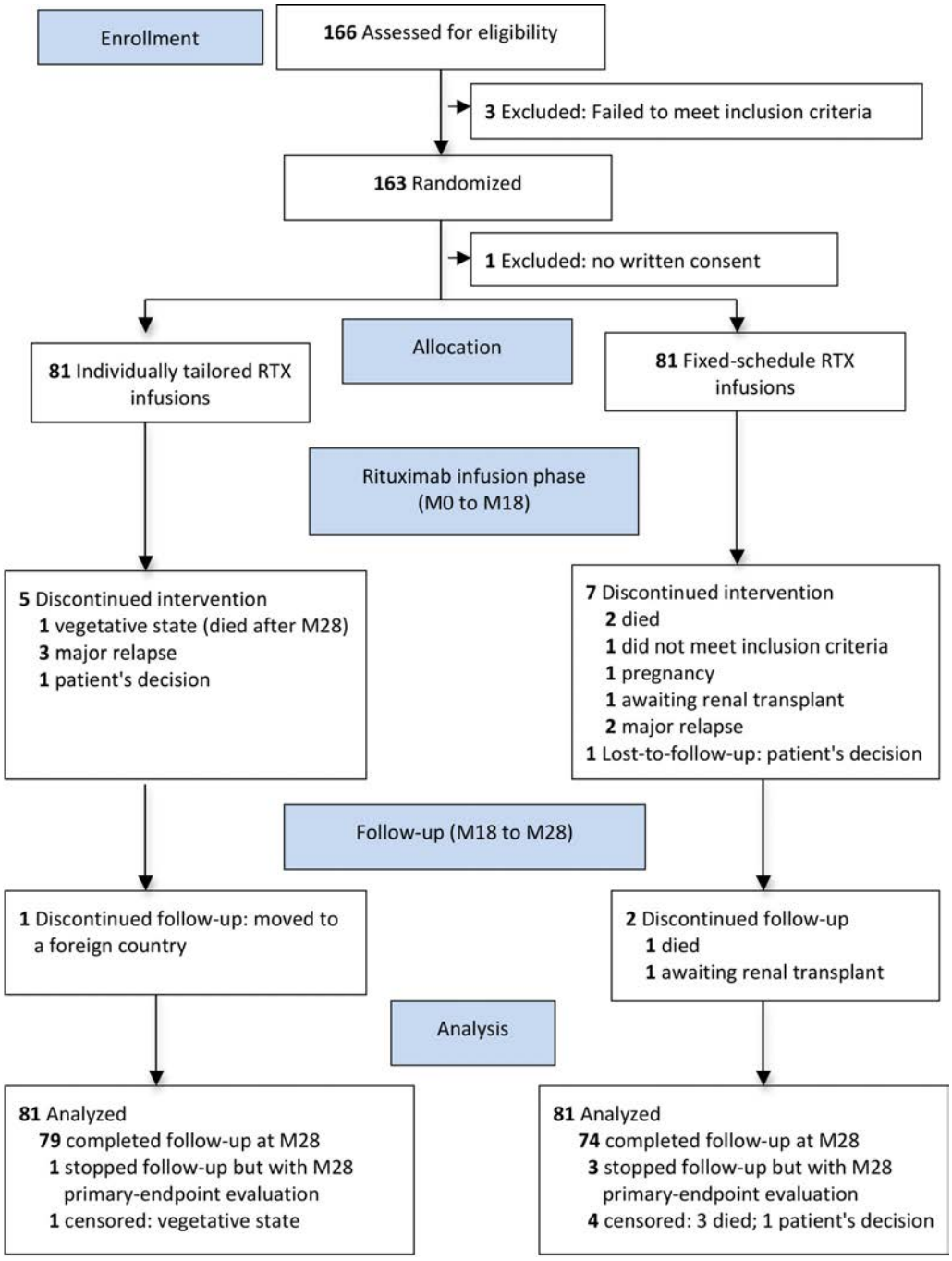


Figure 1 Flowchart of the study. M, month; RTX, rituximab.

28. Kaplan-Meier estimations of the times to first relapse were compared between groups with a Cox regression model, also adjusted for AAV type and supposing within-centre correlation.

Statistical analyses were computed with R V.3.2.2 (R Core Team (2015). R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org)).

RESULTS

Enrolment and baseline characteristics

Among 166 patients enrolled in 59 centres (figure 1) between November 2012 and November 2013, 3 who did not meet inclusion criteria were not randomised. Among the 163 patients randomised, one did not provide written consent and was excluded. Among the 162 randomised patients, 117 (72.2%) had GPA and 45 (28.8%) MPA; 104 (64.2%) and 58 (35.8%), respectively, were in remission after a first flare or at least one relapse. Preinclusion induction treatment included cyclophosphamide for 100 (61.7%), rituximab for 61 (37.6%) and methotrexate for 1 (0.6%). Eighty-one (50%) patients, whose characteristics were comparable (table 1), were randomised to each study arm.

Primary endpoint

At month 28, 21 patients had suffered 22 relapses: 14/81 (17.3%) in 13 tailored-infusion recipients and 8/81 (9.9%) in 8 fixed-schedule–infusion patients (p=0.22). Among the 21 patients who relapsed, 12 had newly diagnosed AAVs, 18 with GPA and 3

Table 1 General characteristics at inclusion

Characteristic	Rituximab infusions	
	Individually tailored (n=81)	Fixed-schedule (n=81)
Age, means±SD, years	62±14	59±13
Female sex, n (%)	31 (38.3)	37 (45.7)
Vasculitis type, n (%)		
GPA	56 (69.1)	61 (75.3)
MPA	25 (30.9)	20 (24.7)
Disease status, n (%)		
Newly diagnosed	53 (65.4)	51 (63.0)
Relapsing	28 (34.6)	30 (37.0)
Induction treatment of last disease flare, n (%)		
Cyclophosphamide	52 (64.2)	49 (60.5)
Rituximab	28 (34.6)	32 (39.5)
Methotrexate	1 (1.2)	0 (0.0)
Prednisone dose (mg); median (IQR)	10 (10–15)]	12 (10–17.3)
Organ involvement at last flare, n (%)		
Ear, nose and throat	46 (56.8)	39 (48.1)
Pulmonary	50 (61.7)	44 (54.3)
Renal	60 (74.1)	56 (69.1)
GFR, mean±SD, mL/min/1.73 m^2 at inclusion	55.6±27.3	58.9±27.0
ANCA-positive at diagnosis, n (%)*	74/77 (96.1)	72/79 (91.1)
Indirect immunofluorescence	68/77 (88.3)	6579 (82.3)
ELISA	64/77 (83.1)	61/79 (77.2)
Anti-PR3	38/77 (49.4)	38/79 (48.1)
Anti-MPO	26/77 (33.8)	24/79 (30.4)
ANCA-positive at inclusion, n (%)†	45/80 (56.3)	58/80 (72.5)
Indirect immunofluorescence	40/80 (50)	54/80 (67.5)
ELISA	28 (35)	43 (53.7)
Anti-PR3	18 (22.5)	21 (26.2)
Anti-MPO	10 (12.5)	23 (28.7)

*Data were missing for four individually tailored-infusion and two fixed-schedule-infusion patients.
†Data were missing for one patient in each group.
ANCA, antineutrophil cytoplasm antibodies; GFR, glomerular filtration rate; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase.; PR3, proteinase-3.

with MPA; 12 were anti-PR3 and 3 were anti-MPO ANCA-positive, 2 were ANCA-positive of unknown specificity and 4 were ANCA-negative. Two relapses (one in each arm) occurred after month 28 and were censured from the main analysis.

Secondary endpoints

Relapses

Comparing tailored versus fixed-schedule rituximab infusions, respectively: relapse-free survival rates were 83.8% (95% CI 76.1% to 92.3%) vs 86.4% (95% CI 79.2 to 94.2) (p=0.58) (figure 2) and major relapses occurred in 6 (7.4%) vs 3 (3.7%) patients (p=0.23). The six major relapses in the tailored-infusion arm manifested as two renal flares, two peripheral neuropathies, one pulmonary nodule and one pachymeningitis with orbital mass. To treat those relapses, two patients received cyclophosphamide and four rituximab. The three major relapses in the control arm (renal flare, myopericarditis with pulmonary infiltrates or subglottic stenosis) were treated with rituximab. All relapses are described in detail in online supplementary tables S1 and S2.

Damage

VDIs (mean ±SD) for the tailored and fixed-schedule rituximab-infusion arms, respectively, were 1.64±1.41 and 1.86±1.70 at inclusion and 1.99±1.57 and 2.09±1.97 at 28 months.

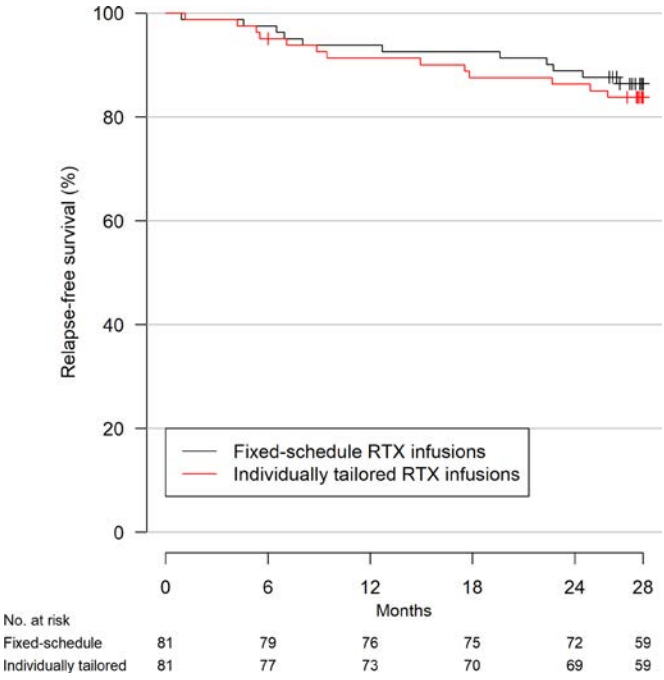


Figure 2 Relapse-free Kaplan-Meier curves according to treatment group. RTX, rituximab.

The damage-accrual difference between the two arms was 0.14 (95% CI –0.07 to 0.35) (p=0.179), using a constrained longitudinal data-analysis model (unplanned analysis required by reviewers).

ANCA-cell and B-cell-repopulation-related AAV relapses

ANCA and circulating CD19+ B cell determinations and their evolutions were available for 161 patients (one relapsing patient's data were missing). Five ANCA-evolution profiles were identified (table 2); none was associated with relapses. At month 28, 46/76 (60.5%) tailored-infusion recipients were ANCA-positive vs 26/71 (36.6%) fixed-schedule patients (p=0.06). In addition, circulating B cells were not detected in 10 (45.4%) patients who relapsed and 4 (18.2%) were ANCA-cell-negative and B-cell-negative. All relapses, including the two censured, were analysed.

Cumulated glucocorticoid dose and duration

Glucocorticoid doses and durations since inclusion did not differ significantly between the two arms. For tailored-infusion versus fixed-schedule regimen, respectively, cumulated glucocorticoid doses (mean ±SD) were 4915±2613 vs 4850±2444 mg (p=0.71) and treatment lasted 25.3 (95% CI 23.9 to 26.6) vs 24.5 (95% CI 22.8 to 36.3) months (p=0.52).

Patients' diaries were collected at each visit (online supplementary table S3).

Twenty-five patients stopped glucocorticoids during the study; that discontinuation was not associated with relapses (online supplementary figure S1).

Rituximab infusions

The tailored-infusion versus fixed-schedule groups, respectively, received 248 vs 381 infusions, with medians (IQR) of 3 (2–4) vs 5 (5–5) administrations. Four (4.9%), 23 (28.4%), 30 (37%), 15 (18.5%), 6 (7.4%), 2 (2.5%) or 1 (1.2%) tailored-regimen patients received, respectively, 1, 2, 3, 4, 5, 6 or 7 rituximab infusions, at a median between-infusion interval of 6.1 (IQR:

Table 2 ANCA evolution and B-cell detection patterns throughout follow-up for patients with ≥1 relapses or none

Parameter profile	Patients with ≥1 relapse(s) (n=22)*	Patients with No relapse (n=139)
ANCA evolution (%)		
Always negative	7 (31.8)	33 (23.7)
Negative at inclusion and became positive	3 (13.6)	14 (10.1)
Positive at inclusion and became negative	2 (9.1)	51 (36.7)
Positive at inclusion and titres rose	1 (4.5)	10 (7.2)
Positive at inclusion and remained stable	9 (40.9)	29 (20.9)
Circulating CD19+ B cell evolution (%)		
Always negative	11 (50)	8 (5.8)
Detected at least once	11 (50)	131 (94.2)
ANCA and circulating CD19+ B cell evolutions (%)		
ANCA-negative and no circulating B cells detected	4 (18.2)	5 (3.6)
Other	18 (81.8)	134 (96.4)

Values are expressed as n (%).
*At the last visit, 23 patients had suffered 24 relapses; 2 relapses that occurred after month 28 were censored in the principal analysis. Thus, with 1 missing value and 1 patient who relapsed twice, we have 22 patients with ≥1 relapses.

Table 3 Numbers of SAEs according to treatment group

SAE	Rituximab infusions: Individually tailored (n=81)	Rituximab infusions: Fixed-schedule (n=81)
Number	37	53
Patients with SAE(s)	26	31
Infection	18	18
Pneumonia	3	6 (1 died)
Bronchitis	6	4
Aspergillus fumigatus colonisation	1	0
Prostatitis	2	0
Septicaemia (from urinary tract)	1	2
Urinary infection	0	1
Septicaemia (fungal)	1	0
Cholecystitis	1	0
Ear, nose and throat	0	1
Sigmoid abscess	0	1
Colitis (*Campylobacter* sp.)	1	0
Others	2	3
Cancer	1	2
Thromboembolic events	1	3
Cardiac events	4	7
Pregnancy	0	1
Neutropaenia	1	0
Others	11	22

SAEs, severe adverse events.

3.1–9.2) months. That group received 168 reinfusions because ANCA became positive or titres rose (22 (13.1%) infusions), circulating B cells reappeared (85 (50.6%) infusions) or both (61 (36.3%) infusions). Ten protocol deviations were observed in the tailored-regimen arm: 10 patients did not receive one reinfusion: eight because the investigator did not follow the reinfusion rules and two patients refused.

Safety

Sixty-nine (85.2%) tailored-infusion recipients vs 74 (91%) controls reported at least one AE (p=0.33), with, respectively, 26 (32.1%) vs 31 (38.3%) experiencing at least one severe AE (SAE) (p=0.51) and 37 vs 53 SAEs per group. Eighteen infectious complications occurred in each arm, with 9 (11.1%) tailored-regimen recipients vs 16 (19.8%) controls experiencing at least one infection. The only episode of neutropaenia occurred in the tailored-infusion arm. SAEs are reported in table 3 and online supplementary table S4.

Four patients died during the study: one tailored-infusion recipient (bronchospasm not related to rituximab infusion) versus three controls (nosocomial pneumonia, carcinomatous meningitis or cardiogenic shock unrelated to AAV or rituximab).

No significant between-group gammaglobulin-level differences or decreases were observed throughout the trial (online supplementary figure S2).

DISCUSSION

The usefulness of monitoring ANCA reappearance/titres and/or circulating CD19+ B cells for AAV treatment is controversial and not evidence-based.[7 8 11 14–16] This prospective trial was designed to determine whether two of the most frequently prescribed laboratory tests during surveillance of AAV remission-maintenance therapy are reliable and could help decide whether or not to reinfuse rituximab during follow-up.

The results of this study confirmed rituximab efficacy for AAV-remission maintenance: relapse rates and major-relapse rates were low in both arms: 6/81 (7.4%) vs 3/81 (3.7%) for tailored and fixed-schedule regimens, respectively. Relapse rates were comparable to that of rituximab-treated patients in the MAINRITSAN trial (3/57 (5.2%) with a major relapse).[5] They should be compared with the 32% observed at 18 months without maintenance therapy after remission induction with rituximab[2] or the 29% major-relapse rate at month 28 under azathioprine after cyclophosphamide-induced remission.[2 5] Moreover, because the high relapse rates observed without treatment[2] or with azathioprine[5] can impact survival and enhance AAV-induced damage,[17 18] on top of iatrogenicity of a new induction treatment, our data further support that it is reasonable to maintain remission with rituximab and not wait to retreat.

This trial's findings also demonstrated that it is indeed possible to maintain remission with fewer infusions, even though a non-significant trend towards more relapses was observed for patients receiving the individually tailored regimen. In that arm, no day-14 infusion was planned and patients received one less reinfusion than the fixed-schedule controls.

Herein, although ANCA evolution and/or circulating CD19+ B cells were not reliable predictors of AAV relapses, combining them achieved fewer infusions in the tailored-infusion arm without significantly more relapses. In the literature, the role of ANCA as a marker of relapse remains a source of debate.[8] In an earlier randomised trial,[19] more than half the patients not given any maintenance therapy and whose ANCA titres rose suffered a major relapse. In a previous retrospective study,[7] we showed that relapse-free survival was longer when anti-PR3 ANCA remained negative, and clinical status and ANCA evolution were closely associated for only 60% of patients with GPA. More recently,[9 16] anti-PR3 ANCA were associated with GPA relapses only in the subgroup of patients with a 'vasculitis phenotype' (eg, renal involvement or alveolar haemorrhage), particularly after receiving rituximab. Herein, five distinct ANCA-evolution profiles were identified, none of which was a good predictor of relapses. Patients receiving individually tailored infusions were more likely to be ANCA-positive at month 28. Among relapsing patients, 10/13 (76.9%) were ANCA-positive at month 28 vs only 3/8 (37.5%) fixed-schedule

controls. The small number of events prevents us from drawing any definitive conclusions.

Pertinently, relapses could also occur in the absence of circulating B cells, perhaps because B-cell repopulation could be only at sites of active disease[20] or because the CD19+ B lymphocyte count is not strictly associated with CD27 +memory B-cell reemergence.[21] Four of our patients were ANCA-negative at relapse without any circulating B cells. This pattern, considered rare,[11] represented 18.2% of the relapses in our study. Thus, we are unable to conclude as to the relevance of monitoring ANCA and/or CD19+ B cells. In the MAINRITSAN2 trial, patients assigned to the tailored-infusion strategy received fewer rituximab infusions and relapses in this group were not significantly different from those of the fixed-schedule controls. Although these laboratory findings seem to have no association with relapse, they are useful markers to guide infusion rhythm during follow-up.

Eighteen infections occurred in each arm, with lung infections being the most frequent. Even though rituximab was not associated with more infectious complications than azathioprine[3] or methotrexate,[4] safety remains an important issue. In large series,[22–25] up to 29% of rituximab-treated patients experienced infectious complications. None of our patients developed *Pneumocystis jiroveci* pneumonia, probably because of mandatory trimethoprim–sulfamethoxazole prophylaxis. Also, progressive multifocal leukoencephalopathy was not seen within the 28 months of observation.

This trial's findings have multiple repercussions for patients and physicians. The low relapse rates observed in the two arms after 28 months of follow-up confirmed that rituximab-maintenance therapy is justified. Moreover, patients were probably overtreated with previously administered regimens[5] because remission could be sustained with a lower rituximab dose. Although ANCA and CD19+ B cell monitoring was not associated with relapse, those values contributed to deciding to reinfuse, with the clear benefit of fewer rituximab infusions in the tailored-infusion arm.

This study had some limitations. It was open-labelled but all relapses were assessed by an independent Adjudication Committee, unaware of treatment arm and the circulating CD19+ B cell count. Biological parameters were assayed in each centre, because it would have been impractical to centralise testing for the 59 participating centres throughout the country and impossible to decide rapidly to reinfuse patients. However, all ANCA-titering and CD19+ B cell counts for a given patient had to be done in the same laboratory. ANCA titres were measured with different techniques according to each centre's practice. Interassay variability was not evaluated before the study. Fixed-schedule infusion-arm patients were more likely to be ANCA-positive at inclusion. It cannot be excluded that this difference might have impacted the relapse risks of patients in the two arms. By protocol design, two-thirds of the patients were included after their first flare; therefore, our results may not completely be fully generalisable to the subgroup of patients with relapsing disease.

Our study also has several strengths. It was a multicentre trial using a reinfusion-decision algorithm applied to the tailored-infusion arm, which received 248 infusions; only 10 protocol deviations were observed, probably because the algorithm included in the eCRF emitted an automatic directive indicating the need for reinfusion.

In conclusion, AAV relapse rates for patients treated according to individually tailored or fixed-schedule rituximab-infusion regimens did not differ significantly. However, those benefitting from personalised patient-centred care received fewer infusions and, hence, lower total rituximab doses.

Author affiliations
[1]Internal Medicine, Referral Center for Rare Systemic and Autoimmune Diseases: Vasculitis and Scleroderma, Cochin Hospital, Paris Descartes University, Paris, France
[2]Department of Internal Medicine, Institut Mutualiste Montsouris, Paris, France
[3]Centre d'Epidémiologie Clinique, Hôpital Hôtel-Dieu, Université Paris Descartes, Sorbonne Paris Cité, INSERM Unité 1153, Paris, France
[4]Département de Néphrologie et Transplantation d'Organes, CHU de Toulouse, Toulouse, France
[5]Service de Médecine Interne, Hôtel-Dieu, Centre Hospitalier Universitaire, Nantes, France
[6]Service de Médecine Interne et Immunologie Clinique, Centre Hospitalier Universitaire de Dijon, INSERM, UMR 1098, University of Bourgogne Franche-Comté, FHU INCREASE, Dijon, France
[7]Unité de Néphrologie, Hôpital Européen Georges-Pompidou, Université Paris Descartes, Paris, France
[8]Aix Marseille Univ, Centre de Néphrologie et de Transplantation Rénale, AP-HM, Hôpital de la Conception, Marseille, France
[9]Service de Médecine Interne Hématologie, Centre Hospitalier de Dax, Dax, France
[10]Pôle médecine, Hôpital Général Henri-Duffaut, Avignon, France
[11]Service de Néphrologie, Dialyse et Transplantation Rénale, Hôpital la Cavale Blanche, CHRU Brest, Brest, France
[12]Département de Médecine Interne, Centre Hospitalier Bretagne Atlantique de Vannes, Vannes, France
[13]Unité de Médecine Interne, CHU de Caen, Caen, France
[14]Service de Médecine Interne, CHU de Toulouse, Toulouse, France
[15]Service de Néphrologie, Hôpital Henri-Mondor, Créteil, France
[16]Service de Médecine Interne, Centre Hospitalier Universitaire, Hôpital Gabriel-Montpied, Clermont-Ferrand, France
[17]Service de Médecine Interne et Maladies Infectieuses, CHU de Bordeaux, Pessac, France
[18]Service de Médecine Interne, Hôpitaux privés de Metz, Metz, France
[19]Service de Médecine Interne, Centre Hospitalier Universitaire Jean-Minjoz, Besançon, France
[20]Service de Médecine Interne, CHU de Montpellier, Montpellier, France
[21]Service de Rhumatologie, Hôpital de Hautepierre, CHU de Strasbourg, Strasbourg, France

Acknowledgements The authors thank URC-CIC Paris Descartes Necker/Cochin (Séverine Aït El Ghaz-Poignant and Charly Larrieu) for implementation, monitoring and data management of the study, DEC-Agence Générale des Equipements et Produits de Santé for their logistics assistance with the study drugs and Janet Jacobson for editorial assistance.

Collaborators Other investigators and members of the French Vasculitis Study Group who participated in the study are listed in the online supplementary.

Contributors PCh and LG conceived and planned the study. ÉP and PR designed and carried out the statistical analyses. PCh wrote the first draft of the manuscript and all authors contributed to subsequent revisions. All other authors entered data, participated in devising the protocol and reviewed all versions of the manuscript.

Funding This study was funded by a research grant from the French Ministry of Health (PHRC National 2011 AOM11145) and sponsored by the Assistance Publique–Hôpitaux de Paris. Hoffmann-La Roche provided rituximab for the study.

Competing interests BT has received consulting and speaking fees (Roche, LFB, Grifols, GSK). MH has received personal fees from Roche. AK has received personal fees and non-financial support from Roche. XP has received speaking fees and honoraria (Pfizer, LFB, Roche) and a research grant (Pfizer).

Patient consent Obtained.

Ethics approval The Ethics Committee (Comité de Protection des Personnes Île-de-France 1 (Paris)) approved the study.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Yates M, Watts RA, Bajema IM, *et al*. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis* 2016;75:1583–94.
2 Stone JH, Merkel PA, Spiera R, *et al*. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010;363:221–32.
3 Jones RB, Tervaert JW, Hauser T, *et al*. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010;363:211–20.
4 Pagnoux C, Mahr A, Hamidou MA, *et al*. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med* 2008;359:2790–803.

5 Guillevin L, Pagnoux C, Karras A, *et al*. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med* 2014;371:1771–80.
6 Jayne DR, Gaskin G, Pusey CD, *et al*. ANCA and predicting relapse in systemic vasculitis. *QJM* 1995;88:127–33.
7 Thai LH, Charles P, Resche-Rigon M, *et al*. Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. *Autoimmun Rev* 2014;13:313–8.
8 Tomasson G, Grayson PC, Mahr AD, *et al*. Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis--a meta-analysis. *Rheumatology* 2012;51:100–9.
9 Fussner LA, Hummel AM, Schroeder DR, *et al*. Factors determining the clinical utility of serial measurements of antineutrophil cytoplasmic antibodies targeting proteinase 3. *Arthritis Rheumatol* 2016;68:1700–10.
10 Charles P, Bienvenu B, Bonnotte B, *et al*. Rituximab: recommendations of the French Vasculitis Study Group (FVSG) for induction and maintenance treatments of adult, antineutrophil cytoplasm antibody-associated necrotizing vasculitides. *La Presse Médicale* 2013;42:1317–30.
11 Specks U, Merkel PA, Seo P, *et al*. Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med* 2013;369:417–27.
12 Jennette JC, Falk RJ, Andrassy K, *et al*. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187–92.
13 Flossmann O, Bacon P, de Groot K, *et al*. Development of comprehensive disease assessment in systemic vasculitis. *Ann Rheum Dis* 2007;66:283–92.
14 Boomsma MM, Stegeman CA, van der Leij MJ, *et al*. Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. *Arthritis Rheum* 2000;43:2025–33.
15 Finkielman JD, Merkel PA, Schroeder D, *et al*. Antiproteinase 3 antineutrophil cytoplasmic antibodies and disease activity in Wegener granulomatosis. *Ann Intern Med* 2007;147:611–9.
16 Kemna MJ, Damoiseaux J, Austen J, *et al*. ANCA as a predictor of relapse: useful in patients with renal involvement but not in patients with nonrenal disease. *J Am Soc Nephrol* 2015;26:537–42.
17 Slot MC, Tervaert JW, Franssen CF, *et al*. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int* 2003;63:670–7.
18 Abdou NI, Kullman GJ, Hoffman GS, *et al*. Wegener's granulomatosis: survey of 701 patients in North America. Changes in outcome in the 1990s. *J Rheumatol* 2002;29:309–16.
19 Tervaert JW, Huitema MG, Hené RJ, *et al*. Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet* 1990;336:709–11.
20 Ferraro AJ, Smith SW, Neil D, *et al*. Relapsed Wegener's granulomatosis after rituximab therapy--B cells are present in new pathological lesions despite persistent "depletion" of peripheral blood. *Nephrol Dial Transplant* 2008;23:3030–2.
21 Lebrun C, Bourg V, Bresch S, *et al*. Therapeutic target of memory B cells depletion helps to tailor administration frequency of rituximab in myasthenia gravis. *J Neuroimmunol* 2016;298:79–81.
22 Holle JU, Dubrau C, Herlyn K, *et al*. Rituximab for refractory granulomatosis with polyangiitis (Wegener's granulomatosis): comparison of efficacy in granulomatous versus vasculitic manifestations. *Ann Rheum Dis* 2012;71:327–33.
23 Charles P, Néel A, Tieulié N, *et al*. Rituximab for induction and maintenance treatment of ANCA-associated vasculitides: a multicentre retrospective study on 80 patients. *Rheumatology* 2014;53:532–9.
24 Cartin-Ceba R, Golbin JM, Keogh KA, *et al*. Rituximab for remission induction and maintenance in refractory granulomatosis with polyangiitis (Wegener's): ten-year experience at a single center. *Arthritis Rheum* 2012;64:3770–8.
25 Alberici F, Smith RM, Jones RB, *et al*. Long-term follow-up of patients who received repeat-dose rituximab as maintenance therapy for ANCA-associated vasculitis. *Rheumatology* 2015;54:1153–60.

EXTENDED REPORT

Long-term efficacy of remission-maintenance regimens for ANCA-associated vasculitides

Benjamin Terrier,[1] Christian Pagnoux,[1,2] Élodie Perrodeau,[3] Adexandre Karras,[4] Chahera Khouatra,[5] Olivier Aumaître,[6] Pascal Cohen,[1] Olivier Decaux,[7] Hélène Desmurs-Clavel,[8] François Maurier,[9] Pierre Gobert,[10] Thomas Quémeneur,[11] Claire Blanchard-Delaunay,[12] Bernard Bonnotte,[13] Pierre-Louis Carron,[14] Eric Daugas,[15] Marize Ducret,[16] Pascal Godmer,[17] Mohamed Hamidou,[18] Olivier Lidove,[19] Nicolas Limal,[20] Xavier Puéchal,[1] Luc Mouthon,[1] Philippe Ravaud,[3] Loïc Guillevin,[1,21] on behalf of the French Vasculitis Study Group

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212768).

For numbered affiliations see end of article.

Correspondence to
Dr Benjamin Terrier, Department of Internal Medicine, Hôpital Cochin, 75014 Paris, France; benjamin.terrier@aphp.fr

Received 29 November 2017
Revised 5 April 2018
Accepted 9 April 2018
Published Online First
3 May 2018

ABSTRACT

Objective To compare long-term efficacy of remission-maintenance regimens in patients with newly diagnosed or relapsing antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitides.

Methods The 28-month Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis trial compared rituximab with azathioprine to maintain remission in patients with newly diagnosed or relapsing granulomatosis with polyangiitis, microscopic polyangiitis or renal-limited ANCA-associated vasculitis. Thereafter, prospective patient follow-up lasted until month 60. The primary endpoint was the major-relapse rate at month 60. Relapse and serious adverse event-free survival were also assessed.

Results Among the 115 enrolled patients, only one was lost to follow-up at month 60. For the azathioprine and rituximab groups, respectively, at month 60, the major relapse-free survival rates were 49.4% (95% CI 38.0% to 64.3%) and 71.9% (95% CI 61.2% to 84.6%) (p=0.003); minor and major relapse-free survival rates were 37.2% (95% CI 26.5% to 52.2%) and 57.9% (95% CI 46.4% to 72.2%) (p=0.012); overall survival rates were 93.0% (95% CI 86.7% to 99.9%) and 100% (p=0.045) and cumulative glucocorticoid use was comparable. Quality-adjusted time without symptoms and toxicity analysis showed that rituximab-treated patients had 12.6 months more without relapse or toxicity than those given azathioprine (p<0.001). Antiproteinase-3-ANCA positivity and azathioprine arm were independently associated with higher risk of relapse. HRs of positive ANCA to predict relapse increased over time.

Conclusion The rate of sustained remission for ANCA-associated vasculitis patients, following rituximab-based or azathioprine-based maintenance regimens, remained superior over 60 months with rituximab, with better overall survival.

Trial registration number NCT00748644.

INTRODUCTION

Antineutrophil cytoplasm antibody (ANCA)-associated vasculitides are necrotising vasculitides affecting small-sized vessels, with potential organ-threatening or life-threatening complications.[1] They include granulomatosis with polyangiitis (Wegener's), microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis (Churg-Strauss). The latter is usually studied separately because of its particularities. Staged therapeutic strategies based on disease severity have dramatically improved overall survival over the last decades.[2–5]

Rituximab was approved, combined with glucocorticoids, for remission-induction treatment of severe granulomatosis with polyangiitis and microscopic polyangiitis, based on the results of the Rituximab in ANCA-Associated Vasculitis (RAVE) trial[6] and the Rituximab versus Cyclophosphamide in ANCA-Associated Vasculitis trial.[7] Follow-up of RAVE trial patients showed the non-inferiority of the single initial rituximab cycle to cyclophosphamide then azathioprine at achieving sustained remissions.[6 8] However, only 39% of the rituximab-treated patients and 33% of the cyclophosphamide–azathioprine group remained in sustained complete remission off glucocorticoids at month 18, highlighting the persistently high relapse rate of ANCA-associated vasculitides.

The prospective, open-label, randomised, controlled Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis (MAINRITSAN) trial compared systematic rituximab infusions to azathioprine for maintenance of remission.[9] Its results demonstrated that the rituximab maintenance regimen was superior to azathioprine at preventing major relapses at 28 months. However, remission duration following rituximab-based or azathioprine-based maintenance regimens and their long-term toxicities are unknown. Herein, we report MAINRITSAN trial patients' 60 month follow-up (online supplementary data).

METHODS

Study oversight

This MAINRITSAN trial was designed by the two coprincipal investigators (CP and LG). Long-term outcome data were collected by the site investigators and analysed by the Data Analysis Committee (BT, CP, EP, PR and LG) that did not include representatives from Hoffmann-La Roche, which

To cite: Terrier B, Pagnoux C, Perrodeau É, *et al*. *Ann Rheum Dis* 2018;**77**:1151–1157.

provided some of the rituximab for the study. Hoffmann-La Roche was not involved in or consulted about the study design, did not review the manuscript and did not have access to the study data or provide any other support.

All manuscript drafts were written by BT, CP and LG, with input as appropriate from coauthors and other-site investigators (see online supplementary appendix). The Hôpital Cochin Comité de Protection des Personnes (Paris) approved the study, which received legal, monitoring and administrative management support from the Assistance Publique-Hôpitaux de Paris and was funded by the French Ministry of Health (NCT00748644; EudraCT 2008-002846-51).

Patients

The MAINRITSAN trial design details were reported previously.[9] Briefly, patients with newly diagnosed or relapsing granulomatosis with polyangiitis, microscopic polyangiitis or renal-limited ANCA-associated vasculitides in complete remission after combined glucocorticoids and 'pulse' intravenous cyclophosphamide were enrolled between October 2008 and June 2010. Patients were followed every 3 months for 28 months. Thereafter, patients were followed prospectively until month 60, every 3–6 months according to their clinical status.

Treatment groups

They were randomly assigned, at a 1:1 ratio, to receive rituximab or azathioprine maintenance and followed for 28 months. After induction therapy until remission, patients were randomised to receive rituximab (500 mg on days 0 and 14, and at months 6, 12 and 18 postinclusion) or azathioprine (dose: 2 mg/kg/day for 12 months; 1.5 mg/kg/day for 6 months; then 1 mg/kg/day for 4 months). Prednisone dose tapering and the decision to stop prednisone after month 18 were left to each site investigator's discretion. Co-trimoxazole prophylaxis was recommended for all patients with <250 CD4+ T cells/mm^3.

Study assessments

At each follow-up visit, information on disease activity, medications and adverse events (AEs) were collected. Each patient's serum samples were tested in each study centre for ANCA by indirect immunofluorescence and for antiproteinase 3 (PR3) and antimyeloperoxidase (MPO) ANCA with ELISAs, according to clinical status. Rituximab-treated patients' CD19+ B lymphocytes (defined as B cell count >0/mm^3) were counted locally at least before each infusion, during the initial 28-month study period, then according to clinical status.

Outcomes

The primary 60-month endpoint was the time to first major relapse (reappearance or worsening of disease with Birmingham Vasculitis Activity Score (BVAS) >0 and involvement of at least one major organ, a life-threatening manifestation or both). Secondary endpoints included time to first relapse, that is, major or minor (reappearance or worsening of disease with BVAS >0, not corresponding to a major relapse but requiring mild treatment intensification), AEs and their severity and mortality. Relapses were initially graded by each patient's site investigator, then reassessed and validated by the Data Committee. Relapses were treated according to the site investigator's decision. Grade 3/4, death (from any cause; grade 5), cancers, cardiovascular events, AEs requiring hospitalisation or infusion reactions that contraindicated further infusions defined severe AEs (SAEs). Quality-adjusted Time Without Symptoms and Toxicity (Q-TWiST) analyses assessed relapse and SAE-free times for the two groups at 60 months.

Statistical analyses

Patients' data were analysed and compared according to the initial randomisation group. Kaplan-Meier survival curves described overall, major and major and minor relapse-free and event-free survival rates for each arm. Survival analyses were censored at 60 months of follow-up. Survival rates were compared using marginal Cox models to consider the centre effect. The comparison was stratified on disease status (newly diagnosed, relapsing), which was a stratification parameter at randomisation. HRs and their 95% CIs were derived from the Cox models and tested with robust-score tests. Because no rituximab-arm patient died, a stratified log-rank test was used to compare overall survival between groups. Q-TWiST analyses were also run (see online supplementary appendix).

For each patient, the cumulative glucocorticoid dose was estimated with the area under the curve (AUC) of glucocorticoid-dose evolution versus time (inclusion to month 60). AUC means were compared between groups using a linear-mixed model with a random effect at the centre level. For patients with incomplete follow-up, the AUC was divided by the real follow-up time and multiplied by 60 months.

Age at disease flare, sex, ANCA-associated vasculitis, PR3-ANCA status at disease flare, creatininemia >2.27 mg/dL (200 μmol/L), ear, nose and throat, pulmonary and/or cardiovascular involvement(s) and ANCA at inclusion were evaluated as potential factors predictive of relapse. Factors with p value <0.20 in univariate analysis were included in the multivariate analysis. These analyses were adjusted on treatment arm.

To analyse changes in ANCA and B cell count over time as predictors of relapses, we used ANCA and B cell count collected at time points s=0, 3, 6, 9, 12, 15, 18, 21, 24, 28, 36, 42 and 48 months. For each time point, we constructed a data set by selecting all individuals at risk at time *s* (ie, no relapse before s, but still followed-up at s). Data recorded at the month 54 visit were not used as there was not enough information after this time point (individuals at risk, relapses). For each data set, we fitted two Cox models, one for ANCA and one for B cell count. Treatment arm was included as an adjustment variable in each model. HRs for each separate model (one per time point) were plotted against time points. All statistical tests were two-sided with p values <0.05 defining significance.

RESULTS

Patients

Figure 1 follows the status of the 115 enrolled patients (58 randomised to azathioprine, 57 to rituximab) over 28 and 60 months. One hundred and ten (96%) patients completed the 60-month follow-up (four died, one was lost to follow-up and censored at last follow-up).

Efficacy assessments

Relapses

As previously reported, for azathioprine and rituximab arms, respectively, 17 (29%) and three (5%) patients suffered major relapses during the first 28 months, and nine (16%) and six (11%) patients had minor relapses.

Between months 28 and 60, for azathioprine and rituximab arms, respectively, among previously major relapse-free patients, 11 (19%) and 13 (23%) additional patients experienced major relapses; while three (5%) and seven (12%) had minor relapses.

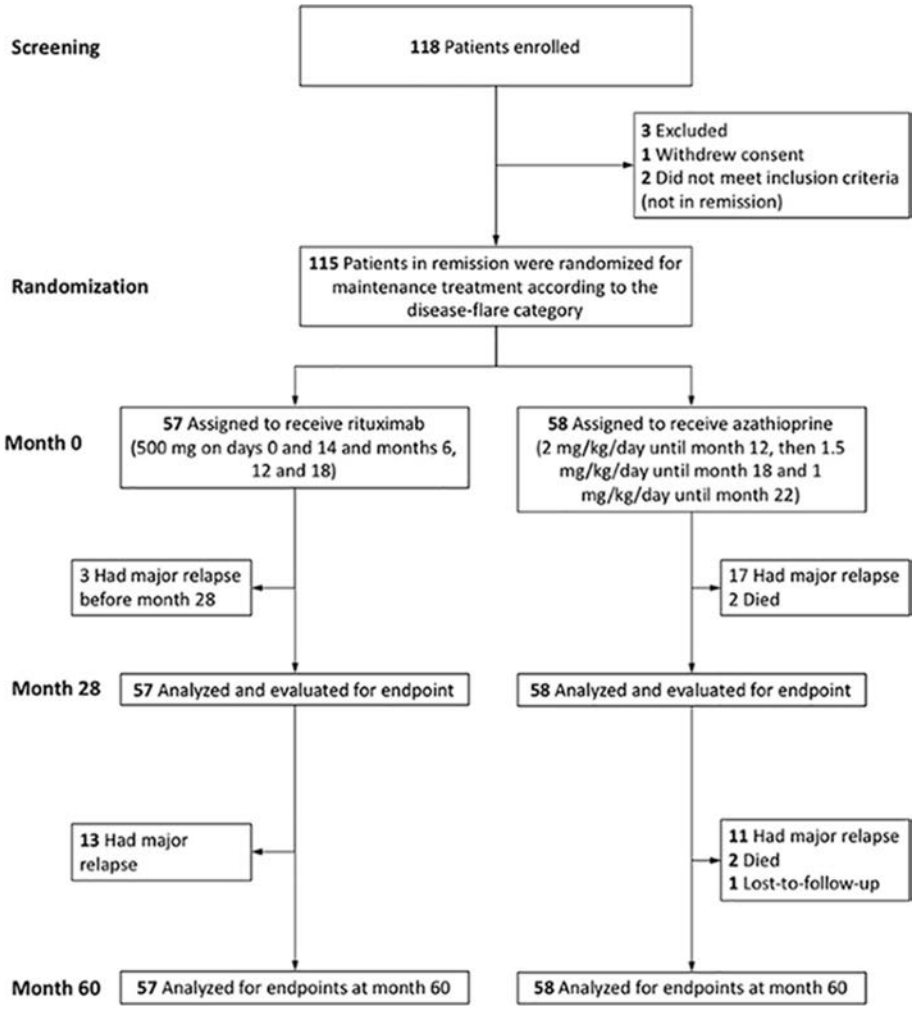


Figure 1 Randomisation and inclusion in the analysis at months 28 and 60. Patients were randomly assigned, at a 1:1 ratio, to receive rituximab or azathioprine maintenance therapy. Randomisation was stratified according to disease-flare category. Among azathioprine-treated patients, four died and one was lost to follow-up after month 28; they were censored at last follow-up. The remaining 110 (96%) patients completed the 60 months of follow-up.

Only one of the 11 azathioprine-group patients with major relapses and two of the 13 rituximab recipients had previously experienced minor relapses during the first 28 months of follow-up. Moreover, all the azathioprine-group patients with minor relapses had prior minor relapses during the first 28 months, versus only two of the seven rituximab recipients.

Hence, at month 60, for the azathioprine and rituximab arms, respectively, the major relapse-free survival rates were 49.4% (95% CI 38.0% to 64.3%) and 71.9% (95% CI 61.2% to 84.6%) (p=0.003) and all relapse-free survival rates were 37.2% (95% CI 26.5% to 52.2%) and 57.9% (95% CI 46.4% to 72.2%) (p=0.012). The azathioprine versus rituximab HRs were 2.51 (95% CI 1.35 to 4.69) (p=0.003) for major relapses and 2.11 (95% CI 1.19 to 3.73) (p=0.012) for major or minor relapses. Kaplan-Meier curves estimated the probability of remaining major or major and minor relapse free (figure 2A, B).

Cumulative glucocorticoid dose

Cumulative glucocorticoid doses, estimated with glucocorticoid-dose versus time (inclusion to month 60) AUCs, were comparable: 11 767 mg (SD 6529 mg) for the azathioprine group and 9841 mg (SD 6557 mg) for rituximab recipients (mean difference 1964 mg; 95% CI –461 to 4388; p=0.110) (online supplementary figure S1).

Adverse events

SAEs are listed in table 1. Sixteen (28%) azathioprine group and 15 (26%) rituximab-arm patients developed severe infections. Infections were mainly respiratory (bronchitis and pneumonia), most frequently in rituximab recipients, while other infections were equally distributed in the two groups. Opportunistic infections included three *Pneumocystis jiroveci* pneumonias, two aspergilloses and two mycobacterial infections. Concerning *P. jiroveci* pneumonia patients (two given rituximab and one taking azathioprine), one had discontinued co-trimoxazole 1 month before infection onset because treatment duration had been considered sufficient, another was allergic to co-trimoxazole and complied poorly with monthly pentamidine aerosolisations and the last received no prophylaxis because of pre-existing co-trimoxazole allergy.

For azathioprine-group and rituximab-group patients, respectively, five (9%) and six (11%) developed cardiovascular events, six (10%) patients had cancers (including non-melanoma skin cancer in four) and two (4%) prostate cancers (men aged 68 years and 73 years).

Overall, SAE-free survival was comparable for the two treatment groups (figure 2D). The azathioprine versus rituximab HR for SAEs was 1.02 (95% CI 0.63 to 1.62) (p=0.951).

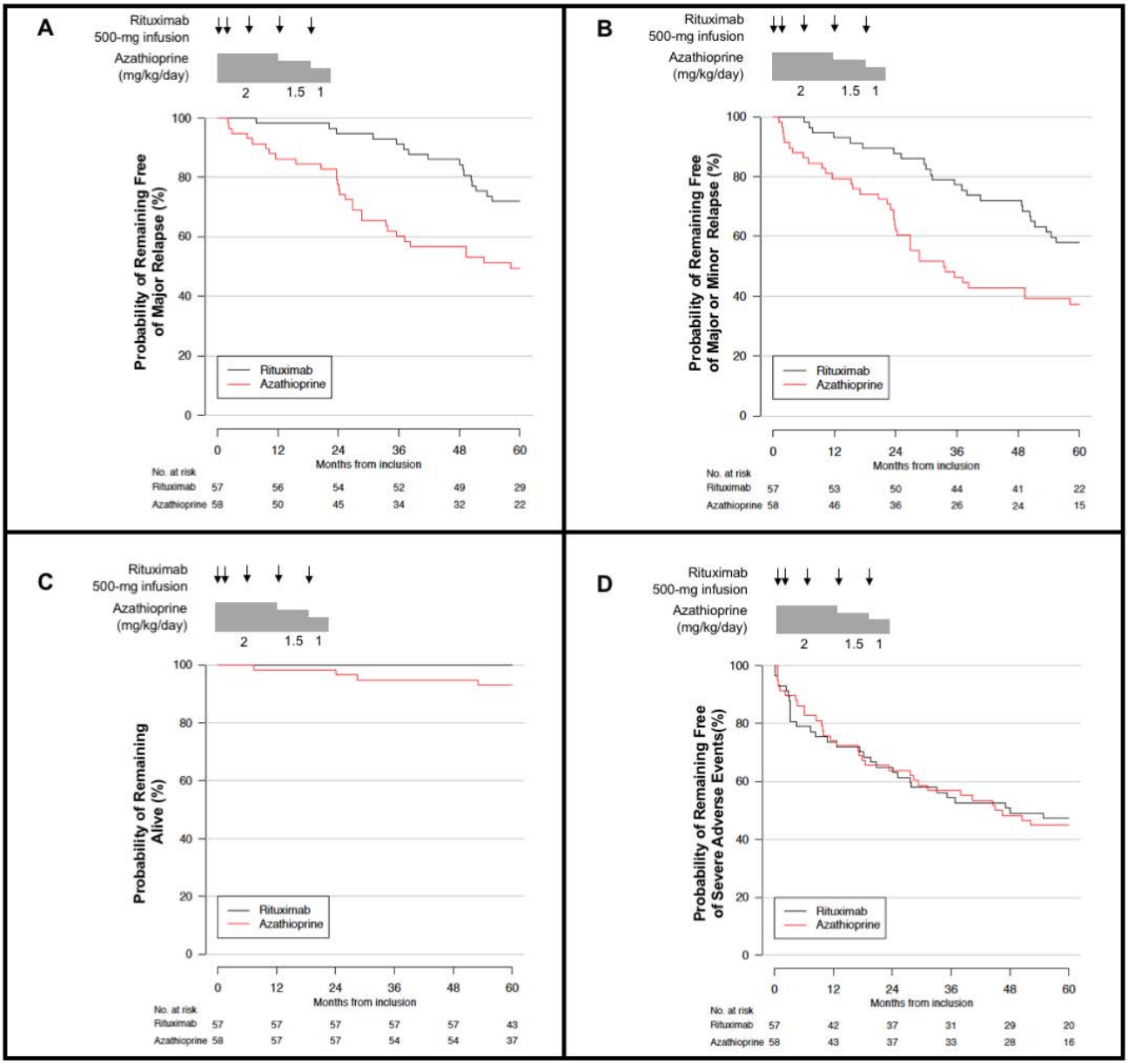


Figure 2 Kaplan-Meier curves for the probability of remaining relapse free according to treatment group. Patients were randomly assigned to receive maintenance therapy with rituximab (500 mg on days 1 and 15 and then months 6, 12 and 18 after the first infusion (arrows)) or azathioprine (2 mg/kg/day from day 1 to month 12, 1.5 mg/kg/day until month 18, then 1 mg/kg/day until the last day of month 22 (horizontal grey bars)). Shown are the postrandomisation probabilities of remaining major relapse free (A) (HR for azathioprine-group patients vs rituximab recipients was 2.51; p=0.003); remaining major or minor relapse free (B) (HR 2.11; p=0.012); surviving (C): because no rituximab-arm patient died, a stratified log-rank test was used to compare between-group overall survival. At 60 months, overall survival rates were 100% for the rituximab group and 93.0% for the azathioprine group (95% CI 86.7% to 99.9%) (p=0.045) and remaining severe adverse event free (D): rates were comparable between the two treatment arms (HR 1.02 (95% CI 0.63 to 1.62; p=0.951).

Deaths

Four azathioprine-group patients died during the trial: three with granulomatosis with polyangiitis and one with microscopic polyangiitis, all newly diagnosed. Two had already been described and occurred before month 28.[9] The third one, a 68-year-old azathioprine-treated man with microscopic polyangiitis with renal involvement (initial serum creatinine: 2.76 mg/dL), in remission after six cyclophosphamide pulses, suffered, at month 10, a major relapse treated with prednisone and rituximab; he died of mesenteric infarction at month 29. The fourth, a 72-year-old azathioprine-treated man with granulomatosis with polyangiitis in remission after six cyclophosphamide pulses developed, at month 28, a major relapse treated with prednisone and rituximab infusions and achieved remission; at month 53, he relapsed again and died of acute heart failure unrelated to vasculitis.

At 60 months, overall survival rates were 93.0% for the azathioprine group (95% CI 86.7% to 99.9%) and 100% for rituximab recipients (p=0.045) (figure 2C).

Q-TWiST analyses

During the 60-month follow-up, rituximab recipients spent 12.6 months more free of relapse or toxicity (p<0.001). The Q-TWiST period was significantly shorter for the azathioprine-treated

Table 1 Severe adverse events according to treatment group

Severe adverse event	Azathioprine group (n=58) No. of events	Rituximab group (n=57)
Infection	20	31
Bronchitis	1	10
Pneumonia with respiratory distress syndrome	3	6
Infectious diarrhoea	4	2
Cholecystitis	2	1
Acute urinary infection	2	1
Pneumocystis jiroveci pneumonia	1	2
Sepsis	1	1
Lung aspergillosis	2	0
Pleural effusion	1	0
Bacterial endocarditis	1	0
Varicella zoster virus infection	1	1
Lung tuberculosis	0	1
Lung atypical mycobacterial infection	1	0
Oesophageal candidiasis	0	1
Colon diverticulitis	0	1
Appendicitis	0	1
Bacterial orchitis	0	1
Infected elbow hygroma	0	1
Unspecified viral infection	0	1
Cardiovascular events	5	6
Cancer	6	2
Skin (non-melanoma)	4	0
Prostate	0	2
Pancreas	1	0
Gastrointestinal stromal tumour	1	0

patients than rituximab recipients (48.0 vs 55.2 months, respectively, p<0.001) (online supplementary table S1 and figure S2). Sensitivity analyses, varying utility coefficients for quality-adjusted health-state duration, yielded the same significant findings (online supplementary table S2).

ANCA testing, CD19+ B cell counts and gammaglobulin levels

Serial ANCA testing (immunofluorescence positive vs negative) and CD19+ Bcell counts for both groups during follow-up are summarised in the online supplementary figures S3 and S4, respectively. Twenty-two (81%) of the 27 azathioprine-treated patients with major relapses were ANCA positive at relapse. None of the three rituximab-arm patients with major relapses (on therapy) before month 28 had CD19+ Bcell reconstitution at the time of relapse, but two were ANCA positive. In contrast, 12 of the 13 rituximab recipients with major relapses between months 28 and 60 (post-therapy) were ANCA positive (data missing for one) at relapse, and all had CD19+ Bcell reconstitution (data missing for two), with CD19+ Bcell counts ranging from 10 to 206/mm^3.

Evolution of gammaglobulin levels was comparable in both groups before month 28 (not recorded after month 28), and is summarised in the online supplementary figure S5.

Factors predictive of relapse

Data from azathioprine and rituximab group were pooled. Risk factors of minor and major relapses were similar in patients from both groups, with no significant interaction between treatment arm and each predictor variable. Table 2 shows the results of the univariate and multivariate analyses. The HRs for relapse for patients with PR3-ANCA specificity and azathioprine arm were 2.04 (95% CI 1.06 to 3.91) (p=0.032) and 2.72 (95% CI 1.55 to 4.76) (p<0.001) in multivariate analysis, respectively.

Table 2 Univariate and multivariate analysis of factors predictive of vasculitis relapse in treated patients

Variables	HR (95% CI)	P values
Univariate analysis		
Age (years)	1.00 (0.98 to 1.02)	0.984
Male (vs female)	1.00 (0.59 to 1.68)	0.997
GPA (vs MPA or renal-limited vasculitis)	2.08 (1.07 to 4.03)	0.030
PR3-ANCA (vs MPO-ANCA or no ANCA)	2.18 (1.18 to 4.00)	0.012
Serum creatinine >2.27 mg/dL	0.58 (0.30 to 1.10)	0.093
Ear, nose and throat involvement	1.59 (0.83 to 3.02)	0.161
Pulmonary involvement	1.04 (0.61 to 1.76)	0.884
Cardiovascular involvement	1.10 (0.60 to 2.00)	0.764
Induction to remission ANCA evolution (persistence vs disappearance)	1.09 (0.65 to 1.82)	0.756
Multivariate analysis		
PR3-ANCA (vs MPO-ANCA or no ANCA)	2.04 (1.06 to 3.91)	0.032
Serum creatinine >2.27 mg/dL	0.58 (0.31 to 1.11)	0.100
Ear, nose and throat involvement	1.18 (0.59 to 2.35)	0.634
Arm (AZA vs RTX)	2.72 (1.55 to 4.76)	<0.001

ANCA, antineutrophil cytoplasmic antibodies; AZA, azathioprine; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, antiproteinase 3; RTX, rituximab.

HRs of detectable B cells to predict relapse was constant over time (figure 3A), whereas HRs of positive ANCA to predict relapse increased over time with a significant linear trend test (p<0.001, compared with p=0.716 for B cell count) (figure 3B).

DISCUSSION

According to this long-term analysis of MAINRITSAN trial patients, rituximab had a superior post-treatment efficacy than azathioprine at maintaining remissions of ANCA-associated vasculitides over 60 months, with a Q-TWiST analysis identified benefit and no safety differences with azathioprine. Our results also showed that rituximab maintenance was associated with better overall survival and that ANCA specificity and positive ANCA over time were associated with higher subsequent relapse risk.

Although the management of ANCA-associated vasculitis patients has dramatically improved since the 2000s, strategies to prevent late relapses, decrease glucocorticoid exposure and reduce disease-related and treatment-related morbidities remain suboptimal. After the 28-month MAINRITSAN trial results, the major question remains rituximab's ability to maintain long term, sustained ANCA-associated vasculitis remissions. More azathioprine-arm patients relapsed during the first 28 months of follow-up and that difference remained significant at month 60, with comparable major relapse rates between months 28 and 60 (17% for the azathioprine group vs 23% for rituximab recipients). The major-relapse frequency increased rapidly over the 12 months following azathioprine discontinuation at 22 months. Most rituximab-arm major relapses occurred 18–24 months after the last infusion (at 36–42 months), still suggesting longer and more sustained efficacy at maintaining remission. These findings suggest that rituximab could delay rather than abrogate relapses and emphasise the need to better identify patients with high-relapse risk that could benefit from longer treatment.

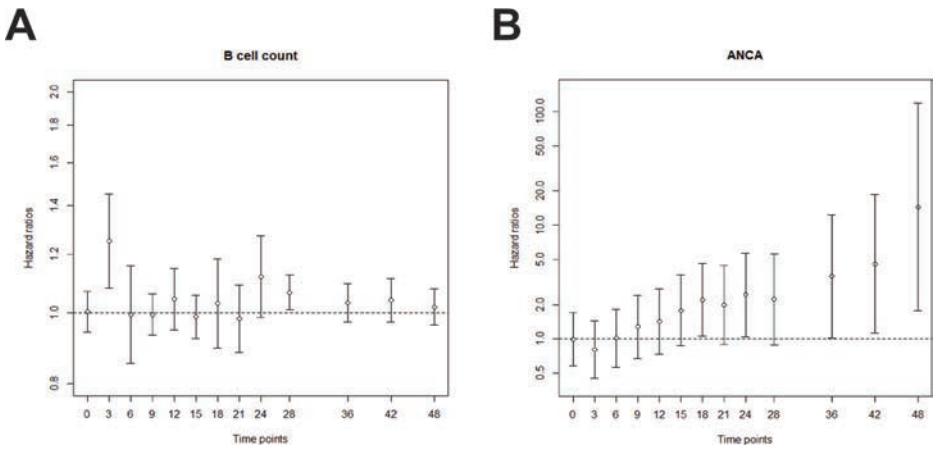


Figure 3 HRs of B cell count and antineutrophil cytoplasmic antibodies (ANCA) to predict vasculitis relapse for each separate model. HRs of detectable B cells to predict relapse was constant over time (A), whereas HRs of positive ANCA to predict relapse increased over time with a significant linear trend test (p<0.001, compared with p=0.716 for B cell count) (B).

The safety profile was comparable for both agents, except for respiratory infections that were slightly more frequent in rituximab recipients. *P. jiroveci* pneumonia occurred in three patients not receiving prophylaxis, further supporting the need for cotrimoxazole or, in case of sulfonamide allergy, pentamidine aerosolisations, oral dapsone or atovaquone to prevent *P. jiroveci* pneumonia in these patients. Cancers were infrequent but were more common in the azathioprine group.

Optimal maintenance therapy doses and durations to further improve long-term outcomes remain major challenges, as is the identification of patients who would benefit the most from prolonged treatment. Whether a rituximab dose exceeding 500 mg for maintenance, as chosen in our trial, could achieve fewer late relapses without increasing AEs, especially severe infections, warrants further investigation. The longest glucocorticoid intakes were associated with fewer relapses in a meta-analysis before rituximab was used to treat ANCA-associated vasculitides,[10] but ongoing vasculitis studies are mostly attempting to develop glucocorticoid-sparing strategies. A retrospective cohort study found that continuing azathioprine or methotrexate maintenance, respectively, for >18 or >36 months, obtained 29% or 66% HR reduction for relapse,[11] and the recently published randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis (REMAIN) trial demonstrated that prolonged remission maintenance therapy with azathioprine to 48 months from diagnosis reduced relapse risk and improves renal survival in AAV.[12] The ongoing MAINRITSAN-3 trial (NCT02433522) compares 46 versus 18 months of rituximab maintenance, like that used herein. Overall, optimal dose and duration of rituximab have still to be defined. Also, data from the Rituximab Vasculitis Maintenance Study (RITAZAREM) trial will show if higher dose of rituximab for almost the same duration show better relapse-free survival.

One secondary MAINRITSAN trial goal was to study correlation between ANCA reappearance and/or B cell reconstitution and the relapse rate. Most patients in each group were ANCA positive at relapse, and B cell reconstitution preceded relapses in most rituximab-treated patients, except for the very few early relapses that occurred during active therapy. Furthermore, our results showed that patients with PR3-ANCA-positive vasculitis were at higher risk of subsequent relapse. Also, positive ANCA over time were able to identify patients who might require longer and repeated maintenance treatment. These findings are consistent with those of previous studies on the impact of PR3-ANCA positivity in ANCA-associated vasculitides.[13–15] However, the role of ANCA monitoring in predicting relapses has always been controversial,[16–19] probably because of the heterogeneity of treatment regimens used in those studies.

This long-term trial follow-up study has several strengths. Its results should be applicable to the broad spectrum of patients seen in routine practice (eg, patients with granulomatosis with polyangiitis, microscopic polyangiitis and renal-limited vasculitis) and patients with newly diagnosed or relapsing disease were included, most with granulomatosis with polyangiitis. However, this latter high percentage of patients with granulomatosis with polyangiitis is relevant in a trial focusing on relapse prevention, because they are at a much higher risk of relapse than those with microscopic polyangiitis.[13–15] Finally, whereas the open-label study design could represent a limitation, major ANCA-associated vasculitis relapses were clearly defined, based on overt clinical manifestations.[20]

In conclusion, the long-term follow-up of MAINRITSAN trial patients showed that the lower risk of major relapses of ANCA-associated vasculitides observed at 28 months with 500 mg rituximab infusions administered on days 1 and 15 then every 6 months until month 18, compared with azathioprine, was sustained over 60 months, especially for patients with granulomatosis with polyangiitis and PR3-ANCA. PR3-ANCA specificity and positive ANCA over time were able to identify patients who might require longer and repeated maintenance treatment.

Author affiliations
[1]Department of Internal Medicine, Hôpital Cochin, Université Paris Descartes, Sorbonne Paris Cité, INSERM Unité 1016, Centre de Référence pour les Maladies Auto-immunes Rares, Paris, France
[2]Department of Rheumatology, Mount Sinai Hospital, Toronto, Ontario, Canada
[3]Centre d'Epidémiologie Clinique, Hôpital Hôtel-Dieu, Université Paris Descartes, INSERM Unité 738, Paris, France
[4]Unité de Néphrologie, Hôpital Européen Georges-Pompidou, Université Paris Descartea, Paris, France
[5]Service de Pneumologie, Centre de Référence pour Maladies Pulmonaires Rares, Hôpital Universitaire Louis Pradel, Lyon, France
[6]Service de Médecine Interne, Centre Hospitalier Universitaire, Hôpital Gabriel Montpied, Clermont-Ferrand, France
[7]Département de Médecine Interne, Hôpitaux Universitaires de Rennes, Hôpital Sud, Université Rennes I, IGDR–UMR 6290, Rennes, France
[8]Service de Médecine Interne, Hôpital Edouard Herriot, Lyon, France
[9]Service de Médecine Interne et d'Immunologie Clinique, Site Belle Isle, HPM, Metz, France

[10]Département de Médecine Interne, Centre Hospitalier Bretagne Atlantique de Vannes, Vannes, France
[11]Département de Néphrologie and Département de Médecine Interne, Centre Hospitalier de Valenciennes, Valenciennes, France
[12]Service de Médecine Interne, Centre Hospitalier Général de Niort, Niort, France
[13]Service de Médecine Interne et d'Immunologie Clinique, Centre Hospitalier Universitaire de Dijon, Université de Bourgogne, IFR100, Dijon, France
[14]Service de Néphrologie, Dialyse et Transplantation, Centre Hospitalier Universitaire de Grenoble, Grenoble, France
[15]Service de Néphrologie, INSERM Unité 699, Département Hospitalo-Universitaire FIRE, Hôpital Bichat, Université Paris Diderot, Paris, France
[16]Département de Néphrologie, Hôpital d'Annecy, Annecy, France
[17]Service de Médecine Interne, Clinique Rhône Durance, Avignon, France
[18]Département de Médecine Interne, Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France
[19]Département de Médecine Interne, Hôpital La Croix Saint-Simon, Paris, France
[20]Service de Médecine Interne, Centre de Référence Labellisé pour la Prise en Charge des Cytopénies Auto-immunes de l'Adulte, Hôpital Henri Mondor, Assistance Publique–Hôpitaux de Paris, Vasculitis Clinic, Créteil, France
[21]Hôpital Cochin, Centre de Référence Maladies Systémiques et Autoimmunes Rares, AP HP, Université Paris Descartes, Service de Médecine Interne, Paris, France

Acknowledgements We thank Séverine Poignant, Émilie Vaillant and Adèle Bellino from the Clinical Research Unit, INSERM CIC P 0901, Cochin University Hospital (Assistance Publique-Hôpitaux de Paris, Université Paris Descartes, Paris) for trial monitoring and handling, preparation and submission of all required research ethics and regulatory documents and Janet Jacobson for editorial assistance.

Collaborators A complete list of additional investigators and members of the French Vasculitis Study Group. The authors' full names and academic degrees are as follows: Florence Vendé, MD, Maxime Samson, MD, PhD, Pierre-Yves Hatron, MD, PhD, Abdeldjallil Koreichi, MD, Alain Ramassamy, MD, Hélène Francois, MD, PhD, Ali Boumallassa, MD, Anne-Bérangère Beucher, MD, Aurélien Delluc, MD, PhD, Bruno Graffin, MD, Catherine Hanrotel-Saliou, MD, Claire Grange, MD, David Launay, MD, PhD, Denis Bagnères, MD, Edouard Begon, MD, Frédéric Grassin, MD, Frédérique Bocquentin, MD, Guillaume Gondran, MD, Isabelle Delacroix, MD, Isabelle Guichard, MD, Isabelle Marie, MD, PhD, Jaques Pourrat, MD, PhD, Jean-François Viallard, MD, PhD, Benoit Wallaert, MD, PhD, Laure Lahaxe, MD, Laurence Vrigneaud, MD, Marc Fabre, MD, Marie Frimat, MD, Marie Lino, MD, Martine Gayraud, MD, Matthias Buchler, MD, PhD, Myriam Niel-Duriez, MD, Nolwenn Rabot, MD, Raphaèle Seror, MD, Ph.D., Roderich Meckenstock, MD, Serge Perrot, MD, PhD, Serge Seiberras, MD, Robin Dhote, MD, PhD, Vincent Poindron, MD, Virginie Rieu, MD, Xavier Delbrel, MD, Xavier Kyndt, MD, Yann Ollivier, MD.

Contributors BT, CP and LG contributed to data collection, data analysis and interpretation, manuscript preparation and review. EP and PR contributed to data analysis and interpretation, manuscript preparation and review. AK, CK, OA, PC, OD, HD-C, FM, PG, TQ, CB-D, BB, P-LC, ED, MD, PG, MH, OL, NL, XP and LM contributed to data generation, analysis and interpretation and manuscript preparation.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests BT has received lecture fees from Roche/Genentech and advisory board fees from ChemoCentryx. CP has received research grants and lecture fees from Roche/Genentech and advisory board fees from ChemoCentryx and Sano.

Patient consent Obtained

Ethics approval Local Institutional Review Board, CPP Paris Ile de France 3.

Provenance and peer review Not commissioned; externally peer reviewed.

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

REFERENCES

1 Jennette JC, Falk RJ, Bacon PA, *et al*. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1–11.
2 Guillevin L, Lhote F, Gayraud M, *et al*. Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. *Medicine* 1996;75:17–28.
3 Ribi C, Cohen P, Pagnoux C, *et al*. Treatment of Churg-Strauss syndrome without poor-prognosis factors: a multicenter, prospective, randomized, open-label study of seventy-two patients. *Arthritis Rheum* 2008;58:586–94.
4 Ribi C, Cohen P, Pagnoux C, *et al*. Treatment of polyarteritis nodosa and microscopic polyangiitis without poor-prognosis factors: a prospective randomized study of one hundred twenty-four patients. *Arthritis Rheum* 2010;62:1186–97.
5 Cohen P, Pagnoux C, Mahr A, *et al*. Churg-Strauss syndrome with poor-prognosis factors: a prospective multicenter trial comparing glucocorticoids and six or twelve cyclophosphamide pulses in forty-eight patients. *Arthritis Rheum* 2007;57:686–93.
6 Stone JH, Merkel PA, Spiera R, *et al*. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010;363:221–32.
7 Jones RB, Tervaert JW, Hauser T, *et al*. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010;363:211–20.
8 Specks U, Merkel PA, Seo P, *et al*. Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med* 2013;369:417–27.
9 Guillevin L, Pagnoux C, Karras A, *et al*. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med* 2014;371:1771–80.
10 Walsh M, Merkel PA, Mahr A, *et al*. Effects of duration of glucocorticoid therapy on relapse rate in antineutrophil cytoplasmic antibody-associated vasculitis: a meta-analysis. *Arthritis Care Res* 2010;62:1166–73.
11 Springer J, Nutter B, Langford CA, *et al*. Granulomatosis with polyangiitis (Wegener's): impact of maintenance therapy duration. *Medicine* 2014;93:82–90.
12 Karras A, Pagnoux C, Haubitz M, *et al*. Randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis. *Ann Rheum Dis* 2017;76:1662–8.
13 Pierrot-Deseilligny Despujol C, Pouchot J, Pagnoux C, *et al*. Predictors at diagnosis of a first Wegener's granulomatosis relapse after obtaining complete remission. *Rheumatology* 2010;49:2181–90.
14 Walsh M, Flossmann O, Berden A, *et al*. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2012;64:542–8.
15 Mahr A, Katsahian S, Varet H, *et al*. Revisiting the classification of clinical phenotypes of anti-neutrophil cytoplasmic antibody-associated vasculitis: a cluster analysis. *Ann Rheum Dis* 2013;72:1003–10.
16 Finkielman JD, Merkel PA, Schroeder D, *et al*. Antiproteinase 3 antineutrophil cytoplasmic antibodies and disease activity in Wegener granulomatosis. *Ann Intern Med* 2007;147:611–9.
17 Sanders J-SF, Huitma MG, Kallenberg CGM, *et al*. Prediction of relapses in PR3-ANCA-associated vasculitis by assessing responses of ANCA titres to treatment. *Rheumatology* 2006;45:724–9.
18 Boomsma MM, Stegeman CA, van der Leij MJ, *et al*. Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. *Arthritis Rheum* 2000;43:2025–33.
19 Thai LH, Charles P, Resche-Rigon M, *et al*. Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. *Autoimmun Rev* 2014;13:313–8.
20 Hellmich B, Flossmann O, Gross WL, *et al*. EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on anti-neutrophil cytoplasm antibody-associated vasculitis. *Ann Rheum Dis* 2007;66:605–17.

EXTENDED REPORT

Validation of the ANCA-associated vasculitis patient-reported outcomes (AAV-PRO) questionnaire

Joanna C Robson,[1,2] Jill Dawson,[3] Helen Doll,[4] Peter F Cronholm,[5] Nataliya Milman,[6] Katherine Kellom,[7] Susan Ashdown,[8] Ebony Easley,[9] Don Gebhart,[10] Georgia Lanier,[11] John Mills,[12] Jacqueline Peck,[8] Raashid Ahmed Luqmani,[13] Judy Shea,[14] Gunnar Tomasson,[15] Peter A Merkel[16]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212713).

For numbered affiliations see end of article.

Correspondence to
Dr Joanna C Robson, Academic Rheumatology Unit, University of the West of England, Bristol BS28HW, UK;
Jo.Robson@uwe.ac.uk

JCR and JD contributed equally.

Received 17 November 2017
Revised 29 March 2018
Accepted 3 April 2018
Published Online First
25 April 2018

ABSTRACT

Objectives To finalise and validate a disease-specific patient-reported outcome (PRO) measure: the ANCA-associated vasculitis patient-reported outcome (AAV-PRO) questionnaire. Using a 35-item candidate questionnaire developed following 50 qualitative interviews in the UK, USA and Canada, a longitudinal survey was conducted to determine the final scale structure and validate the AAV-PRO.

Methods Participants were recruited via Vasculitis UK and the Vasculitis Patient-Powered Research Network. The 35-item candidate questionnaire was completed at baseline and 3 months; UK participants completed the EuroQol-5D-5L (EQ-5D-5L), while US participants completed a test–retest exercise, 3–5 days after baseline. Scale structure was defined using exploratory factor analysis (EFA) and Rasch analysis. Convergent and known groups validity, test–retest reliability and longitudinal construct validity were assessed.

Results There were 626 participants with AAV; >25% reporting 'active disease'. EFA and Rasch analysis supported a 29-item profile measure comprising six domains: 'organ-specific symptoms', 'systemic symptoms', 'treatment side effects', 'social and emotional impact', 'concerns about the future' and 'physical function'. Mean domain scores were higher for participants with 'active disease' versus 'remission' ($p<0.001$). Construct validity was demonstrated by correlations between domain scores and the EQ-5D-5L (range r=−0.55 to 0.78), all $p<0.0001$. In participants reporting 'no change' (n=97) during the test–retest, intraclass correlation coefficient values were high (range 0.89–0.96) for each domain.

Conclusions The AAV-PRO, a new disease-specific PRO measure for AAV, has good face and construct validity, is reliable, feasible and discriminates among disease states.

INTRODUCTION

Granulomatosis with polyangiitis (GPA, Wegener's), eosinophilic granulomatosis with polyangiitis (EGPA, Churg-Strauss) and microscopic polyangiitis (MPA) are life-threatening and organ-threatening disorders affecting the lungs, kidneys, ear, nose, throat, nerves, skin and quality of life of affected patients and are collectively known as the ANCA-associated vasculitides (AAV).[1 2] Despite improvement in mortality and morbidity with newer treatment regimens, the risk of relapse in AAV is 35% over 5 years.[3] Many patients experience persistent disease activity, long-term exposure to toxic therapies[4] and the psychosocial impact of a serious illness.[5]

Health-related quality of life (HRQoL) is impaired in AAV.[6–8] A quarter of patients experience depression and >40% anxiety.[6] Work disability is high with a quarter unemployed due to AAV,[9] and 50% reported their careers had been hindered.[10] Fatigue and pain are important symptoms.[6 11] The opinions of patients and clinicians on the relative importance of outcomes often differ.[12 13]

The Outcome Measures in Rheumatology (OMERACT) core set of outcome measurements for use in clinical trials in AAV included the generic Short-Form 36 (SF-36) patient-reported outcome (PRO) measuring HRQoL.[14–16] Generic PROs can lack specificity,[17] and the OMERACT Vasculitis Working Group identified the need for an AAV-specific PRO to fully capture the patient's perspective.[18] An international steering committee comprising patient partners, methodologists, statisticians and clinicians from the UK, USA and Canada has been developing a new disease-specific PRO, in line with guidance from the US Food and Drug Administration.[19] The project received critical scrutiny and feedback at three successive Vasculitis Workshop sessions at OMERACT conferences.[20 21]

A three-stage approach has been followed. Stage 1: qualitative analysis, item production and testing in the UK, USA and Canada resulting in a 35-iten candidate ANCA-associated vasculitis patient-reported outcomes (AAV-PRO) questionnaire (completed).[22] Stage 2: large-scale parallel survey of people with AAV in the UK and USA, to investigate the underlying scale structure of the AAV-PRO. Stage 3: assessment and validation of the AAV-PRO's measurement properties, including construct validity, reliability, discriminatory ability and ability to detect change. Stages 2 and 3 are reported here.

METHODS AND MATERIALS

An international steering committee, including four patient partners, had oversight of the patient survey materials, working with the patient groups Vasculitis UK and the Vasculitis Patient-Powered Research Network (the VPPRN).

Patients were recruited between June and October 2015.

To cite: Robson JC, Dawson J, Doll H, *et al*. *Ann Rheum Dis* 2018;**77**:1158–1165.

Inclusion/exclusion criteria

Participants were required to have AAV, English speaking, aged ≥18 years and to fulfil the following:

1. Affirm that they had AAV.
2. Received either a positive test result for ANCA or diagnostic biopsy or angiogram.
3. Currently or previously taken glucocorticoids or another immunosuppressant/s.

Participants with AAV were sent a pack by post (Vasculitis UK) or email (the VPPRN in the USA) containing a covering letter, information sheet and forms for demographic (date of birth, location, sex, race, highest educational level employment status) and disease-related data (type of AAV, date of diagnosis, positive ANCA test, current disease state, immunosuppressant medications) and the 35-item candidate AAV-PRO questionnaire. The first 12 items addressed symptom severity; the remaining 23 items addressing the impact of AAV, or its treatment, on HRQoL. Each item has five ordinal integer response options (three formats applying to different items: symptom severity, level of difficulty, frequency of experiencing a problem), scored 0–4; higher scores denoting greater severity or impact. UK participants were also sent an EQ-5D-5L questionnaire[23] at baseline. This five-item generic measure assesses mobility, self-care, usual activities, pain/discomfort and anxiety/depression on a five-point scale. EQ-5D-5L index values were calculated using the cross-walk method.[24]

Three to five days after they provided baseline responses, the US participants were sent a repeat 35-item AAV-PRO candidate questionnaire (test–retest), disease state questions and a transition question concerning change in disease state since baseline questionnaire completion: 'Overall, how are you NOW (in terms of your vasculitis and any treatment side effects), compared with 5 days ago (when you first answered the questionnaire)?'

After 3 months, all UK and US participants were sent the same 35-item candidate AAV-PRO questionnaire and the transition item used for test–retest, but with comparison made with '3 months ago'.

Sample size

Sample size for health status questionnaire development requires at least three respondents per questionnaire item tested.[25] The aim was to recruit at least 500 patients (250 from each country).

Statistical methods

Data were analysed using SPSS release V.20 (PASW Statistics 20© 2015 SPSS). To minimise type I error, the significance level for all analyses was set at two-sided $p<0.01$.

Criteria for questionnaire item reduction

(1) Missing responses>3%[26]; (2) distribution of responses exhibiting ceiling or floor effects (≥50% responses to an item taking either of the two most extreme response categories); (3) high inter-item correlation (≥0.80) or Cronbach's alpha (≥0.93) suggesting redundancy; (4) items poorly correlated with their overall domain/scale score (ie, item-to-total correlations<0.3); (5) cross-loading during factor analysis and (6) particularly poor fit to the model (Item Trait Interaction $p<0.01$) on fitting to a Rasch unidimensional model to any identified domains.

Scale structure and dimensionality

Conceptual framework

The AAV-PRO conceptual framework indicated that the PRO was likely to be multidimensional, that is, containing items addressing symptom severity, and differing aspects of HRQoL (physical, psychological, social and global impact on health).

Factor structure

The formal process of item reduction and determination of scale structure was guided by exploratory factor analysis[27] (EFA), Rasch analysis[28] (RUMM2010 software; RUMM Laboratory, Western Australia 6023) and from insights from the conceptual framework.[26] EFA was conducted using FACTOR,[27] based on a polychoric correlation matrix, using Principal Axis Factoring extraction, with oblique rotation method. Items correlating with a factor of >0.4 were considered to significantly load and the item was assigned to that factor.[29]

Individual item functioning

The polytomous Rasch model (for items with >2 responses) is equivalent to a test of the theoretical construct validity and adequacy of a scale,[30 31] assessing the unidimensionality of items in a scale.[28 32 33]

Scale/domain properties

Internal consistency

Cronbach's alpha coefficients were calculated to assess the internal consistency of questionnaire domains. An alpha ≥0.70 is recommended to claim internal consistency,[34 35] alpha >0.90 may suggest redundancies, requiring item reduction,[31] with 0.80–0.90 considered optimal.[36]

Convergent validity

It was hypothesised that a large Pearson's correlation ($r\geq0.5$) would be obtained between the AAV-PRO domains and generic EQ-5D-5L index scores (UK baseline sample only). It was anticipated that negative correlations would be seen as the two measures are scored in opposite directions.

Test–retest reliability

Intraclass correlation coefficients (ICCs) were used to compare baseline AAV-PRO domain scores, with scores obtained 3–5 days later (US sample only) in those individuals whose condition had remained stable. ICC values>0.60 are recommended.[37]

Meaningful change

The SEM was the error estimated for a single use of the questionnaire and is directly related to the reliability of the scale. The minimal detectable change (MDC) was defined as the smallest amount of change between two time points that indicated a real change in the patient's health status.[38] The MDC_{90} was set to indicate that 90% of stable patients demonstrated random variation of less than this magnitude when assessed on multiple occasions.[39–41]

Known groups validity[26]

It was hypothesised that the AAV-PRO domain scores would differ significantly between patients self-identifying at baseline as having 'active disease' versus patients 'in remission'.

Longitudinal construct validity: responsiveness

Responsiveness was assessed where respondents provided relevant outcomes data at baseline and 3 months. Change scores were calculated as the baseline score minus the 3-month follow-up score for each AAV-PRO domain. Effect sizes (ES) were calculated as the difference between the sample's mean baseline

Table 1 Demographic and clinical characteristics of survey participants

Demographic characteristics	UK n=348 (%)	USA n=278 (%)	All n=626 (%)	X^2	P values
Sex (n=623)					
Male	135 (38.9)	90 (32.7)	225 (36.7)	2.54	0.11
Female	212 (61.1)	185 (67.3)	397 (63.8)		
Age group (years) (n=608)					
≤ 45	25 (7.3)	51 (19.1)	76 (12.5)	40.64	0.00
> 45 ≤ 60	95 (27.9)	90 (33.7)	185 (30.4)		
>60 ≤ 75	166 (48.7)	116 (43.4)	282 (46.4)		
>75	55 (16.1)	10 (3.7)	65 (10.7)		
Ethnicity (n=624)					
Asian	5 (1.4)	7 (2.5)	12 (1.9)	3.77	0.71
Black or African/American	1 (0.3)	2 (0.7)	3 (0.5)		
Black African or Caribbean British	1 (0.3)	0 (0)	1 (0.2)		
White	333 (95.7)	259 (93.8)	592 (94.9)		
American Indian or Alaska Native	0 (0)	1 (0.4)	1 (0.2)		
Multiple	3 (0.9)	3 (1.1)	6 (1.0)		
Other	5 (1.4)	4 (1.4)	9 (1.4)		
Qualifications (highest) (n=623)					
Degree	157 (45.4)	204 (73.6)	361 (57.9)	59.46	0.00
Vocational/employment related	71 (20.5)	26 (9.4)	97 (15.6)		
School/high school qualifications	90 (26.0)	46 (16.6)	136 (21.8)		
None	28 (8.1)	1 (0.4)	29 (4.7)		
Employment status (n=623)					
Disabled	50 (14.5)	48 (17.3)	98 (15.7)	56.68	0.00
Employed with income	78 (22.6)	112 (40.3)	190 (30.5)		
Retired	204 (59.1)	88 (31.7)	292 (46.9)		
Employed without income	3 (0.9)	3 (1.1)	6 (1.0)		
Homemaker/carer	4 (1.2)	9 (3.2)	13 (2.1)		
Unemployed	6 (1.7)	7 (2.5)	13 (2.1)		
Other (eg, student, employed and student)	0 (0.0)	11 (4.0)	11 (1.8)		
Type of AAV					
EGPA	47 (13.5)	48 (17.3)	95 (15.2)	20.37	0.00
GPA	251 (72.1)	184 (66.2)	435 (69.5)		
MPA	28 (8.0)	43 (15.5)	71 (11.3)		
Unspecified AAV	22 (6.3)	3 (1.1)	25 (4.0)		
Positive ANCA test					
Yes	270 (78.3)	222 (79.9)	492 (79.9)	17.66	0.00
No	15 (4.3)	31 (11.2)	46 (7.4)		
Don't know	60 (17.4)	25 (9.0)	85 (13.6)		
Current disease status					
Active disease	100 (29.8)	75 (27.0)	175 (28.5)	0.58	0.45
Remission	236 (70.2)	203 (73.0)	439 (71.5)		
Flare within the last two years					
Yes	135 (40.2)	129 (46.4)	264 (43.0)	5.09	0.17
No	157 (46.7)	112 (40.3)	269 (43.8)		
Don't know	32 (9.5)	21 (7.6)	53 (8.6)		
Never had a flare	12 (3.6)	16 (5.8)	28 (4.6)		
Organs affected by AAV					
Lungs	215 (61.8)	205 (73.7)	420 (67.1)	10.01	0.00
ENT	249 (71.6)	215 (77.3)	464 (74.1)	2.70	0.10
Eyes	135 (38.8)	124 (44.6)	259 (41.4)	2.15	0.14
Kidneys	185 (53.2)	153 (55.0)	338 (54.6)	0.22	0.64
Nerves	139 (39.9)	91 (32.7)	230 (36.7)	3.46	0.06
Skin	128 (36.8)	123 (44.2)	251 (40.1)	3.58	0.06
Joints	192 (55.2)	151 (53.6)	341 (54.5)	0.16	0.70
Time from diagnosis (years)					
Mean (SD)	10.6 (7.5)	7.6 (7.4)	9.3 (7.5)	t=4.89	0.00
Range	0.2–38.8	0.1–44.5	0.1–44.5		

AAV, ANCA-associated vasculitides; EGPA, eosinophilic granulomatosis with polyangiitis; ENT, ear, nose, throat; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis.

score and mean 3-month follow-up score, divided by the SD of baseline score. ES calculates the magnitude of change measured by an instrument in a standardised way allowing comparison between instruments.[42] Change scores and ES were compared with responses on a 3-month transition item regarding change in patients' condition.

RESULTS

Study sample and characteristics

The baseline survey response rate was 74% (n=662/900). Of the 662 respondents, 626 were eligible for inclusion (95%). Demographic and clinical characteristics of participants are shown in table 1 and online supplementary table S1. The mean age was 60.4 years (SD 13.2) and participants were predominantly female (397, 64%). The sample represented the UK (348/626) and the USA (278/626) with 45% and 46% of the sample, respectively; UK respondents were older (mean 63 vs 57 years, p<0.001) and more likely to be retired (59% vs 32%, p<0.001).

Item response distribution: candidate AAV-PRO items

Candidate questionnaire items and baseline distribution of their responses are shown in figure 1. Item response rates were high overall (maximum 1.6% missing data), supporting the feasibility of the questionnaire. One exception concerned 'difficulties with sexual activity or desire' (6.2% missing; 11.8% missing in age group >65). Responses were generally evenly spread across responses, although >50% of respondents endorsed an extreme ('no difficulty') response on two items ('using hands for small careful movements' and 'washing/drying/dressing unaided').

Final dimensionality and scale structure of the AAV-PRO

The final AAV-PRO including 29 individual questionnaire items is shown in figure 2. Details of the Rasch and EFA analyses are given in online supplementary figure S1-3 and online supplementary table S2. The AAV-PRO is a profile measure containing six different domains: 'organ symptoms severity (OSS)', 'systemic symptoms severity (SSS)', 'treatment side effects (TSE)', 'social and emotional impact (SEI)', 'concerns about the future (CAF)' and 'physical function (PF)'. The identified domains each fit the Rasch unidimensional model (Item Trait Interaction p>0.01) and had good internal consistency (Cronbach's alphas 0.77–0.96) (figure 2). Patient partners on the steering committee reviewed the items within each domain and developed the domain titles used above.

Six questionnaire items were identified for rejection based on failure to fit within the Rasch model for a particular domain, plus insights from the Conceptual Framework, EFA and clinical input: 'nerve pain or numbness' reflected damage and felt not suitable for a PRO as would not capture change; 'sexual activity …' obtained poor response rate; 'worried about income' was considered too contextual with responses influenced by differing healthcare; 'using hands for small tasks' response distribution indicating a ceiling effect (skewed towards 'no difficulty'); '… social life is limited' had strong overlap in responses with other better fitting items indicating redundancy; and '… activities essential to your day', 'walking around shops' and 'walking up-stairs' were all highly correlated indicating redundancy (exact meaning of 'essential' flagged as problematic by patient partners).

Scoring of the 29-item AAV-PRO profile measure

Scores for each domain are calculated as the sum of each individual item score (online supplementary figure S4). Examples of

items with response categories are shown in online supplementary figure S5.

Measurement properties

Convergent validity

Correlations (Pearson) between baseline AAV-PRO domains and EQ-5D-5L index scores (UK sample only) were all large (≥0.50): OSS r=−0.55, SSS r=−0.67, TSE r=−0.65, SEI r=−0.73, CAF r=−0.68 and PF r=−0.78 (all P<0.001).

Test–retest reliability

All ICC values between domain scores at baseline and 3–5 days later (US sample) were very good: OSS ICC=0.89 (95% CI 0.84 to 0.93), SSS ICC=0.91 (95% CI 0.86 to 0.94), TSS=0.95 (95% CI 0.93 to 0.97), SEI=0.96 (95% CI 0.94 to 0.97), CAF=0.95 (0.92 to 0.97) and PF=0.96 (0.94 to 0.97) (table 2).

Meaningful change

The SEM and MDC_{90} estimate were calculated based on the ICC and the SD of the baseline score (table 2).

Known groups validity

AAV-PRO domain scores all differed significantly (p<0.001) between patients self-identifying as having 'active disease' versus 'in remission' (see table 3) as was also the case for the EQ-5D-5L.

Longitudinal construct validity

Mean change scores and ES for the AAV-PRO domains were mapped to level of response to the 3-month transition item (table 4). Results showed that respondents reporting 'no change' in their condition exhibited appropriate ES, close to zero, while positive ES range 0.21–0.28 were associated with the response 'much better' for all domains. The response 'slightly better'

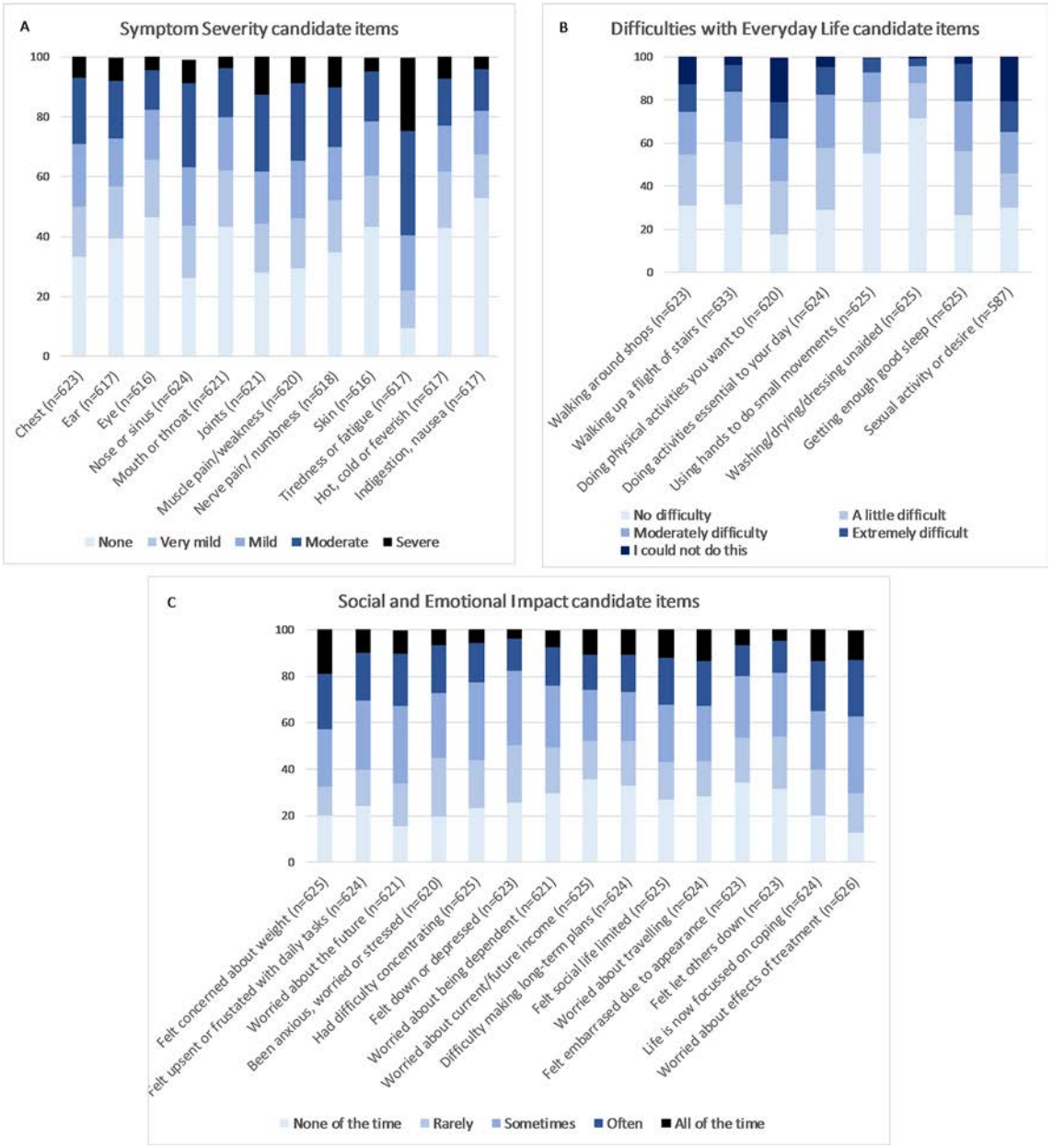


Figure 1 Survey responses at baseline of 35 candidate questionnaire items (n=626). (A) Symptom severity; (B) difficulties with everyday life and (C) social and emotional impact. n=individual response rate for each candidate item.

had ES lying between zero and the value associated with 'much better' responses. In general, responses indicating a worsening health state were associated with negative ES of a magnitude that mirrored results associated with positive responses/improvement. The exception here was the *organ symptom severity* domain with scores lacking a significant linear trend across the transition item responses (all other domains' linear trend $p \leq 0.003$).

Comparison between the AAV-PRO domain scores and demographic and clinical features

There were no differences in mean scores between each of the three AAV (GPA, MPA and EGPA) ($p < 0.01$) and no correlation between length of time from diagnosis and any of the AAV-PRO scales ($p < 0.01$). There were differences between (i) US and UK respondents, with UK scores higher (ie, worse) ($p \leq 0.001$) on all scales, (ii) male and female mean scores, with women scoring higher on all scales ($p < 0.01$), and (iii) younger and older respondents with higher mean scores on the SEI subscale in those in the ≤65-year age group compared with older participants ($p < 0.01$) (see online supplementary tables S3-7).

The final 29-item AAV-PRO is available from the corresponding author and is free for non-commercial academic and clinical use.

DISCUSSION

The AAV-PRO is a new 29-item, disease-specific PRO measure for use in ANCA-associated vasculitis. It has good face, content and construct validity, is reliable, feasible and discriminates among disease states. Patients have played a key role within every stage of development.[22] This article describes the underlying structure of the final AAV-PRO and its validation in terms of reliability, feasibility, discrimination and construct validity.

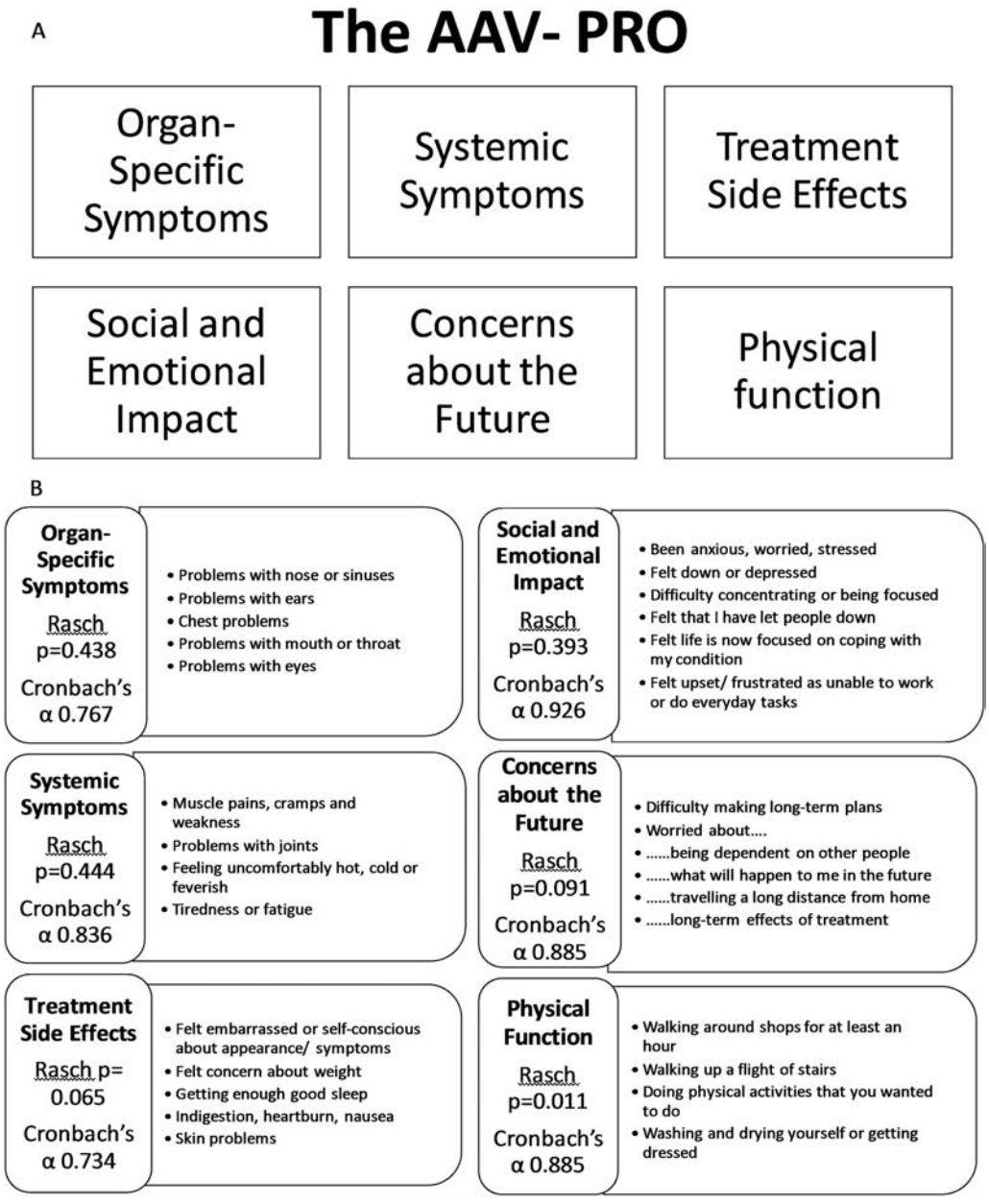


Figure 2 The ANCA-associated vasculitides patient-reported outcomes (AAV-PRO). A profile measure containing six different domains which all individually fit the Rasch model and have good internal consistency. (A) Domains of the AAV-PRO and (B) distribution of the 29 items of the AAV-PRO across the six domains.

Table 2 Test–retest reliability and estimates of meaningful change.

AAV-PRO scale (number of items)	ICC*	95% CI	Baseline mean raw score (SD)	SEM† raw score	SEM† 0–100 scale	MDC_{90}‡ raw score	MDC_{90}‡ 0–100 scale
Organ-specific symptoms (5)	0.89	0.84 to 0.93	6.91 (4.70)	1.56	7.80	3.64	18.20
Systemic symptoms (4)	0.91	0.87 to 0.94	7.21 (4.40)	1.32	8.25	3.08	22.75
Treatment side effects (5)	0.95	0.93 to 0.97	7.17 (4.43)	0.99	4.95	2.31	11.55
Social and emotional impact (6)	0.96	0.94 to 0.97	9.88 (6.24)	1.25	5.21	2.91	12.13
Concerns about the future (5)	0.95	0.92 to 0.97	8.83 (5.35)	1.20	6.00	2.79	13.95
Physical function (4)	0.96	0.94 to 0.97	5.22 (4.17)	0.83	5.19	1.94	12.13

ICCs, SEM and MDC_{90} for the 6 AAV-PRO scales/domains.
Example—for organ symptom severity scale: SEM=4.70 × √1–0.89=1.56 then convert to 0–100 scale: 1.56×100/20=7.80.
Example—for systemic symptom severity scale: MDC_{90}=1.65×1.41× 1.32=3.08 then convert to 0–100 scale: 3.64×100/16=22.75.
*ICC based on US sample only, while SEM uses baseline scores from all US/UK respondents combined.
†SEM=SD × √1 − ICC. Computed using raw scores with scale converted to 0–100 metric at the end.
‡MDC_{90}=1.65 × (√2) × SEM Computed using raw scores with scale converted to 0–100 metric at the end.
AAV-PRO, ANCA-associated vasculitides patient-reported outcomes; ICC, intraclass correlation coefficient; MDC_{90}, minimal detectable change

The final 29-item questionnaire comprises six subscales/domains: 'organ-specific symptoms', 'systemic symptoms', 'TSE', 'SEI', 'CAF' and 'PF'. The identified domains offer a comprehensive profile of the impact of AAV on patients' everyday life and were felt by the patient partners to represent 'what AAV was to them'. Each domain is unidimensional and has good measurement properties including good internal consistency (Cronbach's alphas range 0.77–0.92) and test–retest reliability (ICCs range 0.89–0.96); plus evidence supporting concurrent validity, with moderate to high correlations (range r −0.55 to −0.78, all p<0.0001) with EQ-5D-5L index scores, as hypothesised. All AAV-PRO domain scores distinguished between patients who self-reported having active disease versus disease in remission (p<0.0001), providing support for known groups validity. Length of time from diagnosis alone was not correlated with worse scores, indicating that disease activity, rather than duration of disease, is a key correlate to AAV-PRO scores. There were also no differences in mean scores between the different subtypes of AAV.

Characteristics of the UK and US survey populations differed slightly, participants in the USA were on average younger, with shorter duration of disease and higher educational level. This may reflect the different methods of data collection and may account for the differences seen in subscale scores between countries. Age, educational level and socioeconomic status are associated with computer usage.[43 44]

Women scored higher (ie, worse) on all six subscales of the AAV-PRO. HRQoL is reduced in females in other conditions,[45] and trends towards higher scores for women have been reported in AAV.[15] Younger people (<65) scored higher on the SEI subscale of the AAV-PRO and lower on mental health, a trend also seen in other chronic diseases in this age group.[45]

The design of the survey was to identify the scale structure and measurement properties of the AAV-PRO. As predicted, participants were generally stable regarding self-reported disease activity, with around 70% describing themselves as 'in remission'. Follow-up was 3 months. This somewhat limited the assessment of responsiveness and minimally important change, which are usually assessed over a longer time period in participants expected to change in clinical state, for example, within the context of a clinical trial.[19] The study produced evidence of longitudinal construct validity. Among participants who reported 'no change' ES were appropriately close to zero, and the few participants who reported their condition as 'much better' demonstrated a small amount of change in AAV-PRO scores (ES range 0.21–0.28). Distribution-based estimates of minimal change (SEM and MDC_{90}) which relate to the reliability (ICC) of each scale were all appropriate and will be useful for calculating sample sizes in future studies.[41] Future studies will provide more robust estimates of minimal important differences, further longitudinal construct validity[46] and determine whether summary component scores can be derived.

Table 3 Known groups validity

	Current disease state	N	Mean	SD	t	P values
Organ-specific symptoms	Active	167	47.28	22.55	8.898	<0.0001
	Remission	425	29.35	21.86		
Systemic symptoms	Active	168	60.75	25.37	9.525	<0.0001
	Remission	426	38.53	25.70		
Treatment side effects	Active	171	48.54	22.12	9.565	<0.0001
	Remission	422	30.59	20.09		
Social and emotional impact	Active	172	53.54	24.17	8.079	<0.0001
	Remission	430	35.65	24.69		
Concerns about the future	Active	170	56.76	24.39	7.999	<0.0001
	Remission	431	38.50	25.52		
Physical function	Active	172	44.08	25.76	7.370	<0.0001
	Remission	432	27.56	24.49		

Comparison (using t-tests) of baseline AAV-PRO domain scores according to patient-reported current disease state 'active' versus 'in remission'.
AAV-PRO, ANCA-associated vasculitides patient-reported outcomes.

Table 4 Longitudinal construct validity

Transition item: 'How are you NOW (in terms of your vasculitis and any treatment side effects) compared with 3 months ago (when you first answered the questionnaire)?' Responses:	AAV-PRO domains					
	Organ symptom severity Mean (SD) (ES)	Systemic symptom severity Mean (SD) (ES)	Treatment side effects Mean (SD) (ES)	Social and emotional impact Mean (SD) (ES)	Concerns about the future Mean (SD) (ES)	Physical function Mean (SD) (ES)
Much better	n=38 3.68 (13.79) (0.22)	n=40 6.09 (13.68) (0.21)	n=39 4.62 (12.16) (0.28)	n=40 5.31 (12.27) (0.21)	n=40 6.13 (14.57) (0.23)	n=41 5.79 (15.08) (0.24)
Slightly better	n=69 0.72 (14.86) (0.01)	n=68 2.76 (11.85) (0.07)	n=69 2.03 (12.44) (0.10)	n=72 2.37 (13.36) (0.09)_	n=71 3.87 (16.52) (0.17)	n=73 5.05 (10.66) (0.19)
No change/worse	n=186 0.54 (15.09) (0.01)	n=186 0.44 (15.37) (0.00)	n=185 1.24 (13.12) (0.09)	n=190 1.58 (11.18) (0.06)	n=187 1.66 (13.62) (0.05)	n=193 0.32 (9.43) (0.01)
Slightly worse	n=64* −1.64 (16.67) (−0.07)	n=63 −5.46 (17.84) (−0.19)	n=62 −0.81 (15.92) (−0.01)	n=65* 0.26 (16.02) (0.04)	n=63 −0.87 (17.36) (−0.02)	n=65 −3.08 (18.52) (−0.17)
Much worse	n=14 1.43 (11.17) (0.06)	n=15 −6.25 (18.15) (−0.23)	n=14 −10.71 (16.04) (−0.45)	n=15* −7.78 (15.42) (−0.26)	n=15 −13.67 (16.85) (−0.43)	n=15 −9.58 (27.84) (−0.31)
P values for linear trend	0.185	<0.001	0.001	0.003	<0.001	<0.001
Total	n=374 0.64 (15.06) (0.02)	n=377 0.22 (15.43) (−0.01)	n=373 0.95 (13.71) (0.07)	n=387 1.53 (12.90) (0.06)	n=381 1.44 (15.41) (0.05)	n=392 0.91 (13.69) (0.03)

Mean changes (SD) (using 0–100 metric) and effect sizes for the AAV-PRO domains in relation to patients' responses to a transition item on 3-month follow-up survey.
*The 'n' in each cell varies slightly across the rows reflecting an occasional missing patient's response to an item within the health status scale (hence the particular overall scale would not have been computed for that individual).
AAV-PRO , ANCA-associated vasculitides patient-reported outcomes.

Validated PROs are an important way of accurately measuring the impact and value of new drug treatments on HRQoL by measuring outcomes of importance to patients themselves.[17] PROs can be part of evidence submitted for new drug approvals and can also provide valuable information to clinicians and policymakers asked with making decisions about the use of new treatments.[47] The involvement of patients with AAV-PRO at every stage of development should ensure its face validity and relevance. In addition, it has also been shown that disease-specific instruments may be more responsive to change than generic instruments, which is a crucial characteristic for detecting treatment effect within randomised controlled trials.[48] The AAV-PRO is, therefore, presented as complementary to the SF-36 or EQ5D, which allow comparison with other conditions and population controls, but are not specific to AAV.

The AAV-PRO, a new disease-specific PRO measure for ANCA-associated vasculitis, has good face and construct validity, is reliable, feasible and discriminates among disease states. The AAV-PRO is ready for inclusion within clinical trials and research studies as part of its ongoing validation and exploration of its measurement properties within different populations. The AAV-PRO provides the means to ensure patients' perspectives on their disease are represented in the study of AAV.

Author affiliations
[1]Faculty of Health and Applied Sciences, University of the West of England, Bristol, UK
[2]University of Bristol School of Clinical Science, Bristol, UK
[3]University of Oxford, Nuffield Department of Population Health (HSRU), Oxford, UK
[4]Oxford Outcomes UK, Oxford, UK
[5]University of Pennsylvania Perelman School of Medicine, Department of Family Medicine and Community Health, Philadelphia, Pennsylvania, USA
[6]Division of Rheumatology, Department of Medicine in Ottawa, Ottawa, Ontario, Canada
[7]Children's Hospital of Philadelphia, PolicyLab, Philadelphia, Pennsylvania, USA
[8]Patient Partner, Oxford, UK
[9]University of Pennsylvania, Department of Family Medicine and Community Health, Philadelphia, USA
[10]Patient Partner, Columbus, Ohio, USA
[11]Patient Partner, Boston, Massachusetts, USA
[12]West Bank House, Vasculitis UK, Matlock, UK
[13]Nuffield Department of Orthopaedicx, Rheumatology and Musculoskeletal Science (NDORMs), University of Oxford, Oxford, UK
[14]Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA
[15]University of Iceland, Reykjavik, Iceland
[16]Department of Rheumatology, University of Pennsylvania, Philadelphia, Massachusetts, USA

Correction notice This article has been corrected since it published Online First. The initials for Joanna Robson and Peter Merkel have been added.

Acknowledgements The authors thank all of the patients who contributed their views and valuable time to assist them with this study. They also thank Vasculitis UK, their collaborators on the paper-based UK survey, for their assistance with identifying patients, and for organising and funding postage and packaging. They also thank the Vasculitis Patient-Powered Research Network for its collaboration on the US-based online survey, including identifying patients, building the online version of the questionnaire and compiling a report on patient responses.

Contributors JR and JD contributed equally to this study. JR planned the study, wrote the study protocol and documentation, arranged data entry, clinical input and interpretation of Rasch and EFA analyses, drafted and amended final manuscript. JD planned the study, commented on study protocol and documentation, performed EFA and descriptive statistics and reviewed final document. HD commented on study protocol, performed Rasch analysis and reviewed final document. PFC, NM, KK, SA, EE, DG, JM, JP, RAL, JAS, GT and PAM commented on study protocol and documentation including patient information and design of the survey, commented on results and on all drafts of the manuscript. In addition, JM facilitated UK survey and PAM facilitated US survey.

Funding Sponsored by the University of Oxford and the Vasculitis Clinical Research Consortium (VCRC). Funding for the development of the PRO was received from the Medical Research Fund, Oxford, the Oxfordshire Health Services Research Committee Ref. 1098, and a Patient-Centered Outcomes Research Institute Pilot Project Grant. The VCRC has received support from the US National Institute of Arthritis and Musculoskeletal and Skin Diseases (U54 AR057319 and U01 AR51874), the National Center for Research Resources (U54 RR019497); and the Office of Rare

Diseases Research and the National Center for Advancing Translational Science. The VCRC is part of the Rare Diseases Clinical Research Network (RDCRN). JR and RAL were supported in part by the National Institute for Health Research Musculoskeletal Biomedical Research Unit, Oxford, UK. JR was supported by a National Institute for Health Research (NIHR) clinical lectureship. NM was supported by a UCB/Canadian Rheumatology Association/Arthritis Society postgraduate rheumatology fellowship award and a research fellowship from the Department of Medicine at the Ottawa Hospital. Oxford University Innovation provided funding of translatability assessment.

Competing interests None declared.

Patient consent Obtained

Ethics approval Medical Sciences IDREC, University of Oxford, Oxford, Ref: MS-IDREC-C1-2015-087, for the UK and US survey. In the USA, approval was also given by the Institutional Review Boards at the University of Pennsylvania and the University of South Florida, Ref: Pro00018514.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Jennette JC, Falk RJ, Bacon PA, *et al*. 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis & Rheumatism* 2013;65:1–11.
2 Flossmann O, Berden A, de Groot K, *et al*. Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis* 2011;70:488–94.
3 Rhee RL, Hogan SL, Poulton CJ, *et al*. Trends in long-term outcomes among patients with Antineutrophil Cytoplasmic antibody-associated vasculitis with renal disease. *Arthritis Rheumatol* 2016;68:1711–20.
4 Robson J, Doll H, Suppiah R, *et al*. Glucocorticoid treatment and damage in the anti-neutrophil cytoplasm antibody-associated vasculitides: long-term data from the European Vasculitis Study Group trials. *Rheumatology* 2015;54:471–81.
5 Mooney J, Poland F, Spalding N, *et al*. 'In one ear and out the other - it's a lot to take in': a qualitative study exploring the informational needs of patients with ANCA-associated vasculitis. *Musculoskeletal Care* 2013;11:51–9.
6 Koutantji M, Harrold E, Lane SE, *et al*. Investigation of quality of life, mood, pain, disability, and disease status in primary systemic vasculitis. *Arthritis Rheum* 2003;49:826–37.
7 Basu N, McClean A, Harper L, *et al*. The characterisation and determinants of quality of life in ANCA associated vasculitis. *Ann Rheum Dis* 2014;73:207–11.
8 Fardet L, Flahault A, Kettaneh A, *et al*. Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion. *Br J Dermatol* 2007;157:142–8.
9 Basu N, McClean A, Harper L, *et al*. Markers for work disability in anti-neutrophil cytoplasmic antibody-associated vasculitis. *Rheumatology* 2014;53:953–6.
10 Benarous L, Terrier B, Laborde-Casterot H, *et al*. Employment, work disability and quality of life in patients with ANCA-associated vasculitides. The EXPOVAS study. *Clin Exp Rheumatol* 2017;35.
11 Basu N, McClean A, Harper L, *et al*. Explaining fatigue in ANCA-associated vasculitis. *Rheumatology* 2013;52:1680–5.
12 Herlyn K, Hellmich B, Seo P, *et al*. Patient-reported outcome assessment in vasculitis may provide important data and a unique perspective. *Arthritis Care Res* 2010;62:1639–45.
13 Seo P, Jayne D, Luqmani R, *et al*. Assessment of damage in vasculitis: expert ratings of damage. *Rheumatology* 2009;48:823–7.
14 Merkel PA, Aydin SZ, Boers M, *et al*. The OMERACT core set of outcome measures for use in clinical trials of ANCA-associated vasculitis. *J Rheumatol* 2011;38:1480–6.
15 Walsh M, Mukhtyar C, Mahr A, *et al*. Health-related quality of life in patients with newly diagnosed antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Care Res* 2011;63:1055–61.
16 Tomasson G, Boers M, Walsh M, *et al*. Assessment of health-related quality of life as an outcome measure in granulomatosis with polyangiitis (Wegener's). *Arthritis Care Res* 2012;64:273–9.
17 Fitzpatrick R, Davey C, Buxton MJ, *et al*. Evaluating patient-based outcome measures for use in clinical trials. *Health Technol Assess* 1998;2:1–74.
18 Robson JC, Milman N, Tomasson G, *et al*. Exploration, development, and validation of patient-reported outcomes in Antineutrophil cytoplasmic antibody-associated vasculitis using the OMERACT Process. *J Rheumatol* 2015;42:2204–9.
19 Patrick DL, Burke LB, Powers JH, *et al*. Patient-reported outcomes to support medical product labeling claims: FDA perspective. *Value in Health* 2007;10:S125–S137.
20 Merkel PA, Aydin SZ, Boers M, *et al*. Current status of outcome measure development in vasculitis. *J Rheumatol* 2014;41:593–8.
21 Robson JC, Milman N, Tomasson G, *et al*. Exploration, development, and validation of patient-reported outcomes in antineutrophil cytoplasmic antibody-associated vasculitis using the OMERACT process. *J Rheumatol* 2015.
22 Robson J, Dawson J, Cronholm PF, *et al*. Health related quality of life in ANCA associated vasculitis and item generation for a disease specific patient reported outcome measure. *Patient Related Outcome Measures* 2017.
23 EuroQol G. EuroQol-a new facility for the measurement of health-related quality of life. *Health Policy* 1990;16:199–208.
24 van Hout B, Janssen MF, Feng Y-S, *et al*. Interim scoring for the EQ-5D-5L: mapping the EQ-5D-5L to EQ-5D-3L value sets. *Value in Health* 2012;15:708–15.
25 Barrett P, Kline P. The Observation to Variable Ratio in Factor Analysis. *Personality Study and Group Behaviour* 1981;1:23–33.
26 Fayers P, Machin D. *Quality of Life- Assessment, Analysis and Interpretation*. Chichester: John Wiley & Sons Ltd, 2000.
27 Lorenzo-Seva U, Ferrando PJ. FACTOR: a computer program to fit the exploratory factor analysis model. *Behav Res Methods* 2006;38:88–91.
28 Andrich D. *Rasch Models for Measurement*. Newbury Park, CA: Sage, 1988.
29 Kaiser HF. The application of electronic computers to factor analysis. *Educ Psychol Meas* 1960;20:141–51.
30 Avlund K, Kreiner S, Schultz-Larsen K. Construct validation and the Rasch model: functional ability of healthy elderly people. *Scand J Soc Med* 1993;21:233–46.
31 Streiner DL. Starting at the beginning: an introduction to coefficient alpha and internal consistency. *J Pers Assess* 2003;80:99–103.
32 Jones RW RKH. Comparison of classical test theory and item response theory and their applications to test development. *Educational Measurement Issues and Practice* 1993;12:38–47.
33 Prieto L, Alonso J, Lamarca R. Classical test theory versus Rasch analysis for quality of life questionnaire reduction. *Health Qual Life Outcomes* 2003;1:27.
34 Nunally JC, Bernstein IH. *Psychometric theory*. New York: Columbus OH, 1994.
35 Kline P. *A Handbook of Psychological Testing*. London: Routledge, 1993.
36 Guyatt GH, Feeny DH, Patrick DL. Measuring health-related quality of life. *Ann Intern Med* 1993;118:622–9.
37 Andrews F, Withey S. *Social Indicators of Well-Being: American's Perceptions of Life Quality*. New York: Plenum, 1976.
38 Stratford PW, Binkley JM, Riddle DL. Health status measures: strategies and analytic methods for assessing change scores. *Phys Ther* 1996;76:1109–23.
39 Jaeschke R, Singer J, Guyatt GH. Ascertaining the minimal clinically important difference. *Control Clin Trials* 1989;10:407–15.
40 Wyrwich KW, Tierney WM, Wolinsky FD. Further evidence supporting an SEM-based criterion for identifying meaningful intra-individual changes in health-related quality of life. *J Clin Epidemiol* 1999;52:861–73.
41 de Vet HC, Terwee CB, Ostelo RW, *et al*. Minimal changes in health status questionnaires: distinction between minimally detectable change and minimally important change. *Health Qual Life Outcomes* 2006;4:54.
42 Kazis LE, Anderson JJ, Meenan RF. Effect sizes for interpreting changes in health status. *Med Care* 1989;27:S178–89.
43 Nulty DD. The adequacy of response rates to online and paper surveys: what can be done? *Assessment & Evaluation in Higher Education* 2008;33:301–14.
44 File T, Ryan C. Bureau USC Computer and Internet Use in the United States, 2013. In: ed. *American community survey reports*. 2014. (accessed 29 Sep 2016).
45 Matcham F, Scott IC, Rayner L, *et al*. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: a systematic review and meta-analysis. *Semin Arthritis Rheum* 2014;44:123–30.
46 Revicki DA, Cella D, Hays RD, *et al*. Responsiveness and minimal important differences for patient reported outcomes. *Health Qual Life Outcomes* 2006;4:70.
47 Willke RJ. Measuring the value of treatment to patients: patient-reported outcomes in drug development. *Am Health Drug Benefits* 2008;1:34–40.
48 Wiebe S, Guyatt G, Weaver B, *et al*. Comparative responsiveness of generic and specific quality-of-life instruments. *J Clin Epidemiol* 2003;56:52–60.
49 Muehlhausen W, Doll H, Quadri N, *et al*. Equivalence of electronic and paper administration of patient-reported outcome measures: a systematic review and meta-analysis of studies conducted between 2007 and 2013. *Health Qual Life Outcomes* 2015;13:167.
50 Gwaltney CJ, Shields AL, Shiffman S. Equivalence of electronic and paper-and-pencil administration of patient-reported outcome measures: a meta-analytic review. *Value in Health* 2008;11:322–33.

EXTENDED REPORT

Comparison of magnetic resonance angiography and ^{18}F-fluorodeoxyglucose positron emission tomography in large-vessel vasculitis

Kaitlin A Quinn,[1,2] Mark A Ahlman,[3] Ashkan A Malayeri,[3] Jamie Marko,[3] Ali Cahid Civelek,[3] Joel S Rosenblum,[2] Armin A Bagheri,[2] Peter A Merkel,[4] Elaine Novakovich,[2] Peter C Grayson[2]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2018-213102).

[1]Division of Rheumatology, MedStar Georgetown University Hospital, Washington, District of Columbia, USA
[2]Systemic Autoimmunity Branch, National Institutes of Health, NIAMS, Bethesda, Maryland, USA
[3]National Institutes of Health, Clinical Center, Radiology and Imaging Sciences, Bethesda, Maryland, USA
[4]Division of Rheumatology and Department of Biostatistics, Epidemiology, and Informatics, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence to
Dr Peter C Grayson, Systemic Autoimmunity Branch, National Institutes of Health, NIAMS, Bethesda, MD 20892, USA; peter.grayson@nih.gov

Part of this manuscript was presented at the 2017 ACR/ARHP Annual Meeting.

Received 23 January 2018
Revised 28 March 2018
Accepted 29 March 2018
Published Online First 17 April 2018

To cite: Quinn KA, Ahlman MA, Malayeri AA, *et al*. *Ann Rheum Dis* 2018;**77**:1166–1172.

ABSTRACT

Objectives To assess agreement between interpretation of magnetic resonance angiography (MRA) and ^{18}F-fluorodeoxyglucose positron emission tomography (PET) for disease extent and disease activity in large-vessel vasculitis (LVV) and determine associations between imaging and clinical assessments.

Methods Patients with giant cell arteritis (GCA), Takayasu's arteritis (TAK) and comparators were recruited into a prospective, observational cohort. Imaging and clinical assessments were performed concurrently, blinded to each other. Agreement was assessed by per cent agreement, Cohen's kappa and McNemar's test. Multivariable logistic regression identified MRA features associated with PET scan activity.

Results Eighty-four patients (GCA=35; TAK=30; comparator=19) contributed 133 paired studies. Agreement for disease extent between MRA and PET was 580 out of 966 (60%) arterial territories with Cohen's kappa=0.22. Of 386 territories with disagreement, MRA demonstrated disease in more territories than PET (304vs82, p<0.01). Agreement for disease activity between MRA and PET was 90 studies (68%) with Cohen's kappa=0.30. In studies with disagreement, MRA demonstrated activity in 23 studies and PET in 20 studies (p=0.76). Oedema and wall thickness on MRA were independently associated with PET scan activity. Clinical status was associated with disease activity by PET (p<0.01) but not MRA (p=0.70), yet 35/69 (51%) patients with LVV in clinical remission had active disease by both MRA and PET.

Conclusions In assessment of LVV, MRA and PET contribute unique and complementary information. MRA better captures disease extent, and PET scan is better suited to assess vascular activity. Clinical and imaging-based assessments often do not correlate over the disease course in LVV.

Trial registration number NCT02257866.

INTRODUCTION

Vascular imaging is essential to evaluate patients with giant cell arteritis (GCA) and Takayasu's arteritis (TAK), the two main forms of large-vessel vasculitis (LVV).[1] Temporal artery biopsy is the gold standard to detect cranial forms of GCA, but imaging is necessary to establish the diagnosis for the large-vessel variant of this type of vasculitis and for TAK.[2] Current imaging modalities available for the assessment of LVV include ultrasonography, CT angiography (CTA), catheter-based angiography, magnetic resonance angiography (MRA) and ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET).[3–5]

There remains uncertainty about which imaging technique to choose to evaluate a patient with suspected or established LVV. The extent to which different imaging modalities provide unique versus redundant information about vascular disease is unclear. MRA and CTA are commonly used to detect and monitor arterial anatomical abnormalities such as stenosis and aneurysm; however, MRA is generally preferred over CTA for serial follow-up imaging to avoid radiation and use of iodinated contrast agents. Furthermore, specific imaging sequences on MRA identify arterial wall abnormalities thought to be reflective of ongoing vascular inflammation, including oedema, wall thickness and contrast enhancement. FDG-PET detects abnormal metabolic activity in the wall of inflamed arteries and may, therefore, be more useful than MR to detect and monitor ongoing vascular inflammation in patients with LVV.[3 6]

Most studies that have evaluated the utility of MRA or FDG-PET have focused exclusively on imaging at the time of diagnosis, and there is uncertainty about the utility of these imaging modalities to monitor vascular inflammation in patients with established vasculitis.[3 7–9] Prior reports have demonstrated ongoing vascular inflammation on imaging in periods of apparent clinical remission, highlighting a potential discordance between clinical and imaging based assessment in LVV.[10 11] To what extent vascular inflammation assessed by MRA compared with PET correlates with clinical assessment over the course of disease in LVV is unknown.

The objectives of this study were to assess agreement between interpretation of MRA and PET for disease extent and disease activity, identify features of MRA associated with PET activity and determine the correlation between interpretations of MRA and PET and clinical disease assessments in a prospective, longitudinal cohort of patients with LVV who underwent periodic imaging at different points in the disease course.

METHODS

Study population

Patients with LVV were recruited into a prospective, observational cohort at the National Institutes of Health (NIH) in Bethesda, Maryland, USA. Patients

fulfilled the 1990 American College of Rheumatology (ACR) Classification Criteria for TAK[12] or modified 1990 ACR Criteria for GCA.[13] Patients could be enrolled at the time of diagnosis or later during the disease course.

A comparator group was also studied, consisting mainly of patients with non-inflammatory large-vessel vasculopathies (eg, fibromuscular dysplasia and traumatic stenosis) and other types of vasculitis (eg, polyarteritis nodosa). Details of these patients have been reported elsewhere.[11] The comparator group was included to determine if there was a difference in the performance characteristics of MRA versus PET in assessing patients with diseases that mimic LVV.

Clinical assessment

All patients underwent baseline clinical evaluation, MRA and PET imaging at the NIH Clinical Center. Patients with LVV had follow-up clinical assessments and imaging performed at 6 month intervals, and outside rheumatology records were reviewed between visits. Clinical assessments were performed and recorded by the investigative study team within 24 hours prior to imaging assessment. To enable unbiased comparisons, acute phase reactants and imaging study findings were not incorporated into the definitions of clinical disease activity and remission, an approach that is consistent with clinical definitions of disease activity used in recent randomised controlled trials in LVV.[13] *Active disease* was defined as presence at the time of assessment of any clinical disease feature directly attributed to vasculitis (eg, carotidynia and headache). Fatigue or elevated acute phase reactants alone were not considered sufficient evidence of active disease. *Remission* was defined as the absence of any clinical symptoms directly attributable to vasculitis, regardless of acute phase reactants. Clinical disease was recorded as active or remission, prior to conducting imaging studies.

MRA protocol and assessment

All patients underwent MRA of the aorta and primary branches at each study visit (see online supplementary methods for sequence details). Two vascular radiologists (AAM and JM) interpreted all MRAs included in this study, blinded to clinical data, PET scan assessment and each other's assessment. Disease activity on MRA was defined by global interpretation of each study by the readers based on clinical review of all available sequences. To evaluate disease extent, vascular involvement of 4 segments of the aorta (ascending, arch, descending thoracic and abdominal) and 11 branch arteries (innominate, carotids, subclavians, axillaries, iliacs and femorals) was evaluated in a random subset of 72 paired scans, including TAK, GCA and comparators. Vascular involvement within each territory for the disease extent analyses was defined as the presence of ≥1 of following features: wall thickness, oedema, stenosis, occlusion or aneurysm. Territories that were not adequately visualised or were sites of prior surgical correction were excluded from analysis.

FDG-PET imaging protocol and assessment

Whole-body PET studies were performed on the same morning as MRA imaging (see online supplementary methods for details). Two nuclear medicine physicians (MMA and CC) interpreted all PET scans included in this study. Readers were blinded to clinical data, angiogram assessment and each other's assessment. Consensus between the readers was used to determine whether each scan was consistent with active or inactive vasculitis based on visual inspection of arterial FDG uptake. As previously reported, there was excellent inter-rater agreement (kappa=0.84) between the readers.[11] To evaluate disease extent, FDG uptake was assessed by a single reader in 4 segments of the aorta (ascending, arch, descending thoracic and abdominal) and in 11 branch arteries (innominate, carotids, subclavians, axillaries, iliacs and femorals). The degree of arterial FDG uptake within each territory was visually assessed relative to liver. Vascular involvement within each territory was defined as FDG uptake greater than the liver by visual assessment.

Statistical analysis

Agreement between PET scan assessment and MRA assessment was evaluated by per cent overall agreement, Cohen's kappa and McNemar's test. In cases of disagreement, McNemar's test is useful to determine whether there is equal disagreement in both directions. Multivariable logistic regression analysis was performed to evaluate which features on MRA (oedema, wall thickness, stenosis, occlusion or aneurysm) were associated with reader impression of active vasculitis on PET scan and MRA.

Ethics and informed consent

All patients provided written informed consent. An institutional review board and radiation safety committee at the NIH approved the research.

RESULTS

Study population

A total of 84 patients were recruited into the study. There were 35 patients with GCA, 30 patients with TAK and 19 disease comparators. Luminal abnormalities (stenosis, occlusion and aneurysm) were observed in 30/30 patients with TAK and 19/35 patients with GCA. A total of 133 MRA/PET paired studies were included, as some patients underwent multiple paired studies, performed at 6-month intervals. Baseline demographics of the study population are shown in table 1.

Assessment of disease extent on imaging

A total of 966 vascular territories were assessed from 72 MRA/PET paired scans, as outlined in table 2. Agreement for disease extent between MRA and PET was seen in 580 territories, where 206 territories were involved on both MRA and PET, and 374 territories were not involved on either modality. This corresponded to a per cent overall agreement of 60%, with Cohen's kappa=0.22, consistent with fair strength of agreement. Of the 386 territories where there was disagreement between MRA and PET, territories were more likely to be involved on MRA than PET (304 vs 82, McNemar's p<0.01).

Table 1 Study population baseline demographics

	GCA	TAK	Comparator group	Total
Patients (n)	35	30	19	84
MRA/PET study				
Total (n)	67	47	19	113
1 study	20	18	19	57
2 studies	5	7	0	12
3 studies	10	5	0	15
Age (years±SD)	68.3±8.3	32.5±12.7	46.0±21.6	48.9±14.2
Sex (female, %)	28 (80)	20 (67)	14 (74)	62 (74)
Body mass index (±SD)	27.7±4.3	26.4±6.9	26.5±6.4	26.9±5.9
Disease duration (years±SD)	2.64±2.42	10.60±10.4	N/A	6.62±6.4

GCA, giant cell arteritis; MRA, magnetic resonance angiography; PET, ^{18}F-fluorodeoxyglucose positron emission tomography; TAK, Takayasu's arteritis.

Table 2 Assessment of extent of disease on MRA and PET

	PET territory involved	PET territory not involved	Total
MRA territory involved	206	304	510
MRA territory not involved	82	374	456
Total	288	678	966

MRA, magnetic resonance angiography; PET, ^{18}F-fluorodeoxyglucose positron emission tomography.

Assessment of disease activity on imaging

There was moderate agreement on interpretation of MRA disease activity between the two readers (kappa=0.58). To evaluate disease activity, 133 paired MRA and PET studies were assessed, as displayed in table 3. There was agreement between MRA and PET interpretation of disease activity in 90 studies (62 studies MRA+/PET+ and 28 studies MRA−/PET−). Per cent overall agreement was 68% with Cohen's kappa=0.30, indicating fair agreement. In the 43 studies where there was disagreement, MRA demonstrated disease activity in 23 studies and PET in 20 studies (McNemar's test p=0.76), indicating in cases of disagreement MRA and PET were equally likely to be interpreted as active disease.

Disease activity was also assessed by subgroup, as shown in table 3. Per cent overall agreement was similar among the three groups (TAK 64%, Cohen's kappa=0.24; GCA 72%, Cohen's kappa=0.27; comparator group 63%, Cohen's kappa=0.23). However, for cases of disagreement, there was a difference in pattern among the three groups. For the TAK and GCA subgroups, McNemar's test was p=1.0 and p=0.36, respectively, indicating in cases of disagreement MRA and PET were equally likely to be interpreted as active disease. For the comparator group, which included non-inflammatory vasculopathies, MRA was more likely to be interpreted as active vasculitis than PET (McNemar's test p=0.02).

Features of MRA associated with imaging interpretation

Multivariable logistic regression was performed to evaluate which features of MRA were directly associated with reader interpretation of disease activity on MRA. An increasing number of vascular territories on MRA showing oedema (OR=2.29, 95% CI 1.45 to 3.60, p<0.01) was associated with MRA interpretation of disease activity, whereas an increasing number of territories with increased wall thickness (OR=1.1, 95% CI 0.93 to 1.33, p=0.24) and stenosis (OR=0.90, 95% CI 0.75 to 1.07), p=0.24) was not significantly associated with interpretation of disease activity on MRA.

The association of features on MRA with reader interpretation of activity on a paired PET scan was also assessed. An increasing number of vascular territories on MRA showing oedema (OR=1.36, 95% CI 1.10 to 1.70, p<0.01) and wall thickness (OR=1.17, 95% CI 1.01 to 1.37, p=0.04) were each independently associated with PET scan interpretation of disease activity. Stenosis was not associated with PET scan interpretation of disease activity (OR=0.93, 95% CI 0.81 to 1.07, p=0.33). Of the territories with oedema, wall thickness and stenosis on MRA, 64%, 48% and 35%, respectively, had associated PET scan activity in the corresponding vascular territory. Patterns of agreement between MRA and PET were consistent across the 15 arterial territories under investigation.

Patients in whom both MRA and PET were interpreted as active disease had the highest number of territories with oedema (MRA+/PET+ median five territories, MRA+/PET− median of one territory, MRA−/PET+ median of 0 territories and MRA−/PET− median of 0 territories), as shown in figure 1A. Patients who were active on both MRA and PET also had the greatest number of territories with increased wall thickness (MRA+/PET+ median nine territories, MRA+/PET− median five territories, MRA−/PET+ median of four territories and MRA−/PET− median of two territories), as shown in figure 1B. There were few significant differences between PET scan activity and median number of territories with stenosis, as shown in figure 1C. Representative images showing the association between oedema and wall thickness on MRA, and corresponding vascular FDG uptake on PET, are shown in online supplementary figure.

Association of imaging and clinical features

When assessing activity on imaging across the entire cohort, clinical disease activity assessment was significantly associated with PET scan interpretation of disease activity (55% concordance vs 45% discordance, p<0.01) but not with MRA interpretation (46% concordance vs 54% discordance, p=0.70) (figure 2A). Increased mean age was significantly associated with disease activity by MRA (63 vs 54 years, p=0.03) but not by PET (62 vs 52 years, p=0.16) (figure 2B). Type of vasculitis, prednisone dose, erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) values did not significantly differ between patients with active versus normal MRA or PET studies. (figure 2C–F).

Additional details about the associations between clinical, serological and imaging features of disease in patients with LVV are shown in online supplementary tables 1–3. Notably, 94% patients with clinically active disease and 78% of patients in clinical remission had imaging activity on either PET, MRA or both. Among 69 paired studies performed during clinical remission, PET scan activity was detected in 43 (62%) studies, MRA activity was detected in 46 (67%) studies and activity was concurrently detected by PET and MRA in 35 (51%) studies. Acute phase reactants were not associated with imaging-based disease activity during active disease or clinical remission. Marked disease activity by PET and MRA was observed in patients with LVV during clinical remission with normal acute phase reactants (figure 3).

DISCUSSION

Complex associations between MRA, PET and clinical assessment were identified in a prospective cohort of patients with LVV assessed at different time points in the disease course. When assessing disease extent, there was fair agreement between MRA

Table 3 Assessment of disease activity on MRA and PET

	Total paired studies			Takayasu's arteritis			Giant cell arteritis			Control		
	PET+	PET−	Total	PET+	PET−	Total	PET+	PET−	Total	PET+	PET−	Total
MRA+	62	23	85	20	9	29	40	7	47	2	7	9
MRA−	20	28	48	8	10	18	12	8	20	0	10	10
Total	82	51	133	28	19	47	52	15	67	2	17	19

MRA, magnetic resonance angiography; PET, positron emission tomography.

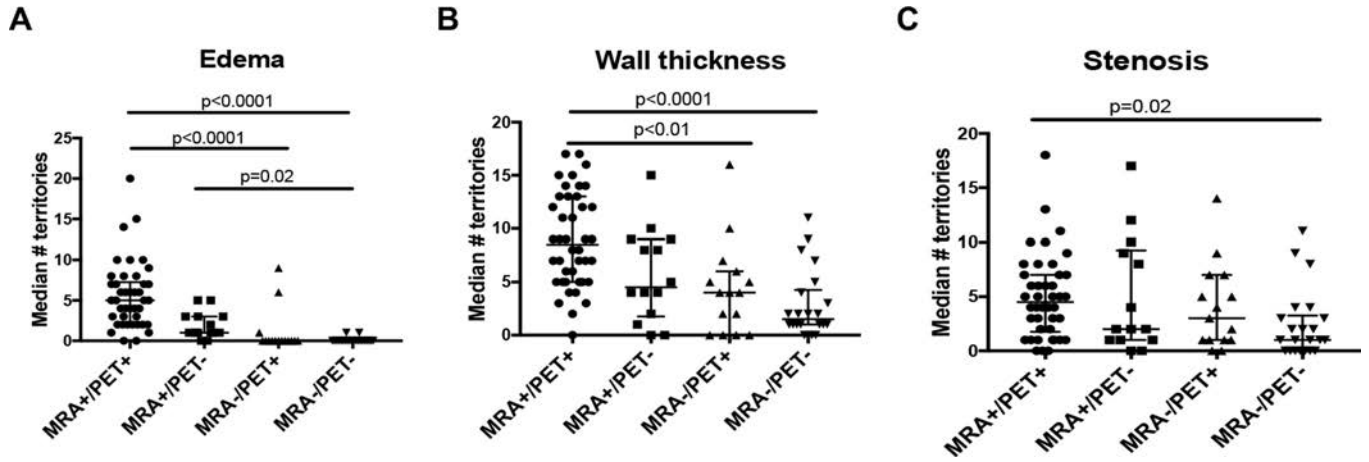


Figure 1 Associations between features of MRA and imaging activity assessed by MRA and PET. Patients were divided based on imaging assessment of disease activity (active vs inactive) by MRA and PET into four subgroups (MRA+/PET+, MRA+/PET−, MRA−/PET+ and MRA−/PET). Associations of the four subgroups with the MRA features of oedema, wall thickness and stenosis are displayed. Patients with active disease on both MRA and PET (MRA+/PET+) had the greatest median number of territories with oedema (A) and wall thickness (B), while there were fewer significant associations between number of territories with stenosis and imaging activity (C). MRA, magnetic resonance angiography; PET, positron emission tomography

and PET, but MRA identified a greater extent of vascular involvement than PET due to detection of both arterial wall abnormalities (wall thickness and oedema) and luminal abnormalities (occlusion, aneurysm and stenosis). When assessing disease activity, inter-rater agreement was greater for PET scan reads compared with MRA reads (kappa=0.84 vs kappa=0.58), indicating that assessment of disease activity by PET is more reliable than MRA. Agreement in disease activity assessment between

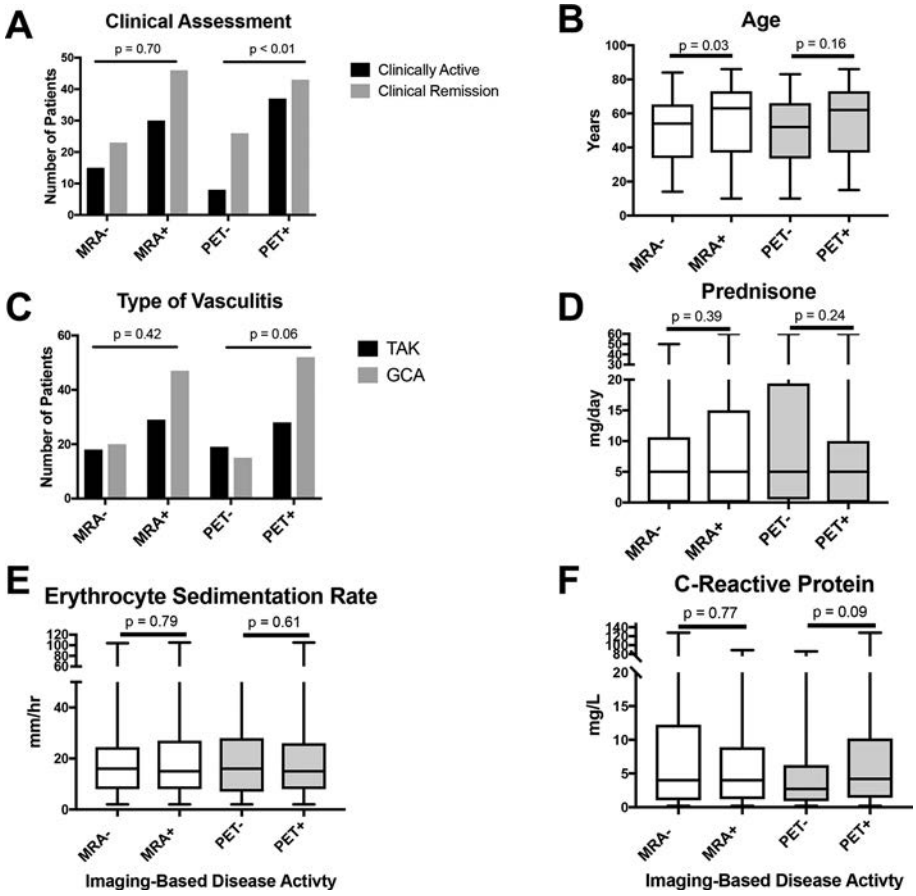


Figure 2 Association of imaging-based interpretation of vasculitis disease activity and clinical features of disease in large-vessel vasculitis. There were few significant clinical differences between patients whose imaging studies (magnetic resonance angiography (MRA) or positron emission tomography (PET)) were interpreted as active vasculitis versus normal. Clinically active disease compared with clinical remission was associated with increased PET interpretation of active vasculitis (A), and older age was significantly associated with increased MRA interpretation of active vasculitis (B). Type of vasculitis (giant cell arteritis (GCA) vs Takayasu's arteritis (TAK)), daily prednisone dose and acute phase reactant levels were not significantly associated with image interpretation by MRA or PET (C–F).

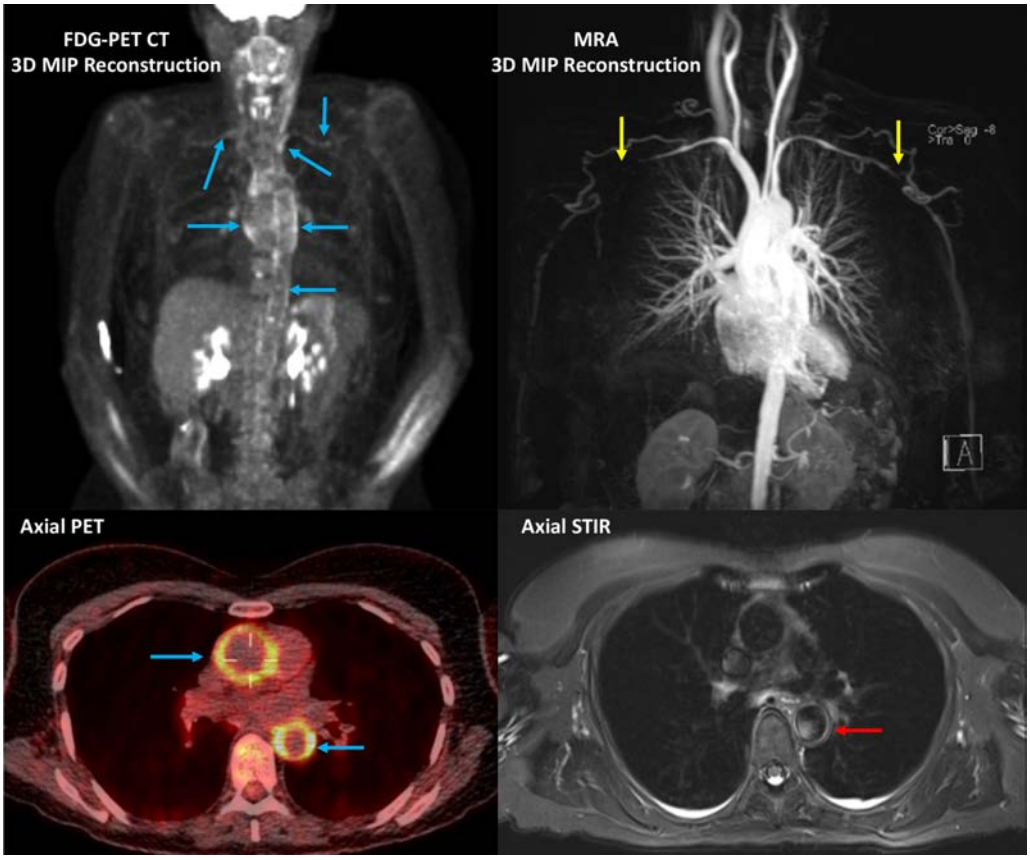


Figure 3 Representative abnormal FDG-PET and MRA studies in a patient with giant cell arteritis in clinical remission. A 72-year-old woman with giant cell arteritis underwent imaging studies 5 years after diagnosis. At the time of imaging, the patient was in clinical remission and had been tapered off all vasculitis-related medications. Erythrocyte sedimentation rate was within normal limits at 25 mm/hour and C reactive protein was 4.6 mg/L (normal <5 mg/L). On FDG-PET, she had severe FDG uptake throughout the aorta and branch arteries (blue arrows), including the ascending and descending aorta (blue arrows on axial PET). On corresponding whole body MRA obtained on the same day as the PET scan, there were severe, bilateral stenoses in the subclavian/axillary arteries (yellow arrows) and increased wall thickness and oedema throughout the aorta on axial short tau inversion recovery (STIR) images (red arrow). These images highlight that patients with LVV can have marked disease activity on imaging studies while in clinical remission with normal acute phase reactants. LVV, large-vessel vasculitis; MRA, magnetic resonance angiography; FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography.

MRA and PET was observed in two-thirds of paired imaging studies; however, only disease activity assessment by PET, and not MRA, was associated with clinical assessment. Despite the significant association between PET and clinical assessment of disease activity, 51% of patients with LVV in clinical remission had evidence of ongoing disease activity by both PET and MRA. Thus, these findings support the concepts that MRA and PET capture complementary but different aspects of vascular biology and that imaging-based assessment of disease activity often differs from clinical assessment.

Specific features on MRA were associated with interpretation of vascular disease activity by MRA and PET. Oedema, in increasing number of arterial territories, was the strongest risk factor for the increased likelihood that an MRA would be interpreted as active. Compared with wall thickness and stenosis, oedema also most strongly correlated with PET scan activity within specific arterial territories. Wall thickness and oedema on MRA were independently associated with global PET scan interpretation of disease activity. PET scan findings are therefore associated with abnormalities of the arterial wall and are not associated with features of damage to the arterial lumen. Vascular oedema and increased wall thickness could be used as a proxy for PET scan activity when access to FDG-PET is not available.

Prior studies report conflicting results about the associations between clinical, serological and imaging-based assessments of disease activity in LVV.[6 8 9 14–33] Interpretation of these studies are frequently limited due to small sample sizes, retrospective study design, lack of standardised imaging protocols applied to all study participants and delay between imaging and clinical assessment with potential intervening treatment changes. Additionally, most of these studies focus on the time of initial diagnosis with relatively little imaging data available at later points in the disease course. The present study addresses these limitations in study design and shows that imaging findings, clinical assessment and acute phase reactants do not necessarily correlate over the course of disease.

Although some disease activity assessment indices in LVV incorporate acute phase reactants into clinical definitions of disease activity,[10 34] this study defined clinical disease activity by symptoms alone without considering acute phase reactants. Using these definitions, ESR and CRP were not associated with imaging-based disease activity in patients with LVV during periods of clinically active disease or remission. Acute phase reactants, particularly at later time points in disease, are not useful to identify subsets of patients with LVV with active disease by PET or MRA.

Previous studies have demonstrated ongoing vascular disease activity by MRA[6 18 35] and by PET[7 11 26] in patients with LVV otherwise in apparent clinical remission. This study is the first to show that approximately half of patients with LVV have what appears to be ongoing disease activity on MRA and PET studies obtained concurrently during clinical remission. In absence of corresponding histology, a limitation inherent to most imaging studies in LVV, it is unclear whether the imaging abnormalities observed during clinical remission represent active vascular inflammation or non-specific changes related to vascular damage. However, these findings align with autopsy data[36 37] and temporal artery biopsies performed during periods of apparent clinical remission,[11] which demonstrate subclinical vascular inflammation in LVV. Both disease-specific factors (eg, type of vasculitis and treatment status) and non-specific factors (eg, age) were associated with imaging activity during clinical remission. These results suggest that vascular imaging abnormalities observed during clinical remission are likely driven by multiple factors including subclinical vasculitis, vascular repair and secondary processes such as atherosclerosis in ageing populations.

This study has some potential limitations. This was a single-centre study, meaning reproducibility of these findings across other cohorts remains unknown. The study population was not an inception cohort, and most patients were enrolled later into the disease course. While this limits the ability to extrapolate the findings to patients with newly diagnosed LVV and likely influences the strength of associations between clinical, serological and imaging assessments, it is more similar to everyday clinical practice, where patients may be seen for the first time at any point in their disease course. Similarly, many patients were taking various immunosuppressive medications at the time of assessment that could impact imaging activity, particularly in the studies performed during clinical remission. Differentiating angiographic and PET findings in LVV versus atherosclerosis can sometimes be challenging. The methods used to define disease activity by clinical and imaging-based approaches were consistent with general approaches employed in prior studies.[8 11 15 19 38 39] Development of validated clinical definitions of disease activity and standardised definitions for imaging-based measurements of vascular activity are major unmet needs in LVV.

The primary objective of this study was to define the strength of agreement between MRA, PET and clinical assessment to detect disease activity in LVV. This study does not address whether MRA and PET have clinical utility to guide medical decision making in the ongoing management of patients with LVV. Previous studies have suggested PET predicts relapse and angiographic progression of disease,[11 40] whereas other studies have refuted this idea.[7 8] Prospective, longitudinal studies that examine the prognostic value of imaging findings in relationship to long-term clinical and angiographic outcomes are needed. Studies that examine the clinical utility of vascular imaging as a biomarker in LVV weighed against the potential costs and safety concerns of serial imaging should be conducted prior to the incorporation of specific forms of advanced imaging into clinical practice.

While there is much complexity in evaluating disease activity in LVV, findings from this study suggest that MRA and PET provide unique and complementary information in the assessment of LVV. PET scans are better suited to assess disease activity than MRA, and MRA studies are better than PET to identify disease extent including vascular damage. In clinical situations where PET imaging is not available or when radiation exposure is a concern, an increasing number of arterial territories with oedema on MRA, and to a lesser extent wall thickness, could be used as a surrogate for PET scan activity. Approximately half of patients with LVV in clinical remission have evidence of vascular disease activity on concomitant PET and MRA studies, indicating a potential disconnect between clinical and imaging assessment of disease activity. Ultimately, prospective longitudinal studies are needed, ideally performed within randomised clinical trials, to determine the utility of incorporating these imaging modalities into clinical practice as a serial marker of disease activity in LVV.

Contributors KAQ, MAA, AAM, PAM and PCG contributed to the conception and design of this study. KAQ, MAA, AAM, JM, ACC, JSR, AAB, EN and PCG recruited patients into the study and participated in data collection. KAQ, MAA, JSR, AAB and PCG contributed to the data analysis. All authors contributed to data interpretation, critically reviewed the article for important intellectual content and approved the final draft for submission.

Funding This work was supported by the Intramural Research Program at the National Institute of Arthritis and Musculoskeletal and Skin Diseases. KAQ received funding from a Vasculitis Clinical Research Consortium (VCRC)/Vasculitis Foundation Fellowship. The VCRC is part of the Rare Diseases Clinical Research Network, an initiative of the Office of Rare Diseases Research, National Center for Advancing Translational Science (NCATS). The VCRC is funded through collaboration between NCATS and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (U54 AR057319).

Competing interests None declared.

Patient consent Obtained

Ethics approval An institutional review board and radiation safety committee at the NIH approved the research (NCT 02257866).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There are no additional data available.

REFERENCES

1 Jennette JC, Falk RJ, Bacon PA, *et al*. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1–11.
2 Blockmans D. The use of (18F)fluoro-deoxyglucose positron emission tomography in the assessment of large vessel vasculitis. *Clin Exp Rheumatol* 2003;21:S15–22.
3 Blockmans D, Bley T, Schmidt W. Imaging for large-vessel vasculitis. *Curr Opin Rheumatol* 2009;21:19–28.
4 Cimmino MA, Camellino D. Large vessel vasculitis: which imaging method? *Swiss Med Wkly* 2017;147:w14405.
5 Prieto-González S, Depetris M, García-Martínez A, *et al*. Positron emission tomography assessment of large vessel inflammation in patients with newly diagnosed, biopsy-proven giant cell arteritis: a prospective, case-control study. *Ann Rheum Dis* 2014;73:1388–92.
6 Scheel AK, Meller J, Vosshenrich R, *et al*. Diagnosis and follow up of aortitis in the elderly. *Ann Rheum Dis* 2004;63:1507–10.
7 Blockmans D, de Ceuninck L, Vanderschueren S, *et al*. Repetitive 18F-fluorodeoxyglucose positron emission tomography in giant cell arteritis: a prospective study of 35 patients. *Arthritis Rheum* 2006;55:131–7.
8 Both M, Ahmadi-Simab K, Reuter M, *et al*. MRI and FDG-PET in the assessment of inflammatory aortic arch syndrome in complicated courses of giant cell arteritis. *Ann Rheum Dis* 2008;67:1030–3.
9 Lee KH, Cho A, Choi YJ, *et al*. The role of (18) F-fluorodeoxyglucose-positron emission tomography in the assessment of disease activity in patients with takayasu arteritis. *Arthritis Rheum* 2012;64:866–75.
10 Kerr GS, Hallahan CW, Giordano J, *et al*. Takayasu arteritis. *Ann Intern Med* 1994;120:919–29.
11 Grayson PC, Alehashemi S, Bagheri AA, *et al*. ^{18}F-fluorodeoxyglucose-positron emission tomography as an imaging biomarker in a prospective, longitudinal cohort of patients with large vessel vasculitis. *Arthritis Rheumatol* 2018;70:439–49.
12 Arend WP, Michel BA, Bloch DA, *et al*. The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 1990;33:1129–34.
13 Langford CA, Cuthbertson D, Ytterberg SR, *et al*. A randomized, double-blind trial of abatacept (CTLA-4Ig) for the treatment of giant cell arteritis. *Arthritis Rheumatol* 2017;69:837–45.
14 Meller J, Grabbe E, Becker W, *et al*. Value of F-18 FDG hybrid camera PET and MRI in early takayasu aortitis. *Eur Radiol* 2003;13:400–5.
15 Meller J, Strutz F, Siefker U, *et al*. Early diagnosis and follow-up of aortitis with [(18)F] FDG PET and MRI. *Eur J Nucl Med Mol Imaging* 2003;30:730–6.

16 Einspieler I, Thürmel K, Pyka T, *et al*. Imaging large vessel vasculitis with fully integrated PET/MRI: a pilot study. *Eur J Nucl Med Mol Imaging* 2015;42:1012–24.
17 de Leeuw K, Bijl M, Jager PL. Additional value of positron emission tomography in diagnosis and follow-up of patients with large vessel vasculitides. *Clin Exp Rheumatol* 2004;22:S21–6.
18 Newman KA, Ahlman MA, Hughes M, *et al*. Diagnosis of giant cell arteritis in an asymptomatic patient. *Arthritis Rheumatol* 2016;68:1135.
19 Andrews J, Al-Nahhas A, Pennell DJ, *et al*. Non-invasive imaging in the diagnosis and management of Takayasu's arteritis. *Ann Rheum Dis* 2004;63:995–1000.
20 Bruschi M, De Leonardis F, Govoni M, *et al*. [18FDG-PET and large vessel vasculitis: preliminary data on 25 patients]. *Reumatismo* 2008;60:212–6.
21 Fuchs M, Briel M, Daikeler T, *et al*. The impact of 18F-FDG PET on the management of patients with suspected large vessel vasculitis. *Eur J Nucl Med Mol Imaging* 2012;39:344–53.
22 Karapolat I, Kalfa M, Keser G, *et al*. Comparison of F18-FDG PET/CT findings with current clinical disease status in patients with Takayasu's arteritis. *Clin Exp Rheumatol* 2013;31:S15–21.
23 Kobayashi Y, Ishii K, Oda K, *et al*. Aortic wall inflammation due to Takayasu arteritis imaged with 18F-FDG PET coregistered with enhanced CT. *J Nucl Med* 2005;46:917–22.
24 Papathanasiou ND, Du Y, Menezes LJ, *et al*. 18F-Fludeoxyglucose PET/CT in the evaluation of large-vessel vasculitis: diagnostic performance and correlation with clinical and laboratory parameters. *Br J Radiol* 2012;85:e188–94.
25 Yamada I, Nakagawa T, Himeno Y, *et al*. Takayasu arteritis: diagnosis with breath-hold contrast-enhanced three-dimensional MR angiography. *J Magn Reson Imaging* 2000;11:481–7.
26 Arnaud L, Haroche J, Malek Z, *et al*. Is (18)F-fluorodeoxyglucose positron emission tomography scanning a reliable way to assess disease activity in Takayasu arteritis? *Arthritis Rheum* 2009;60:1193–200.
27 Magnani L, Versari A, Salvo D, *et al*. [Disease activity assessment in large vessel vasculitis]. *Reumatismo* 2011;63:86–90.
28 Besson FL, Parienti JJ, Bienvenu B, *et al*. Diagnostic performance of ^{18}F-fluorodeoxyglucose positron emission tomography in giant cell arteritis: a systematic review and meta-analysis. *Eur J Nucl Med Mol Imaging* 2011;38:1764–72.
29 Blockmans D, Stroobants S, Maes A, *et al*. Positron emission tomography in giant cell arteritis and polymyalgia rheumatica: evidence for inflammation of the aortic arch. *Am J Med* 2000;108:246–9.
30 Walter MA, Melzer RA, Schindler C, *et al*. The value of [18F]FDG-PET in the diagnosis of large-vessel vasculitis and the assessment of activity and extent of disease. *Eur J Nucl Med Mol Imaging* 2005;32:674–81.
31 Incerti E, Tombetti E, Fallanca F, *et al*. ^{18}F-FDG PET reveals unique features of large vessel inflammation in patients with Takayasu's arteritis. *Eur J Nucl Med Mol Imaging* 2017;44:1109–18.
32 Webb M, Chambers A, AL-Nahhas A, *et al*. The role of 18F-FDG PET in characterising disease activity in Takayasu arteritis. *Eur J Nucl Med Mol Imaging* 2004;31:627–34.
33 Barra L, Kanji T, Malette J, *et al*. Imaging modalities for the diagnosis and disease activity assessment of Takayasu's arteritis: A systematic review and meta-analysis. *Autoimmun Rev* 2018;17:175–87.
34 Misra R, Danda D, Rajappa SM, *et al*. Development and initial validation of the Indian Takayasu Clinical Activity Score (ITAS2010). *Rheumatology* 2013;52:1795–801.
35 Tso E, Flamm SD, White RD, *et al*. Takayasu arteritis: utility and limitations of magnetic resonance imaging in diagnosis and treatment. *Arthritis Rheum* 2002;46:1634–42.
36 Dellavedova L, Carletto M, Faggioli P, *et al*. The prognostic value of baseline (18) F-FDG PET/CT in steroid-naïve large-vessel vasculitis: introduction of volume-based parameters. *Eur J Nucl Med Mol Imaging* 2016;43:340–8.
37 Ostberg G. Morphological changes in the large arteries in polymyalgia arteritica. *Acta Med Scand Suppl* 1972;533:135–59.
38 Soussan M, Nicolas P, Schramm C, *et al*. Management of large-vessel vasculitis with FDG-PET: a systematic literature review and meta-analysis. *Medicine* 2015;94:e622.
39 Muratore F, Pipitone N, Salvarani C, *et al*. Imaging of vasculitis: state of the art. *Best Pract Res Clin Rheumatol* 2016;30:688–706.
40 Eshet Y, Pauzner R, Goitein O, *et al*. The limited role of MRI in long-term follow-up of patients with Takayasu's arteritis. *Autoimmun Rev* 2011;11:132–6.

EXTENDED REPORT

Efficacy and safety of biologics in relapsing polychondritis: a French national multicentre study

Guillaume Moulis,[1,2,3] Grégory Pugnet,[1,2] Nathalie Costedoat-Chalumeau,[4,5] Alexis Mathian,[6] Gaëlle Leroux,[7,8] Jonathan Boutémy,[9] Olivier Espitia,[10] Laurence Bouillet,[11] Sabine Berthier,[12] Jean-Baptiste Gaultier,[13] Pierre-Yves Jeandel,[14] Amadou Konaté,[15] Arsène Mékinian,[16] Elisabeth Solau-Gervais,[17,18] Benjamin Terrier,[4] Daniel Wendling,[19] Fanny Andry,[11] Camille Garnier,[2] Pascal Cathébras,[13] Laurent Arnaud,[20,21] Aurore Palmaro,[1,3] Patrice Cacoub,[7,8] Zahir Amoura,[6] Jean-Charles Piette,[7,8] Philippe Arlet,[2] Maryse Lapeyre-Mestre,[1,3,22] Laurent Sailler[1,2,3]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212705).

For numbered affiliations see end of article.

Correspondence to
Dr Guillaume Moulis, UMR 1027 INSERM-UPS, Pharmacoepidemiology unit, Faculté de Médecine, Toulouse 31000, France; moulis.g@chu-toulouse.fr

Received 15 November 2017
Accepted 1 March 2018
Published Online First 13 March 2018

ABSTRACT

Objectives To assess the efficacy and the safety of biologics in a cohort of patients with relapsing polychondritis (RP).

Methods We conducted a French multicentre retrospective cohort study including patients treated with biologics for RP. Efficacy outcomes were clinical response (partial or complete) and complete response during the first 6 months of exposure, plus daily corticosteroid dose at 6 months. Other outcomes were adverse drug reactions (ADRs), persistence of biologics and factors associated with a response.

Results This study included 41 patients exposed to 105 biologics (tumour-necrosis factor (TNF) inhibitors, n=60; tocilizumab, n=17; anakinra, n=15; rituximab, n=7; abatacept, n=6). Overall response rate during the first 6 months of exposure was 62.9%. Complete response rate was 19.0%. Reduced corticosteroid doses were highly variable among patients. ADRs were mostly infections (n=42). Reasons for biologic withdrawal (73.3%) were insufficient efficacy (34.3%; ranging from 23.5% for tocilizumab to 72.7% for etanercept), loss of efficacy (18.1%) and ADRs (20.9%; mostly for anakinra: 46.7%). Persistence was comparable among biologic classes. Among TNF inhibitors, the highest persistence was observed with adalimumab. Differences in clinical response rates were observed depending on biologics and organ involvement. There were trends towards a lower response rate in cases with associated myelodysplastic syndrome and for a higher response rate for nasal/auricular chondritis, sternal chondritis and concomitant exposure to non-biologic disease-modifying antirheumatic drugs.

Conclusions This study describes the efficacy of biologics for refractory RP. However, the number of complete responses was low and there were concerns about the risk of ADRs, particularly infections.

INTRODUCTION

Relapsing polychondritis (RP) is a rare autoimmune disease that affects cartilaginous tissues, with a risk of both cartilaginous (e.g., respiratory tract) or non-cartilaginous organ involvement.[1] Its incidence has been estimated to be <5/million people/year.[2–4] The main features are nasal and auricular chondritis, seronegative polyarthritis, sternal and costal cartilage inflammation, and laryngotracheal, ocular, cochlear and vestibular involvement. Other features (mainly cardiovascular and central nervous system inflammation) are rare.[1 5] Patients with myelodysplastic syndrome associated with RP or laryngotracheal involvement may have a worse prognosis.[5]

The disease course is characterised by flares and remissions. To this day, corticosteroids are the first-line treatment for this disease. Because the usual course takes many years to evolve, severe involvement or an inadequate response to high-dose corticosteroids (≥1 mg/kg/day of prednisone equivalent),[1] corticosteroid-sparing agents are often prescribed, such as immunosuppressive or immunomodulatory drugs (e.g., dapsone, methotrexate, azathioprine, cyclophosphamide, ciclosporin and mycophenolate).[1]

The pathophysiology of RP is poorly understood. The roles of both humoral and cellular immune responses have been described. Many cytokines may be also involved, like tumour necrosis factor (TNF)-α, interleukin-1 and interleukin-6.[6] Consequently, the use of biologics in corticosteroid-dependent patients and in patients with an inadequate response to high doses of corticosteroids have been increasingly reported during the last decade.[1 7] However, these have been mainly single case reports and, hence, are subject to potential publication bias (favouring positive outcomes). Few single-centre series have been reported until now: that is, nine refractory patients treated with rituximab,[8] nine patients exposed to 22 biologics (mainly TNF inhibitors),[9] and four and three patients treated with abatacept.[10 11] Moreover, end points for the assessment of biologic efficacy have been highly variable across these reports; therefore, it was very difficult to estimate the efficacy of biologics to treat RP from a literature review.[1]

Thus, this study aimed to assess the efficacy and safety of biologics in a large cohort of patients with RP.

To cite: Moulis G, Pugnet G, Costedoat-Chalumeau N, *et al*. *Ann Rheum Dis* 2018;**77**:1172–1178.

METHODS

Patients

This national, multicentre, retrospective study included adult patients treated with biologics for RP between 2001 and July 2015 in France. Rheumatologists and internal medicine physicians were contacted by the French National Society of Internal Medicine (n=1200) and by the *Club Rhumatisme et Inflammation* (n=2500 rheumatologists and internal medicine practitioners) networks. Inclusion criteria were being an adult (≥18 years) patient with RP who satisfied the McAdam, Damiani and Levine as well as the Michet diagnosis criteria[12–14] and exposed to at least one biologic, including TNF inhibitors, anakinra, abatacept, tocilizumab and rituximab. The exclusion criterion was a patient's opposition to data being collected.

Data collection

Demographic, clinical and biological data were recorded on a standardised form at the time of exposure to the biologic (T0), then at 3 and 6 months, and then every 6 months. The follow-up ended with discontinuation of the biologic or the last date when the patient was still receiving the biologic. For rituximab patients, we recorded data for up to 12 months after exposure.

Outcomes

Efficacy outcomes were the rates of complete response (CR, defined by no clinical activity) and of response (defined by at least a partial clinical decrease in disease activity, including CR) obtained in at least one assessment during the first 6 months of exposure. Due to the retrospective design and the subsequent unavailability of systematic paraclinical examinations, investigators were requested to categorise the clinical signs of disease activity at each visit compared with the signs of RP when the biologic was started: that is, as worsening, stable disease, partial improvement (ie, response) or no activity (ie, CR). The rates of response and CR during the first 6 months of exposure were presented according to the biologics in the overall population as a global measurement, but also according to organ involvement (ie, auricular/nasal, joint, ocular, cochleovestibular or respiratory involvement). In the analyses according to organ involvement, response was defined as partial or complete improvement in the clinical signs of disease activity in the given organ. To assess corticosteroid-sparing, we compared daily corticosteroid doses (prednisone equivalent) between T0 and month 6 for patients who had ≥6 months exposure to a biologic. Adverse drug reactions (ADRs) were described. We also compared the persistence (time under treatment) of biologics (excluding rituximab) and the reasons for discontinuing a biologic.

Statistical analyses

Descriptive analyses are presented using frequencies and percentages for qualitative variables, and medians or means (as appropriate) with ranges for continuous variables. We analysed the persistence of biologics using Kaplan-Meier curves. Comparisons were made using the log-rank test (α=5%).

We also assessed the factors associated with achieving a response during the first 6 months of exposure to a first-line biologic using a univariate logistic regression model. Odds ratios (ORs) and their 95% confidence intervals (CIs) were computed. The following variables were tested: age, gender, the presence of an associated disease (except myelodysplastic syndrome), the presence of a myelodysplastic syndrome, disease duration before exposure to a biologic, Charlson's score,[15] history of organ involvement due to RP, organ involvement due to RP at initiation of the biologic and concomitant exposure to a non-biologic disease-modifying antirheumatic drug (NBDMARD). From multiple testing, the alpha threshold value was 0.0024 (Bonferroni's correction). This was an explanatory model. No multivariate model was conducted due to the low number of patients.

All statistical analyses were performed using SAS V.9.4 software.

RESULTS

Patients

Forty-one patients were included from 14 centres; the patients were exposed to 105 biologics in total. Baseline characteristics are detailed in table 1. The mean age was 46.9±12.5 years and 53.6% were women. Median time from RP diagnosis to first-line initiation of a biologic was 26.5 months. The most frequent involvements were nasal chondritis, arthralgia and auricular chondritis. All but two patients had an active disease at first exposure to a biologic, and all but three patients had previous exposure to NBDMARDs (mostly methotrexate, n=30).

Exposure to biologics

The reasons for initiating a biologic were corticosteroid dependence (n=28), an inadequate response to corticosteroids as judged by the prescriber (n=11) and ADRs to methotrexate (n=3: one hepatitis, one neutropaenia and one skin rash). First-line biologics were TNF inhibitors (n=30), tocilizumab (n=5), rituximab (n=4), and anakinra and abatacept (n=1 each). Twenty-eight patients were exposed to at least two lines of biologics (because of insufficient efficacy in 14, relapses in 8 and ADRs in 9).

In total, 105 exposures to biologics were recorded: TNF inhibitors, n=60 in 32 patients; tocilizumab, n=17 in 15 patients (2 patients had been re-exposed to tocilizumab after an intermediate biologic); anakinra, n=15 in 13 patients (2 patients had been re-exposed to anakinra after an intermediate biologic); rituximab, n=7 to 7 patients; abatacept, n=6 in 5 patients (2 patients had been re-exposed to abatacept after an intermediate biologic). The details of the biologic lines are shown in the online supplementary table 1. All biologics were used at the same dosage as given for rheumatoid arthritis. Abatacept and tocilizumab were given intravenously to all patients. At initiation of the biologics, corticosteroids were ongoing in 88 cases (83.8%; mean dose: 23.9 mg prednisone equivalent, range: 5–80) and NBDMARDs in 64 cases (60.9%), mostly methotrexate (42 cases, 40.0%; NBDMARDs used concomitantly to first-line biologics are detailed in table 1).

Overall, only slight differences in the patients' characteristics between biologics were observed (online supplementary table 2).

Overall response and CR rates

The outcomes considering the 105 exposures to biologics are presented in table 2. Rates of response and of CR achievement during the first 6 months were 62.9% and 19.0%, respectively. Response rates were the lowest for abatacept (50.0%) and anakinra (53.3%). They were 63.3%, 70.6% and 71.4% for TNF inhibitors, tocilizumab and rituximab, respectively. There were similar response rates across the TNF inhibitors. Analysis restricted to first-line biologics led to similar results for TNF inhibitors, with few patients exposed to other biologics (table 3).

Efficacy according to organ involvement

Achieving a response during the first 6 months with a biologic and according to organ involvement is shown in table 4. The

Table 1 Baseline characteristics of patients (n=41)

Variable	Value
Age at diagnosis, mean±SD, years	46.9±12.5
Females, n (%)	22 (53.6)
Associated disease, n (%)*	20 (48.8)
Charlson's comorbidity score, median (range)	1 (1–4)
Organ involvement before biologic exposure	
Fever, n (%)	19 (46.3)
Rheumatological manifestations, n (%)	34 (82.9)
Arthralgia, n (%)	34 (82.9)
Arthritis, n (%)	19 (46.3)
Auricular and nasal chondritis, n (%)	41 (100)
Auricular, n (%)	31 (75.6)
Nasal, n (%)	34 (82.9)
Sternal chondritis, n (%)	22 (53.7)
Manubriosternal, n (%)	6 (14.6)
Sternoclavicular, n (%)	14 (34.1)
Costosternal, n (%)	15 (36.6)
Ophthalmological manifestations, n (%)	18 (43.9)
Episcleritis, n (%)	11 (26.8)
Scleritis, n (%)	4 (9.8)
Uveitis, n (%)	8 (19.5)
Retinal vasculitis, n (%)	1 (2.4)
Respiratory manifestations, n (%)	24 (58.5)
Without acute respiratory failure, n (%)	24 (58.5)
With acute respiratory failure, n (%)	1 (2.4)
Vestibular and cochlear manifestations, n (%)	15 (36.6)
Sensorineural deafness, n (%)	15 (36.6)
Vestibular dysfunction, n (%)	7 (17.1)
Skin manifestations, n (%)	6 (14.6)
Purpura, n (%)	4 (9.8)
Erythema nodosum, n (%)	2 (4.9)
Cardiovascular manifestations, n (%)	5 (12.2)
Pericarditis, n (%)	3 (7.3)
Myocarditis, n (%)	1 (2.4)
Valvular disease, n (%)	2 (4.9)
Peripheral neuropathy, n (%)	3 (7.3)
Autoantibodies	
Rheumatoid factor, n (%)	4/33 (12.1)
ACPA, n (%)	1/26 (3.8)
Antinuclear antibodies, n (%)	19/35 (54.3%)
ANCA, n (%)	6/37 (16.2)
Anti-collagen II n (%)	2/10 (20.0)
Anti-matrilin 1	0/2 (0)
Inflammation or fibrosis on histology, n (%)	6/6 (100)
Non-corticosteroids treatments before biologic exposure n (%)	38 (92.7)
Methotrexate, n (%)	30 (73.2)
Cyclophosphamide, n (%)	11 (26.9)
Azathioprine, n (%)	10 (24.4)
Dapsone, n (%)	7 (17.1)
Mycophenolate, n (%)	5 (12.2)
Concomitant corticosteroids at the time of first exposure to a biologic	
Number of patients, n (%)	35 (85.4)
Mean dose (range), mg prednisone equivalent	24.0 (10–80)
Concomitant NBDMARDs at the time of first exposure to a biologic †	
Methotrexate, n (%)	17 (41.5)
Hydoxychloroquine, n (%)	6 (14.6)
Azathioprine, n (%)	3 (7.3)
Mycophenolate, n (%)	3 (7.3)
Dapsone, n (%)	2 (4.9)
Colchicine, n (%)	2 (4.9)

*Including notably four myelodysplastic syndromes, four cases of spondyloarthritis, three of inflammatory bowel diseases, three of neutrophilic dermatitis and two of rheumatoid arthritis. In one patient who had associated Crohn's disease, both diseases were in flare-up at the first exposure to adalimumab.
†One patient was exposed to methotrexate and hydroxychloroquine, one patient received dapsone and hydroxychloroquine, one patient received methotrexate and colchicine and one patient received mycophenolate and hydroxychloroquine.
ACPA, anti-citrullinated protein antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; NBDMARDs, non-biologic disease-modifying antirheumatic drugs

efficacy of biologics for nasal/auricular chondritides were the highest with tocilizumab and the few patients treated with abatacept or rituximab. TNF inhibitors were the most effective biologic for joint inflammation, particularly adalimumab. Infliximab, tocilizumab and rituximab were more effective than anakinra for ocular involvement. Tocilizumab and TNF inhibitors were the most effective biologics for respiratory-tract involvement (along with abatacept, but only two observations were described with this biologic). No conclusion could be drawn for cochlea–vestibular involvement because almost all of these patients were exposed to TNF inhibitors.

Corticosteroid-sparing effect

Among the patients exposed to biologics for at least 6 months, there was only a modest reduction in median daily corticosteroid dose (5 mg of prednisone equivalent) between T0 and month 6. However, there was huge variability between individuals (tables 2 and 3, figure 1). Similar results were observed in analyses restricted to first-line biologics (figure not shown).

Adverse drug reactions

Overall, 20.9% of biologics were withdrawn due to ADRs. All ADRs associated with biologics are detailed in the online supplementary table 3. The most frequent ADRs were infections (n=42) and reactions at the site of injecting subcutaneous biologics (n=12). Three opportunistic infections were described: two recurrences of herpes occurred with anakinra and one zoster that occurred with tocilizumab. One case of cancer was observed: a lung carcinoma in a patient who smoked tobacco and after 19 months of exposure to anakinra. No deaths occurred during exposure to a biologic.

Persistence of biologics

Persistence was comparable among biologic classes (figure 2, panel A, p=0.77). Among the TNF inhibitors, the highest persistence was observed with adalimumab and the lowest with etanercept (figure 2, panel B: adalimumab vs etanercept: p=0.02).

Factors associated with achieving a response to first-line biologics during the first 6 months of treatment

No variable achieved significance (table 5). There was a non-significant trend towards a decreased rate of response in cases of associated myelodysplastic syndrome (OR, 0.14; 95% CI 0.01 to 1.51). Conversely, there was a significant trend towards an increased rate of response for cases of associated NBDMARDs (OR, 2.14; 95% CI 0.55 to 8.38), a history of sternal chondritis

Table 2 Efficacy and ADRs to the 105 exposures to biologics prescribed for relapsing polychondritis in 41 patients

Exposures to biologics	Response achieved during the first 6 months , n (%)	CR achieved during the first 6 months , n (%)	Variation in CS dose at M6, mg PEQ, median (range)*	Follow-up, months, median (range)	Discontinuation of biologic				
					Overall n (%)	Insufficient efficacy n (%)	Loss of efficacy n (%)	ADR n (%)	Stable CR
Overall (n=105)	66 (62.9)	20 (19.0)	−5.0 (−72.5;+70.0)	6.0 (0.1–80.8)	77 (73.3)	36 (34.3)	19 (18.1)	22 (20.9)	1 (0.9)
TNF antagonists (n=60)	38 (63.3)	14 (23.3)	−5 (−53;+70)	6.0 (0.4–80.8)	47 (78.3)	23 (38.3)	15 (25.0)	8 (13.3)	1 (1.7)
Infliximab (n=20)	12 (60.0)	7 (35.0)	−5 (−50;+70)	6.5 (0.4–80.8)	16 (80.0)	7 (35.0)	6 (30.0)	3 (15.0)	0
Adalimumab (n=25)	16 (64.0)	5 (20.0)	−7.5 (−53;+10)	8.0 (0.4–71.7)	18 (72.0)	6 (24.0)	7 (28.0)	5 (20.0)	1 (4.0)
Etanercept (n=11)	8 (72.7)	0	−5 (−50;+0)	5.5 (0.7–36.7)	11 (100)	8 (72.7)	2 (18.2)	2 (18.2)	0
Golimumab (n=3)	2 (66.7)	2 (66.7)	−20	3.8 (3.4–7.2)	1 (33.3)	1 (33.3)	0	0	0
Certolizumab (n=1)	0	0	–	2.9	1 (100)	1 (100)	0	1 (100)	0
Tocilizumab (n=17)	12 (70.6)	2 (11.8)	−1 (−72.5;+0)	3.7 (0.4–36.2)	10 (58.8)	4 (23.5)	2 (11.7)	4 (23.5)	0
Anakinra (n=15)	8 (53.3)	2 (13.3)	−12.5 (-20;+0)	2.6 (0.3–63.8)	13 (86.7)	5 (33.3)	0	7 (46.7)	0
Rituximab (n=7)	5 (71.4)	1 (14.3)	−3 (−30;+5)	6.0	3 (42.8)	3 (42.8)	0	0	0
Abatacept (n=6)	3 (50.0)	1 (16.7)	−16 (−40;+0)	9.5 (0.1–37.1)	6 (100)	3 (50.0)	2 (33.3)	1 (16.7)	0

*Data available for 13 patients on infliximab, 16 on adalimumab, 7 on etanercept, 1 on golimumab, none on certolizumab pegol, 4 on anakinra, 7 on rituximab, 10 on tocilizumab and 4 on abatacept.
ADR, adverse drug reaction; CR, complete response; CS, corticosteroids; PEQ, prednisone equivalent; TNF, tumour necrosis factor.

(OR, 5.00; 95% CI 1.22 to 20.45), nasal or auricular chondritis at biologic initiation (OR, 3.64; 95% CI 0.90 to 14.61) or sternal chondritis when the biologic was started (OR, 3.95; 95% CI 0.90 to 17.40).

DISCUSSION

This study reports on the efficacy and safety of biologics to treat RP in the largest cohort assessed to date. In total, we described 60 exposures to TNF inhibitors, 17 to tocilizumab, 15 to anakinra, 7 to rituximab and 6 to abatacept in 41 patients.

The study population consisted of patients with severe RP, with 43.9%, 58.5%, 36.6% and 12.2% having experienced ophthalmological, respiratory tract, vestibular or cochlear, and cardiovascular involvements before initiating a biologic, respectively. Ninety-three per cent of patients had been exposed to NBDMARDs before receiving a biologic.

Overall, the biologics showed an overall response rate of 62.9%, but a low rate of CR (19.0%). The response was transient, leading to biologic withdrawal in 18.1% of cases. In contrast with previous case reports, the corticosteroid-sparing effect of biologics was highly variable between patients in our study, with only a mild effect overall.

Due to this study's retrospective observational design, we cannot exclude that unmeasured factors related to the patients or physicians influenced treatment choice and the outcomes. However, the baseline characteristics of patients were relatively similar between the biologics. Hence, this study provides better understanding of the efficacy and safety of each biologic in a real-life setting and suggests some differences between biologics.

It is very difficult to compare these data with published case reports because of the differences in patients' characteristics, outcome definitions and publication bias. Indeed, all biologics have been reported to be effective against RP, including for severe features such as respiratory and eye involvement.[1 7]

This study confirms the efficacy of TNF inhibitors with a low rate of withdrawal because of ADRs.[1 7] However, etanercept did have a high rate of discontinuation due to insufficient efficacy. In conclusion, this study suggests that infliximab and adalimumab should be preferred among the TNF-α antagonists. Too few patients were exposed to golimumab and certolizumab pegol to draw any conclusions regarding their risk–benefit profiles. Adalimumab had the highest persistence rate, suggesting a good overall risk–benefit ratio.

Table 3 Efficacy and ADRs to first-line biologics prescribed for relapsing polychondritis in 41 patients

Exposures to biologics	Response achieved during first 6 months, n (%)	CR achieved during first 6 months, n (%)	Decrease in CS dose at M6, mg PEQ, median (range)*	Follow-up, months, median (range)	Discontinuation of biologic			
					Overall n (%)	Insufficient efficacy n (%)	Loss of efficacy n (%)	ADR n (%)
Overall (n=41)	27 (65.8)	12 (29.3)	−5.0 (−72.5;+10.0)	6.0 (0.4–80.8)	29 (70.7)	14 (34.1)	8 (27.6)	9 (21.9)
TNF antagonists (n=30)	19 (63.3)	9 (30.0)	−5 (−53;+10)	6.5 (0.4–80.8)	25 (83.3)	12 (40.0)	8 (26.7)	7 (23.3)
Infliximab (n=11)	6 (54.5)	5 (45.4)	−2.5 (−15;+0)	7.0 (0.7–80.8)	9 (81.8)	5 (45.4)	2 (18.2)	1 (9.1)
Adalimumab (n=12)	8 (66.7)	4 (33.3)	−10 (−53;+10)	13.6 (0.4–71.7)	10 (83.3)	3 (25.0)	4 (33.3)	3 (25.0)
Etanercept (n=7)	5 (71.4)	0	−2.5 (−5;+0)	5.5 (0.7–36.7)	7 (100)	4 (57.1)	2 (85.7)	3 (42.9)
Tocilizumab (n=5)	4 (80.0)	2 (40.0)	−37.2 (−72.5;+2)	1.8 (0.4–11.5)	3 (60.0)	1 (20.0)	0	1 (20.0)
Rituximab (n=4)	3 (75.0)	0	−1 (−5;+5)	6.0	2 (50.0)	2 (50.0)	–	–
Anakinra (n=1)	1 (100)	1 (100)	−25	63.8	0	0	0	0
Abatacept (n=1)	0	0	–	1.8	1 (100)	1 (100)	0	0

*Data available for seven patients receiving infliximab, nine on adalimumab, four on etanercept, one on anakinra, four on rituximab, two on tocilizumab and none on abatacept.
ADR, adverse drug reaction; CR, complete response; CS, corticosteroids; PEQ, prednisone equivalent;TNF, tumour necrosis factor.

Table 4 Achieved response during the first 6 months of exposure to a biologic by involvement of organs (105 exposures to biologics in total)

Exposures to biologics	Nasal or auricular chondritis, n (%)	Joints, n (%)	Sternal chondritis, n (%)	Ocular inflammation, n (%)	Vestibular or cochlear manifestation, n (%)	Respiratory manifestations, n (%)
Overall (n=105)	31/57 (54.4)	28/74 (37.8)	31/57 (54.4)	12/17 (70.6)	6/9 (66.6)	27/38 (71.0)
TNF antagonists (n=60)	15/34 (44.1)	17/43 (39.5)	16/24 (66.7)	6/8 (75.0)	5/8 (62.5)	17/24 (70.8)
Infliximab (n=20)	4/9 (44.4)	4/14 (28.56)	2/7 (28.6)	4/4 (100)	1/3 (33.3)	6/8 (75.0)
Adalimumab (n=25)	6/12 (50.0)	8/16 (50.0)	9/10 (90.0)	2/4 (50.0)	3/4 (75.0)	7/10 (70.0)
Etanercept (n=11)	3/9 (33.3)	4/10 (40)	5/5 (100)	–	1/1 (100)	4/4 (100)
Golimumab (n=3)	2/3 (66.7)	1/2 (50.0)	0/1 (0)	–	–	0/1 (0)
Certolizumab (n=1)	0/1 (0)	0/1 (0)	0/1 (0)	–	–	0/1 (0)
Tocilizumab (n=17)	7/9 (77.8)	4/11 (36.4)	7/8 (87.5)	3/4 (75.0)	–	6/6 (100.0)
Anakinra (n=15)	4/8 (50.0)	4/10 (40.0)	2/3 (66.7)	1/3 (33.3)	1/1 (100.0)	1/3 (33.3)
Rituximab (n=7)	2/2 (100)	1/4 (25.0)	1/1 (100)	2/2 (100)	–	1/3 (33.3)
Abatacept (n=6)	3/4 (75.0)	2/6 (33.3)	2/2 (100)	–	–	2/2 (100)

TNF, tumour-necrosis factor.

This study confirms the low efficacy and high rate of withdrawal because of ADRs associated with anakinra,[9] suggesting that this drug should not be preferred as a first-line biologic. In contrast, tocilizumab was highly effective for almost all features of RP, but with a 23.5% rate of withdrawal due to ADRs. This study also confirms the mild overall effectiveness of abatacept for RP, as suggested by the small open-label trial of four patients by Peng and Rodriguez.[10] Two of our patients with tracheal symptoms responded to abatacept, but none of our six patients exposed to abatacept had parenchymal pulmonary or central nervous system involvement, which worsened with abatacept in the trial by Peng and Rodriguez.[10]

Conversely, our study showed the good efficacy of rituximab in contrast to the study by Leroux *et al*,[8] but supports previous case reports.[7] Of note, three patients in the present cohort had been previously included in Leroux's analysis. Unfortunately, it was not possible to access the medical charts of all nine patients included in this series. In our study, the efficacy of rituximab was notable for nasal or auricular chondritis, sternal chondritis and eye involvement. Of note, in the study by Leroux *et al*, all nine patients were refractory to high-dose steroids and to at least two immunosuppressive drugs and, therefore, may have had a more resistant disease. Lastly, only two patients in the study by Leroux *et al* had eye involvement (including one also included in our study), but achieved stability by 6 months after starting rituximab.[8]

As previously suggested,[9] the rotation of biologics is widely used in practice. The low number of patients and the heterogeneity of biologic exposures have prevented further analyses according to lines of treatments. We found no factor significantly associated with an achieved response at 6 months. As expected, there was a trend towards a lower response rate in cases of associated myelodysplastic syndrome, as suggested in a recent case-series.[16] Interestingly, the concomitant use of NBDMARDs tended to be associated with an achieved response, as were nasal/auricular or sternal chondritis. None of these associations reached significance and we cannot exclude trends found by chance due to multiple testing. However, these associations are clinically relevant and the non-significance may be preferentially caused by a lack of statistical power.

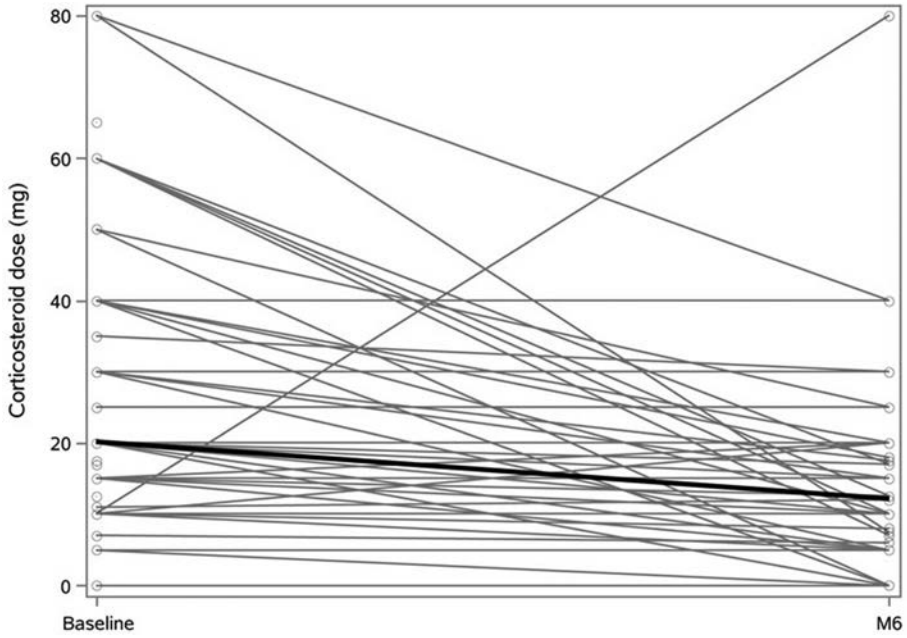


Figure 1 Effect of corticosteroid sparing between the initiation of the biologics and month 6 in patients who were exposed to a biologic for at least 6 months. Mean is shown in bold.

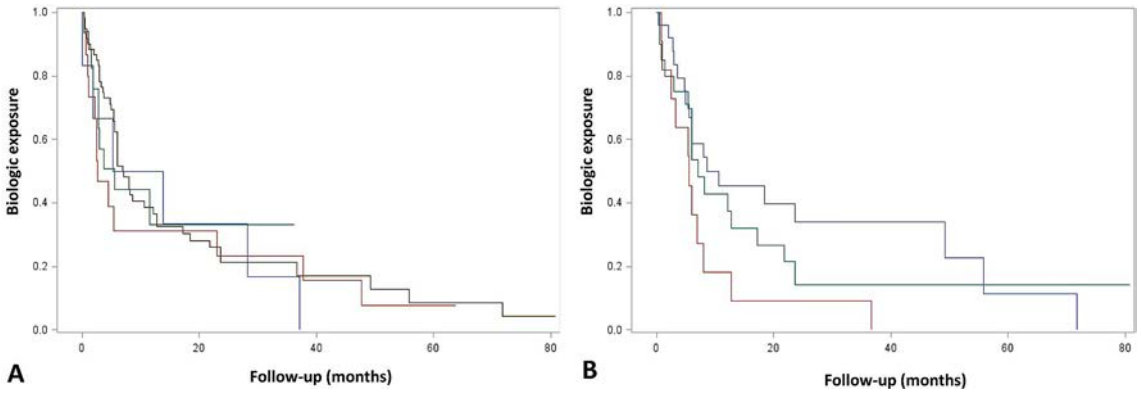


Figure 2 Persistence of biologics (except rituximab) considering the 105 exposures to biologics: (panel A) according to pharmacological class: abatacept (blue curve), anakinra (red curve), tocilizumab (green curve) and TNF inhibitors (brown curve); (panel B) among the three most frequently used TNF inhibitors: adalimumab (blue curve), etanercept (red curve) and infliximab (green curve). TNF, tumour-necrosis factor.

This study demonstrated the high drop-out rate from biologics in real-life practice because of insufficient efficacy, loss of efficacy or an ADR, ranging from 33.3% to 86.7% across the biologics. Overall, about three-quarters of the biologics were discontinued within a mean follow-up time of 6 months, including one-fifth for ADRs. Among these, infections (notably respiratory tract infection) were common. Of note, most patients were concomitantly exposed to corticosteroids and non-biologic immunosuppressive drugs. Unfortunately, due to the retrospective design of our study, no data could be recorded regarding vaccinations prior to exposure to biologics.

This study has some limitations, mostly because of its retrospective design. Fourteen University centres participated in the study; thus, case recording is incomplete and may not reflect all patients treated for RP with biologics in France. Assessment of disease activity was made according to clinical activity, with no standardised paraclinical examination available to improve documentation of organ involvement. As previously stated, the multiplicity of lines of biologics and of unmeasured factors that may have impacted on the choice of biologic or the outcomes prevent from making any definitive direct comparisons, the few patients included and the heterogeneity of biologics used limited interpretation of the risk–benefit ratio for each organ and for each biologic. Similarly, 41.5% of the patients were concomitantly exposed to various NBDMARDs. Due to the heterogeneity of NBDARDs, no comparison could be made. Of note, no patient was concomitantly exposed to oral cyclophosphamide because, in France, cyclophosphamide is almost exclusively given intravenously for severe flares.

As stated above, the assessment of factors associated with a response in univariate analyses should be considered as exploratory; the lack of statistical power led to suggesting trends only. Altogether, this study suggests the need for registries on patients with RP, in particular to compare biologics with NBDMARDs, which is an important question that was not addressed in this study. Indeed, despite the findings on various cytokine expressions,[6] the pathophysiology of this disease is widely unknown and, thus, the rationale for using biologics as a first-line treatment is insufficient.

In conclusion, this retrospective study showed the efficacy of biologics to treat patients with RP who were resistant to NBDMARDs. It also suggested differences in efficacies depending on the biologic and organ involvement, as well as differences in safety profiles. Prospective studies with head-to-head comparisons of biologics for RP are needed to confirm these results.

Table 5 Factors associated with an achieved response during the first 6 months of receiving a first-line biologic

Variables	OR (95% CI)	P values
Age at initiation of the biologic, >50 years vs ≤50 years	0.78 (0.21 to 2.92)	0.72
Female sex	1.94 (0.52 to 7.17)	0.32
Associated disease (excl. myelodysplastic syndrome)	0.93 (0.25 to 3.34)	0.91
Myelodysplastic syndrome	0.14 (0.01 to 1.51)	0.10
Disease duration from diagnosis to receiving a biologic, ≥24 vs <24 months (median)	0.60 (0.16 to 2.21)	0.44
Associated non-biologic disease-modifying antirheumatic drug	2.14 (0.55 to 8.38)	0.27
Charlson's score >0	0.70 (0.19 to 2.56)	0.58
Previous manifestations before biologic		
Fever	0.80 (0.22 to 2.92)	0.73
Arthralgia/arthritis	0.64 (0.11 to 3.69)	0.61
Sternal, sternocostal and sternoclavicular chondritides	5.00 (1.22 to 20.45)	0.02
Ophthalmological	0.44 (0.12 to 1.64)	0.22
Respiratory tract	0.69 (0.18 to 2.63)	0.56
Vestibular and cochlear	1.06 (0.28 to 4.06)	0.93
Antibodies		
Rheumatoid factors	1.58 (0.14 to 17.21)	0.71
Antinuclear antibodies	1.68 (0.40 to 7.07)	0.48
Disease manifestations at onset of a biologic		
Nasal/auricular	3.64 (0.90 to 14.61)	0.07
Arthralgia/arthritis	0.58 (0.14 to 2.34)	0.44
Sternal, sternocostal and sternoclavicular chondritis	3.95 (0.90 to 17.40)	0.07
Ophthalmological	0.43 (0.09 to 2.09)	0.29
Respiratory tract	1.07 (0.29 to 3.92)	0.92
Vestibular and cochlear	0.83 (0.17 to 4.14)	0.82

It is a univariate analyses.

Author affiliations
[1]UMR 1027, INSERM, University of Toulouse, Toulouse, France
[2]Department of Internal Medicine, Toulouse University Hospital, Toulouse, France
[3]Clinical Investigation Center 1436, Toulouse University Hospital, Toulouse, France
[4]Department of Internal Medicine, National Referral Center for Rare and Systemic Autoimmune Diseases, Cochin Hospital, Assistance Publique - Hôpitaux de Paris, University Paris Descartes, Paris, France
[5]INSERM U 1153, Center for Epidemiology and Statistics Sorbonne Paris Cité (CRESS), Paris, France

[6]Department of Internal Medicine 2, Pitié-Salpêtrière University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France
[7]Department of Internal Medicine and Clinical Immunology, Assistance Publique-Hôpitaux de Paris, Groupe Hospitalier Pitié-Salpêtrière, Paris, France
[8]UMR 7211, Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Sorbonne Université, UPMC Université Paris 06, Paris, France
[9]Department of Internal Medicine, Caen University Hospital, Caen, France
[10]Department of Internal Medicine, Nantes University Hospital, Nantes, France
[11]Department of Internal Medicine, Grenoble University Hospital, Grenoble, France
[12]Department of Internal Medicine, Dijon University Hospital, Dijon, France
[13]Department of Internal Medicine, Saint-Etienne University Hospital, Saint-Priest-en-Jarez, France
[14]Department of Internal Medicine, Nice University Hospital, Nice, France
[15]Department of Internal Medicine, Montpellier University Hospital, Montpellier, France
[16]Department of Internal Medicine and Clinical Immunology, Saint-Antoine University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France
[17]Department of Rheumatology, Poitiers University Hospital, Poitiers, France
[18]Laboratoire Inflammation, Tissus Epithéliaux et Cytokines, Poitiers University, Poitiers, France
[19]Department of Rheumatology, Besançon University Hospital, Besançon, France
[20]Department of Rheumatology, Strasbourg University Hospital, Strasbourg, France
[21]Laboratoire d'ImmunoRhumatologie Moléculaire, INSERM UMR_S1109, Strasbourg University, Strasbourg, France
[22]Department of Clinical and Medical Pharmacology, Toulouse University Hospital, Toulouse, France

Correction notice This article has been corrected since it published Online First. Author Arsène Mékinian's name has been corrected.

Acknowledgements The authors thank the Société Nationale Française de Médecine Interne and the Club Rhumatisme et inflammation for having informed the physicians about this study. This work has been presented at the 2016 American College of Rheumatology meeting (11–16 November 2016, Washington, DC, USA).

Contributors GM, GP, ML-M and LS designed the study. All other authors included patients into the study. GM and AP conducted the statistical analyses. GM, GP, ML-M and LS interpreted the results and drafted the manuscript. All authors reviewed the manuscript and gave their approval for submission.

Funding This study was funded by Toulouse University Hospital.

Competing interests GM received a travel grant from Abbvie in 2013 and Amgen in 2017 and received research grants from Novartis, CSL Behring and the Institut Servier in 2016 and 2017. GP received travel support and lecture fees from Abbvie. DW received speaking fees and membership on the advisory boards of the following societies: AbbVie, BMS, MSD, Pfizer, Roche Chugai, Amgen, Nordic Pharma, UCB, SOBI, Sanofi Aventis, Novartis, Janssen, Celgene, Hospira, Lilly and Sandoz; he received grants/hospitality from Abbvie, Pfizer, Roche Chugai, MSD and UCB. BT received travel support from Roche and LFB, and received consulting fees from Roche, GSK, LFB and Grifols. PC received consulting and lecturing fees from Abbvie, Astra Zeneca, Bristol-Myers Squibb, Gilead, Glaxo Smith Kline, Janssen, Merck Sharp Dohme, Roche, Servier and Vifor.

Patient consent Not required.

Ethics approval This study received approval from the Toulouse University Ethics Committee in 2013, and according to French law from the Comité Consultatif du Traitement de l'Information et de la Recherche en Santé (n°13.251) in 2013 and then authorisation from the Commission Nationale de l'Informatique et des Libertés (n°DR-2013-378) in 2013.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Mathian A, Miyara M, Cohen-Aubart F, *et al*. Relapsing polychondritis: A 2016 update on clinical features, diagnostic tools, treatment and biological drug use. *Best Pract Res Clin Rheumatol* 2016;30:316–33.
2 Kent PD, Michet CJ, Luthra HS. Relapsing polychondritis. *Curr Opin Rheumatol* 2004;16:56–61.
3 Hazra N, Dregan A, Charlton J, *et al*. Incidence and mortality of relapsing polychondritis in the UK: a population-based cohort study. *Rheumatology* 2015;54:kev240–187.
4 Horváth A, Páll N, Molnár K, *et al*. A nationwide study of the epidemiology of relapsing polychondritis. *Clin Epidemiol* 2016;8:211–30.
5 Dion J, Costedoat-Chalumeau N, Sène D, *et al*. Relapsing Polychondritis Can Be Characterized by Three Different Clinical Phenotypes: Analysis of a Recent Series of 142 Patients. *Arthritis Rheumatol* 2016;68:2992–3001.
6 Arnaud L, Mathian A, Haroche J, *et al*. Pathogenesis of relapsing polychondritis: a 2013 update. *Autoimmun Rev* 2014;13:90–5.
7 Kemta Lekpa F, Kraus VB, Chevalier X. Biologics in relapsing polychondritis: a literature review. *Semin Arthritis Rheum* 2012;41:712–9.
8 Leroux G, Costedoat-Chalumeau N, Brihaye B, *et al*. Treatment of relapsing polychondritis with rituximab: a retrospective study of nine patients. *Arthritis Rheum* 2009;61:577–82.
9 Moulis G, Sailler L, Pugnet G, *et al*. Biologics in relapsing polychondritis: a case series. *Clin Exp Rheumatol* 2013;31:937–9.
10 Peng SL, Rodriguez D. Abatacept in relapsing polychondritis. *Ann Rheum Dis* 2013;72:1427–9.
11 Moulis G, Pugnet G, Sailler L, *et al*. Abatacept in relapsing polychondritis. *Ann Rheum Dis* 2013;72:e27.
12 McAdam LP, O'Hanlan MA, Bluestone R, *et al*. Relapsing polychondritis: prospective study of 23 patients and a review of the literature. *Medicine* 1976;55:193–215.
13 Michet CJ, McKenna CH, Luthra HS, *et al*. Relapsing polychondritis. Survival and predictive role of early disease manifestations. *Ann Intern Med* 1986;104:74–8.
14 Damiani JM, Levine HL. Relapsing polychondritis--report of ten cases. *Laryngoscope* 1979;89(6 Pt 1):929–46.
15 Charlson ME, Pompei P, Ales KL, *et al*. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;40:373–83.
16 Mekinian A, Dervin G, Lapidus N, *et al*. Biologics in myelodysplastic syndrome-related systemic inflammatory and autoimmune diseases: French multicenter retrospective study of 29 patients. *Autoimmun Rev* 2017;16:903–10.

EXTENDED REPORT

Autoantibodies and scleroderma phenotype define subgroups at high-risk and low-risk for cancer

Takeru Igusa,[1] Laura K Hummers,[2] Kala Visvanathan,[3] Carrie Richardson,[2] Fredrick M Wigley,[2] Livia Casciola-Rosen, Antony Rosen,[2] Ami A Shah[2]

Handling editor Josef S Smolen

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2018-212999).

[1]Departments of Civil Engineering and Applied Mathematics and Statistics, Johns Hopkins University, Baltimore, Maryland, USA
[2]Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[3]Departments of Epidemiology and Medical Oncology, Johns Hopkins Bloomberg School of Public Health and Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Correspondence to
Dr. Ami A Shah, Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD 21224, USA; Ami.Shah@jhmi.edu

Ar and AAS are joint senior authors
TI, LKH and KV are joint first authors

Received 11 January 2018
Revised 20 March 2018
Accepted 29 March 2018
Published Online First 20 April 2018

To cite: Igusa T, Hummers LK, Visvanathan K, *et al*. *Ann Rheum Dis* 2018;**77**:1180–1187.

ABSTRACT

Objectives Recent studies demonstrate autoantibodies are powerful tools to interrogate molecular events linking cancer and the development of autoimmunity in scleroderma. Investigating cancer risk in these biologically relevant subsets may provide an opportunity to develop personalised cancer screening guidelines. In this study, we examined cancer risk in distinct serologic and phenotypic scleroderma subsets and compared estimates with the general population.

Methods Patients in the Johns Hopkins Scleroderma Center observational cohort were studied. Overall and site-specific cancer incidence was calculated in distinct autoantibody and scleroderma phenotypic subsets, and compared with the Surveillance, Epidemiology and End Results registry, a representative sample of the US population.

Results 2383 patients with scleroderma contributing 37 686 person-years were studied. 205 patients (8.6%) had a diagnosis of cancer. Within 3 years of scleroderma onset, cancer risk was increased in patients with RNA polymerase III autoantibodies (antipol; standardised incidence ratio (SIR) 2.84, 95% CI 1.89 to 4.10) and those lacking centromere, topoisomerase-1 and pol antibodies (SIR 1.83, 95% CI 1.10 to 2.86). Among antipol-positive patients, cancer-specific risk may vary by scleroderma subtype; those with diffuse scleroderma had an increased breast cancer risk, whereas those with limited scleroderma had high lung cancer risk. In contrast, patients with anticentromere antibodies had a lower risk of cancer during follow-up (SIR 0.59, 95% CI 0.44 to 0.76).

Conclusions Autoantibody specificity and disease subtype are biologically meaningful filters that may inform cancer risk stratification in patients with scleroderma. Future research testing the value of targeted cancer screening strategies in patients with scleroderma is needed.

INTRODUCTION

Prior investigations have demonstrated an increase in cancer risk in patients with systemic sclerosis (scleroderma) compared with the general population.[1–11] In a study of patients with scleroderma and cancer, our group showed that patients with RNA polymerase III autoantibodies (antipol) had cancer occur within a short interval of scleroderma onset.[12] Subsequent studies demonstrated that these patients have genetic alterations (somatic mutations and/or loss of heterozygosity) at the *POLR3A* locus that encodes for RNA polymerase III in their cancers, with both mutation-specific and cross-reactive immune responses seen.[13] These data strongly suggest that alterations of autoantigen sequence in cancers may trigger antitumour immune responses that spread to the wild-type molecule, resulting in autoimmunity.[14]

Many international scleroderma cohorts have similarly observed that patients with scleroderma and antipol have a significantly increased risk of cancer at the time of scleroderma onset compared with scleroderma patients without these antibodies.[15–19] In addition, patients lacking antibodies against centromere, topoisomerase-1 and RNA polymerase III (hereafter referred to as 'CTP-negative') also have more cancer diagnosed within a short interval of scleroderma onset, suggesting there may be other serologic subsets of cancer-associated scleroderma.[17 20 21] Case reports suggest that therapy of coincident cancer may induce scleroderma remission,[22–24] raising the possibility that early cancer detection and therapy in patients with new-onset scleroderma might improve scleroderma outcomes.

Our prior work suggests that investigating cancer risk in scleroderma as a group, without differentiating between serologically relevant subsets or using the cancer-scleroderma interval as a filter, may mask important differences in the relationship between cancer and autoimmunity. In the current study, we examined overall and site-specific cancer risk at scleroderma onset in distinct serologic and phenotypic subsets and for the first time compared these estimates with the general population.

METHODS

Study population

Patients seen at the Johns Hopkins Scleroderma Center for their first visit between 1 January 2000 and 31 December 2015 were eligible for the study if they consented to participate in our IRB-approved cohort database and had a diagnosis of scleroderma. Scleroderma was defined by 1980 or 2013 American College of Rheumatology/European League Against Rheumatism classification criteria,[25 26] at least three of five CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome criteria or having definite Raynaud's, abnormal nailfold capillaries and a scleroderma-specific autoantibody. Clinical and serological data are collected prospectively at baseline and at 6 months interval. Patients were classified as having limited or diffuse scleroderma by established criteria.[27] Four autoantibody categories were assessed: anticentromere A/B (cenp),

antitopoisomerase-1 (topo), antipol and CTP-negative. Patients were considered positive for an autoantibody if they were ever positive based on clinically obtained assays. Double autoantibody positivity was infrequent: 14 patients were positive for both anticenp and antipol, 6 for anticenp and antitopo and 14 for antipol and antitopo. Patients who could not be classified into an autoantibody subset because of missing autoantibody data were only included in the overall scleroderma cohort analyses. For all analyses, the timing of scleroderma onset was defined by the first scleroderma symptom, either Raynaud's or non-Raynaud's. Patient-reported cancer diagnoses and dates of diagnosis, obtained at the enrolment visit and during follow-up, were confirmed by medical record review and pathology reports if available.[17] Electronic medical records were comprehensively reviewed to ensure that all cancer cases were captured during follow-up.

Examination of cancer risk in scleroderma compared with the general population

Cancer risk was determined by comparing cancer incidence in our cohort with the Surveillance, Epidemiology and End Results (SEER) registry, a nationally representative sample of the US population. Cancer incidence was examined in the overall scleroderma cohort, and in autoantibody and cutaneous subsets. We computed standardised incidence ratios (SIR) for cancer overall and individual cancer types. Our cancer subtype analyses focused on breast and lung cancers as these are the most prevalent cancers in scleroderma, but other cancer sites were also examined (see online supplementary appendix 1). The observed number of cancers in our cohort was compared with the expected number of cancer cases for the US population by identifying the crude rate of incident cancers corresponding to each patient's age (within 5-year intervals), gender, race, ethnicity and the calendar year of exposure in SEER.[28] Person time prior to 1973 was not examined as SEER data began in 1973. At the time of analysis, SEER data were complete through 2014. SEER crude rates for 2014 were used as a surrogate for person time after 2014. The sum of the crude rates for all years of exposure for all patients yielded the expected number of cancer cases. To find the 95% confidence limits, we followed standard procedure.[28 29]

Because we are interested in cancer diagnosed close to the time of scleroderma onset (defined as time zero) that may be suggestive of cancer-induced autoimmunity, we examined two time windows for our primary analyses: (i) 3 years before scleroderma onset until cancer diagnosis date or the last visit date (termed 'overall cancer risk' during follow-up) and (ii) 3 years before scleroderma onset until 3 years after scleroderma onset (±3 years, 'cancer-associated scleroderma'). Patients with cancers preceding these time windows were excluded from our analysis. Administrative censoring occurred at the cancer diagnosis date or last visit date, whichever came first. The study population for our primary analyses comprised 2383 patients with scleroderma.

Since including individuals with cancers diagnosed a few years before joining the cohort may introduce a form of immortal person time bias, we performed two additional analyses restricting our study population to patients who presented to our centre within 5 years of their first scleroderma symptom ('recent-onset scleroderma'). In the first analysis, we only included cancer diagnoses that occurred after the first visit to our centre. As referral to a tertiary centre is often delayed, we also performed an analysis involving patients with recent-onset scleroderma and examined cancer diagnoses after scleroderma symptom onset. This time point better reflects presentation to a community rheumatology practice.

Finally, we graphically examined cancer risk over time (starting 3 years before scleroderma onset) in patients with scleroderma compared with the general population. The expected cancer incidence was computed using SEER data for each patient-year of exposure. Observed and expected numbers of cancer cases,

Table 1 Risk for all cancers*

Analysis time	Antibody	Subtype	Sample size	Person-years	No. observed	No. expected	SIR (95% CI)	P value
Overall risk	All	Limited	1470	26 624	128	182.0	0.70 (0.59 to 0.84)	<0.001†
		Diffuse	913	11 062	77	69.0	1.12 (0.88 to 1.39)	0.36
	Cenp	Limited	570	11 857	53	90.0	0.59 (0.44 to 0.77)	<0.001†
		Diffuse	38	754	3	5.5	0.55 (0.11 to 1.60)	0.41
	Topo	Limited	241	4035	20	25.5	0.78 (0.48 to 1.21)	0.32
		Diffuse	240	3134	17	17.7	0.96 (0.56 to 1.53)	0.99
	Pol III	Limited	59	962	9	6.8	1.33 (0.61 to 2.53)	0.48
		Diffuse	219	2509	36	17.6	2.05 (1.44 to 2.84)	<0.001†
	CTP-negative	Limited	242	4065	31	25.5	1.21 (0.82 to 1.72)	0.33
		Diffuse	137	1709	8	10.6	0.75 (0.32 to 1.48)	0.53
±3 years	All	Limited	1470	7935	35	41.4	0.84 (0.59 to 1.18)	0.36
		Diffuse	913	5210	44	28.2	1.56 (1.13 to 2.10)	0.007
	Cenp	Limited	570	3003	10	16.7	0.60 (0.29 to 1.10)	0.111
		Diffuse	38	212	0	1.1	0.00 (0.00 to 3.34)	0.66
	Topo	Limited	241	1353	4	6.6	0.60 (0.16 to 1.54)	0.42
		Diffuse	240	1393	10	6.4	1.55 (0.75 to 2.86)	0.23
	Pol III	Limited	59	305	3	1.9	1.59 (0.33 to 4.66)	0.58
		Diffuse	219	1209	25	8.0	3.13 (2.03 to 4.62)	<0.001†
	CTP-negative	Limited	242	1335	15	6.2	2.43 (1.36 to 4.00)	0.004†
		Diffuse	137	808	4	4.2	0.95 (0.26 to 2.44)	0.99

*Excluding non-melanoma skin cancers.
†Statistically significant P value after adjustment for multiple (10) comparisons per analysis.
cenp, centromere; CTP, centromere, topoisomerase-1 and RNA polymerase III; pol, polymerase; topo, topoisomerase-1.

and the corresponding SIR, were plotted in 6-year time windows (ie, ±3-year increments with time zero denoting scleroderma onset). For each patient, cancer risk exposure ended on the date of cancer diagnosis, last visit or at the end of the 6-year window. The cumulative incidence of cancer was also plotted over time for patients with scleroderma overall and in each autoantibody subgroup.

Analyses were performed using MATLAB R2016b (MathWorks, Natick, Massachusetts, USA) and R V.3.4.0 (R Foundation, Vienna, Austria). Bonferroni adjustment for multiple (10) comparisons was performed, as each time window and tumour type had 10 autoantibody-subtype comparisons. Therefore, P≤0.05/10 or P≤0.005 was considered to be statistically significant.

RESULTS

The study population for our primary analyses consisted of 2383 patients with scleroderma contributing 37 686 person-years (table 1). The mean age at scleroderma onset was 42.4±15.1 years. Sixty per cent of patients had limited scleroderma, 83% were female and 76% self-identified as white race. Among the 1712 patients with autoantibody data, 608 (35.5%) were positive for anticenp, 481 (28.1%) for antitopo and 278 (16.2%) for antipol; 379 patients (22.1%) were CTP-negative. An additional 671 patients could not be classified into an antibody subset because of missing data. Approximately 9% of patients (205/2383) had a history of cancer (see online supplementary appendix figure 1 for tumour sites). Additional scleroderma characteristics of this population are detailed in online supplementary appendix table 1.

Determination of cancer risk relative to the general population: all cancers

Patients with diffuse scleroderma did not have an increased risk of cancer (SIR 1.12, 95% CI 0.88 to 1.39; table 1, *overall cancer risk*). In contrast, an increased risk of cancer was observed among antipol patients with diffuse disease (SIR 2.05, 95% CI 1.44 to 2.84). Patients with limited scleroderma had a 30% lower risk of cancer (SIR 0.70, 95% CI 0.59 to 0.84), and this was notable in anticenp-positive patients (SIR 0.59, 95% CI 0.44 to 0.77).

Next, we sought to determine the risk of cancer within 3 years of scleroderma onset ('cancer-associated scleroderma') compared with individuals in the general population. While

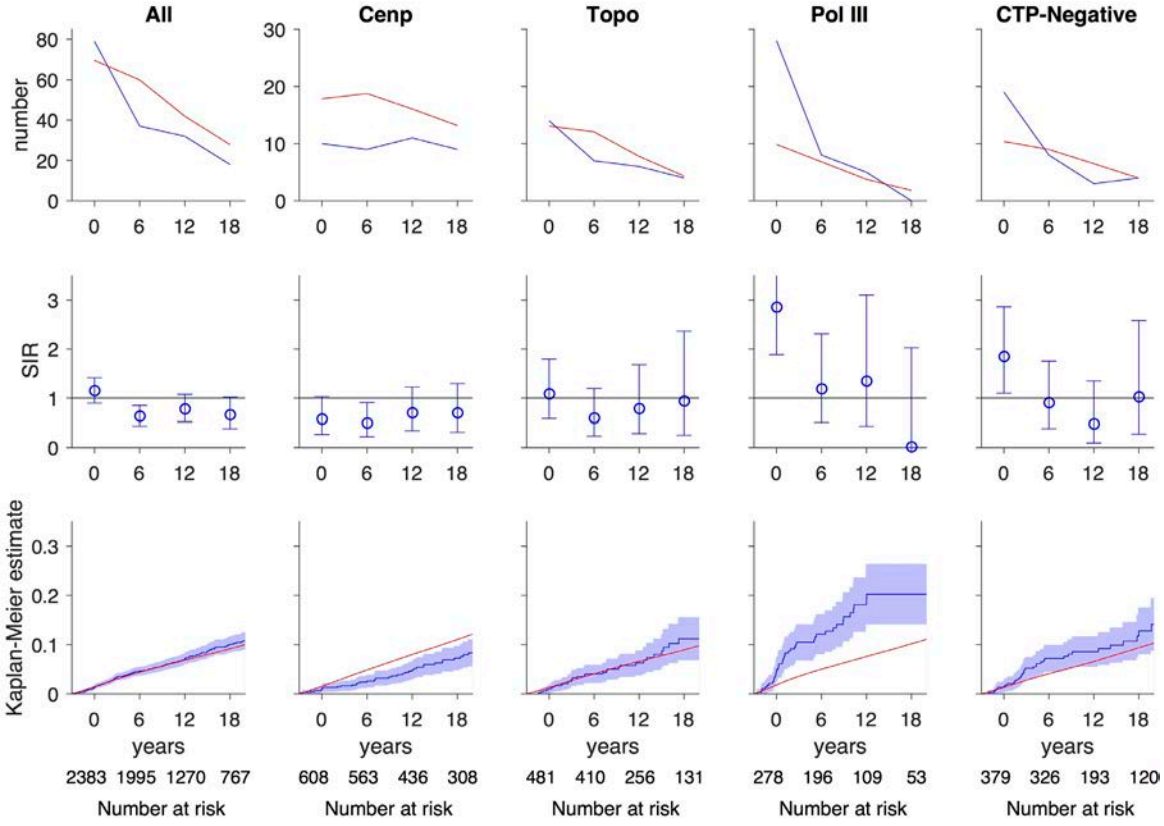


Figure 1 Risk of all cancers over time. In each graph, the x-axis reflects time from scleroderma onset (defined as time zero). *Top and middle rows*, each time window represents a 6-year period (±3 years), eg, data plotted at time zero reflects cancer risk within ±3 years of scleroderma onset. The number at risk for each time window is denoted at the bottom of the graph. *Top row*, the observed number of cancer cases (blue) is presented in comparison with the number of cancer cases that are expected based on Surveillance, Epidemiology and End Results (SEER) data (red). *Middle row*, the ratio between the observed and expected cancer cases is presented as a standardised incidence ratio (SIR) along with its 95% CI. Values of 1 denote a cancer risk equivalent to that of the background population. *Bottom row*, the cumulative incidence of cancer among patients with scleroderma (solid blue line) starting at 3 years before scleroderma onset is presented with 95% CIs (shaded blue region). Red lines represent the expected cumulative incidence of cancer based on SEER data for the general population. Patients with scleroderma with anticentromere (cenp) antibodies appear to have a decreased risk of cancer over time. Patients with scleroderma with RNA polymerase (pol) III antibodies and the centromere, topoisomerase-1 (topo) and RNA polymerase III (CTP)-negative group have an increased risk of cancer that is prominent at scleroderma onset. The cumulative incidence of cancer is significantly higher than that observed in the general population among patients with pol III autoantibodies.

Table 2 Risk for breast cancer

Analysis time	Antibody	Subtype	Person-years	No. observed	No. expected	SIR (95% CI)	P value
Overall risk	All	Limited	26 624	42	57.6	0.73 (0.53 to 0.99)	0.039
		Diffuse	11 062	28	20.3	1.38 (0.91 to 1.99)	0.123
	Cenp	Limited	11 857	18	29.9	0.60 (0.36 to 0.95)	0.027
		Diffuse	754	3	1.6	1.84 (0.38 to 5.39)	0.45
	Topo	Limited	4035	8	8.2	0.97 (0.42 to 1.92)	0.99
		Diffuse	3134	5	5.3	0.95 (0.31 to 2.21)	0.99
	Pol III	Limited	962	1	1.9	0.52 (0.01 to 2.91)	0.86
		Diffuse	2509	16	5.2	3.06 (1.75 to 4.98)	<0.001*
	CTP-negative	Limited	4065	12	7.2	1.66 (0.86 to 2.89)	0.130
		Diffuse	1709	1	3	0.33 (0.01 to 1.83)	0.39
±3 years	All	Limited	7935	15	12.8	1.17 (0.66 to 1.94)	0.60
		Diffuse	5210	17	8.3	2.06 (1.20 to 3.29)	0.010
	Cenp	Limited	3003	4	5.4	0.75 (0.20 to 1.91)	0.76
		Diffuse	212	0	0.4	0.00 (0.00 to 9.33)	0.99
	Topo	Limited	1353	1	2.1	0.48 (0.01 to 2.69)	0.78
		Diffuse	1393	3	2	1.51 (0.31 to 4.41)	0.64
	Pol III	Limited	305	0	0.6	0.00 (0.00 to 6.66)	0.99
		Diffuse	1209	12	2.3	5.14 (2.66 to 8.98)	<0.001*
	CTP-negative	Limited	1335	8	1.8	4.44 (1.92 to 8.74)	0.001*
		Diffuse	808	1	1.1	0.87 (0.02 to 4.86)	0.99

*Statistically significant P value after adjustment for multiple (10) comparisons per analysis.
cenp, centromere; CTP, centromere, topoisomerase-1 and RNA polymerase III; pol, polymerase; topo, topoisomerase-1.

patients with limited scleroderma did not have an increased risk of cancer-associated scleroderma (table 1, ±3 years), patients with diffuse scleroderma had a 56% increased risk compared with the general population (SIR 1.56, 95% CI 1.13 to 2.10). This risk increase was notable among antipol patients with diffuse disease (SIR 3.13, 95% CI 2.03 to 4.62). Additionally, CTP-negative patients with limited scleroderma had an increased risk of cancer-associated scleroderma (SIR 2.43, 95% CI 1.36 to 4.00).

The increased risk of cancer at scleroderma onset among antipol-positive and CTP-negative patients is illustrated in figure 1. The number of cancer cases observed around the time of scleroderma onset (top row, blue curve) is greater than the number of expected cancer cases based on SEER data (red curve) in these two autoantibody subsets. The relative risk of cancer compared with the general population is presented in time-dependent SIRs (middle row) and was increased for antipol-positive and CTP-negative groups close to scleroderma onset. The cumulative incidence of cancer was significantly higher among antipol patients (blue lines, blue dashed lines 95% CI) compared with that expected in the general population (red line) (bottom row, figure 1). In contrast, the cumulative incidence of cancer was lower than expected in the anticenp group.

Cancer risk in patients with recent-onset scleroderma

We performed two additional analyses restricting our study population to patients who presented to our scleroderma centre within 5 years of their first scleroderma symptom and examined cancer diagnoses (i) after first visit to our tertiary referral centre or (ii) after the first scleroderma symptom. Our findings of an increased risk of cancer among antipol-positive patients with diffuse scleroderma remained unchanged in both analyses, although in these restricted analyses this was statistically significant only after first symptom when adjusting for multiple comparisons (see online supplementary appendix table 2).

Breast cancer

Antipol-positive patients with diffuse scleroderma had an increased risk of breast cancer overall (SIR 3.06, 95% CI 1.75 to 4.98) and within 3 years of scleroderma onset (SIR 5.14, 95% CI 2.66 to 8.98; table 2). Within 3 years of scleroderma onset, CTP-negative patients with limited disease also have an increased risk of breast cancer (SIR 4.44, 95% CI 1.92 to 8.74). The marked increased risk of breast cancer at scleroderma onset in these two autoantibody subsets is illustrated in figure 2 (top and middle rows). The cumulative incidence of breast cancer is significantly higher among antipol patients compared with the general population (bottom row, figure 2).

Lung cancer

The number of lung cancer cases was small (n=30 overall). However, in an exploratory analysis, an increased risk of lung cancer was seen in antipol patients with limited disease within 3 years of scleroderma onset (SIR 10.4, 95% CI 1.26 to 37.7; table 3; figure 3).

CONCLUSIONS

In this investigation, we used autoantibodies, cutaneous subtype and temporal clustering as biologically relevant filters to investigate cancer risk and type in patients with scleroderma compared with the general population. We made several novel findings that, if confirmed by others, will inform our approach to early cancer detection in scleroderma, and also provide additional insights into mechanistic connections between cancer and scleroderma. First, while patients with scleroderma did not have an increased overall risk of cancer compared with the general population, antipol-positive patients with diffuse scleroderma and CTP-negative patients with limited scleroderma are at increased risk for cancer at scleroderma onset. Second, scleroderma patients with antipol antibodies may have increased risk of different types of cancers depending on whether they have limited or diffuse

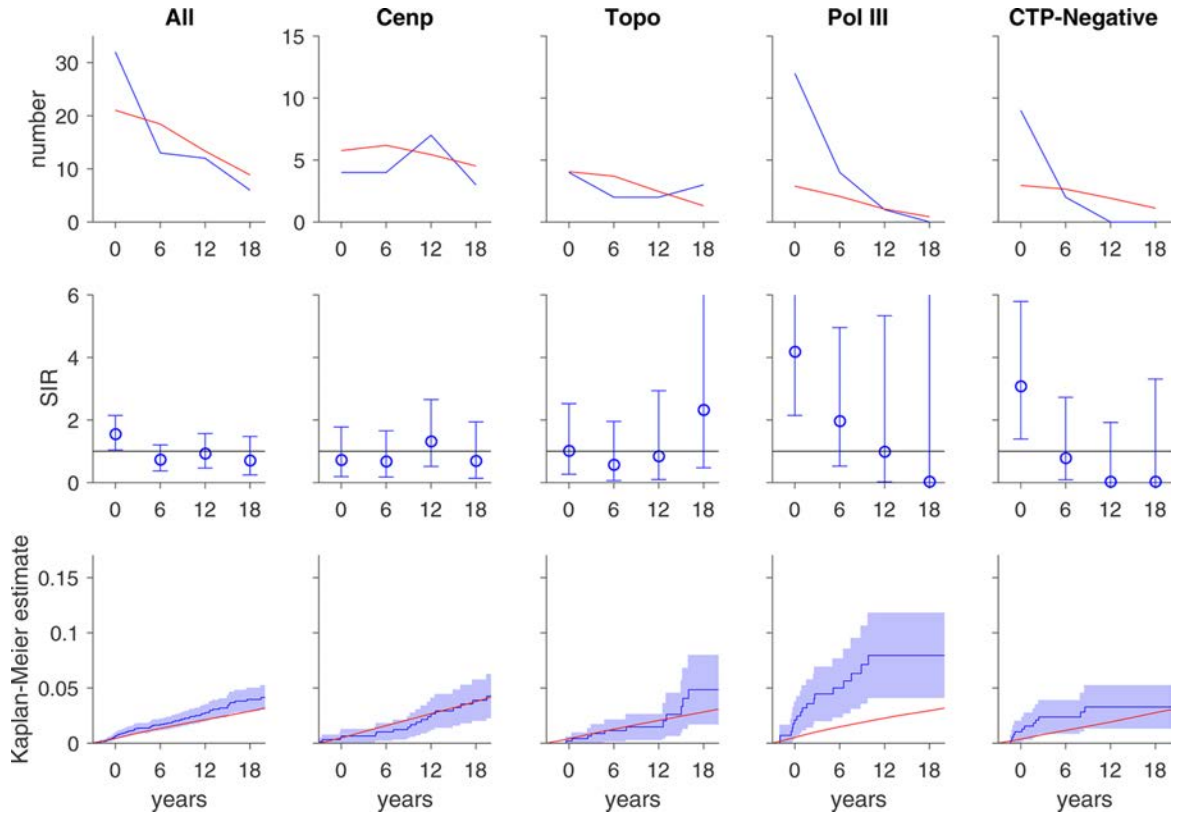


Figure 2 Risk of breast cancers over time. In each graph, the x-axis reflects time from scleroderma onset (defined as time zero). *Top and middle rows*, each time window represents a 6-year period (±3 years), eg, data plotted at time zero reflects breast cancer risk within ±3 years of scleroderma onset. *Top row*, the observed number of breast cancer cases (blue) is presented in comparison with the number of breast cancer cases that are expected based on Surveillance, Epidemiology and End Results (SEER) data (red). *Middle row*, the ratio between the observed and expected breast cancer cases is presented as a standardised incidence ratio (SIR) along with its 95% CI. Values of 1 denote a breast cancer risk equivalent to that of the background population. *Bottom row*, the cumulative incidence of breast cancer among patients with scleroderma (solid blue line) starting at 3 years before scleroderma onset is presented with 95% CIs (shaded blue region). Red lines represent the expected cumulative incidence of breast cancer based on SEER data for the general population. Patients with topoisomerase (topo) and centromere (cenp) antibodies do not have an increased risk of breast cancer. Patients with scleroderma with polymerase (pol) III antibodies and the centromere , topoisomerase-1 and RNA polymerase III (CTP)-negative group have an increased risk of breast cancer that is prominent at scleroderma onset. The cumulative incidence of breast cancer is significantly higher than that observed in the general population among patients with pol III autoantibodies.

cutaneous disease. Third, patients with anticenp antibodies may have a decreased risk of cancer. Overall, these data suggest that autoantibodies could be useful tools for cancer risk stratification to maximise detection of cancer through enhanced screening of high-risk groups, while minimising the harms and costs from overscreening.

Prior studies investigating cancer incidence in patients with scleroderma relative to the general population have used study populations from national centralised registries or cohorts similar to ours.[1–6 8–10 30] However, most of these studies excluded patients with cancer diagnoses shortly before scleroderma onset, and many lacked data on autoantibody status or phenotypic subtype. In contrast, we studied cancer risk within (±) 3 years of scleroderma onset, and investigated whether this varied by autoantibody specificity, cutaneous subtype and cancer type. We included the time before scleroderma diagnosis because our recent data demonstrated that POLR3A is genetically altered in short-interval cancers associated with an immune response to that protein, where both mutation-specific and cross-reactive immune responses were seen.[13] These data strongly support a biological model in which cancer precedes scleroderma, and initiates a scleroderma immune response and clinical disease.[13 14] The fact that 27% of patients with scleroderma with antipol antibodies and cancer have cancer shortly preceding scleroderma onset highlights the frequency of this subgroup, and the importance to include them. Our current study demonstrates that the risk of cancer around the time of scleroderma onset in anti-pol-positive patients is manyfold higher than that expected in the general population, supporting the idea that these patients may require more aggressive cancer screening at disease onset.

Interestingly, our data suggest that cancer risk may differ among antipol patients depending on their cutaneous subtype, as those with diffuse scleroderma had a higher risk of breast cancer and those with limited scleroderma may have an increased risk of lung cancer. These findings, particularly for lung cancer, require validation in other scleroderma cohorts given the small numbers of lung cancer cases in each autoantibody-subtype stratum. While prior studies have identified an increased risk of breast cancer concomitant with scleroderma onset in antipol-positive patients, these studies included patients with scleroderma without antipol as comparator groups, limiting the ability to determine excess risk compared with the general population.[15 31] Our data suggest that enhanced breast cancer screening, incorporating sensitive measures such as MRI, may be warranted in antipol-positive women with diffuse scleroderma, but this needs further evaluation. Our exploratory analyses, if confirmed in other cohorts, suggest that antipol-positive patients may also require increased

Table 3 Risk for lung cancer

Analysis time	Antibody	Subtype	Person-years	No. observed	No. expected	SIR (95% CI)	P value
Overall risk	All	Limited	26 624	24	18.9	1.27 (0.81 to 1.89)	0.29
		Diffuse	11 062	6	6.6	0.91 (0.34 to 1.99)	0.99
	Cenp	Limited	11 857	8	9.6	0.83 (0.36 to 1.63)	0.75
		Diffuse	754	0	0.6	0.00 (0.00 to 6.27)	0.99
	Topo	Limited	4035	6	2.5	2.40 (0.88 to 5.23)	0.084
		Diffuse	3134	2	1.7	1.19 (0.14 to 4.31)	0.99
	Pol III	Limited	962	3	0.7	4.31 (0.89 to 12.61)	0.067
		Diffuse	2509	2	1.6	1.28 (0.15 to 4.62)	0.93
	CTP-negative	Limited	4065	2	2.5	0.81 (0.10 to 2.91)	0.99
		Diffuse	1709	0	0.9	0.00 (0.00 to 3.91)	0.78
±3 years	All	Limited	7935	5	3.9	1.27 (0.41 to 2.96)	0.72
		Diffuse	5210	2	2.7	0.75 (0.09 to 2.73)	0.99
	Cenp	Limited	3003	0	1.6	0.00 (0.00 to 2.28)	0.40
		Diffuse	212	0	0.1	0.00 (0.00 to 35.12)	0.99
	Topo	Limited	1353	2	0.6	3.42 (0.41 to 12.34)	0.23
		Diffuse	1393	1	0.6	1.69 (0.04 to 9.41)	0.89
	Pol III	Limited	305	2	0.2	10.43 (1.26 to 37.67)	0.032
		Diffuse	1209	2	0.7	2.80 (0.34 to 10.11)	0.32
	CTP-negative	Limited	1335	0	0.5	0.00 (0.00 to 6.88)	0.99
		Diffuse	808	0	0.4	0.00 (0.00 to 10.48)	0.99

*Statistically significant P value after adjustment for multiple (10) comparisons per analysis.
cenp, centromere; CTP, centromere, topoisomerase-1 and RNA polymerase III; pol, polymerase; topo, topoisomerase-1.

vigilance in monitoring for lung, tongue and prostate malignancies (see online supplementary appendix 1).

Our prior work demonstrated that CTP-negative patients may also be at risk of cancer-associated scleroderma.[17 20 21] In this study, CTP-negative patients with limited scleroderma had an increased risk of breast cancer and melanoma (see online supplementary appendix 1) at scleroderma onset, suggesting that vigilance for breast cancer and comprehensive skin examination is most important. Of note, the CTP-negative group is likely heterogeneous, with several novel unrecognised immune responses.[17 20 21] Identifying distinct autoantibodies in this subgroup (eg, anti-RNPC3)[20 21] associated with an increased risk of cancer could facilitate development of a cancer risk prediction model in scleroderma.

Our study showed for the first time that patients with scleroderma with anticenp antibodies may have a substantially decreased risk of cancer compared with the general population. This unexpected finding possibly explains the different cancer risks observed in scleroderma cohorts internationally because the ratios of anticenp-positive to antipol-positive patients in cohorts dramatically impact the blended cancer risk. The finding that distinct serologic subgroups have different cancer risks suggests that cancer immunity may be a common principle across the scleroderma spectrum, with cancer emergence influenced by the different immune responses such that for anticenp, cancer emergence may be inhibited, while inhibition is only partial for antipol. Prior studies in small cohorts of patients with breast cancer have demonstrated that anticenp antibodies may be present and associate with improved disease-free and overall survival.[32–34] Intriguing recent data also suggest that anti-DNA antibodies can have direct anticancer effects in cells with DNA repair defects,[35] possibly explaining the decreased risk of breast and other cancers among patients with systemic lupus erythematosus.[36] While it is possible that anticenp immune responses exert a similar anticancer effect in scleroderma, other possibilities exist, and mechanistic studies are needed.

This was a prospective study using a large, well-defined scleroderma cohort to investigate whether scleroderma-specific immune responses and clinical phenotypes associate with a higher risk of certain cancer types. These findings require validation in other scleroderma cohorts given the observational study design and smaller sample sizes in each subgroup as patients are divided into finer classification schemes. Our primary analyses focused on cancers that were detected up to 3 years before the clinical onset of scleroderma, as we were interested in cancer-induced autoimmunity. We recognise that including person time prior to the first visit to our centre raises concerns about immortal person time biases due to mortality from cancer diagnosis prior to presentation. To address this, we performed sensitivity analyses only including patients with recent onset scleroderma and examined cancer diagnoses after first visit to our centre. Our primary findings for antipol were similar. Our findings in the other autoantibody subsets were attenuated, likely due to decreased statistical power. Several patients were missing sufficient autoantibody data to be classified into a serologic subset; on average, these patients presented for their first visit 3 years before those who could be classified into an autoantibody category, suggesting a period effect due to limited availability of certain commercial autoantibody assays in earlier years. These differences may affect the generalisability of our findings. We do not think surveillance bias plays a major role in our findings, as historically all clinical cancer screening in our centre has been based on age and gender and was not influenced by scleroderma diagnosis or features. However, we recognise that incidental malignancies may be detected during testing performed for scleroderma; conversely, patients with early cancer or scleroderma may face a competing risk of death from either process before diagnosis of the other disease, resulting in an underestimation of cancer cases at the time of scleroderma onset. Stratified analyses suggested that smoking and interstitial lung disease were effect modifiers for lung cancer risk (data not shown). Unfortunately, smoking information was unavailable in

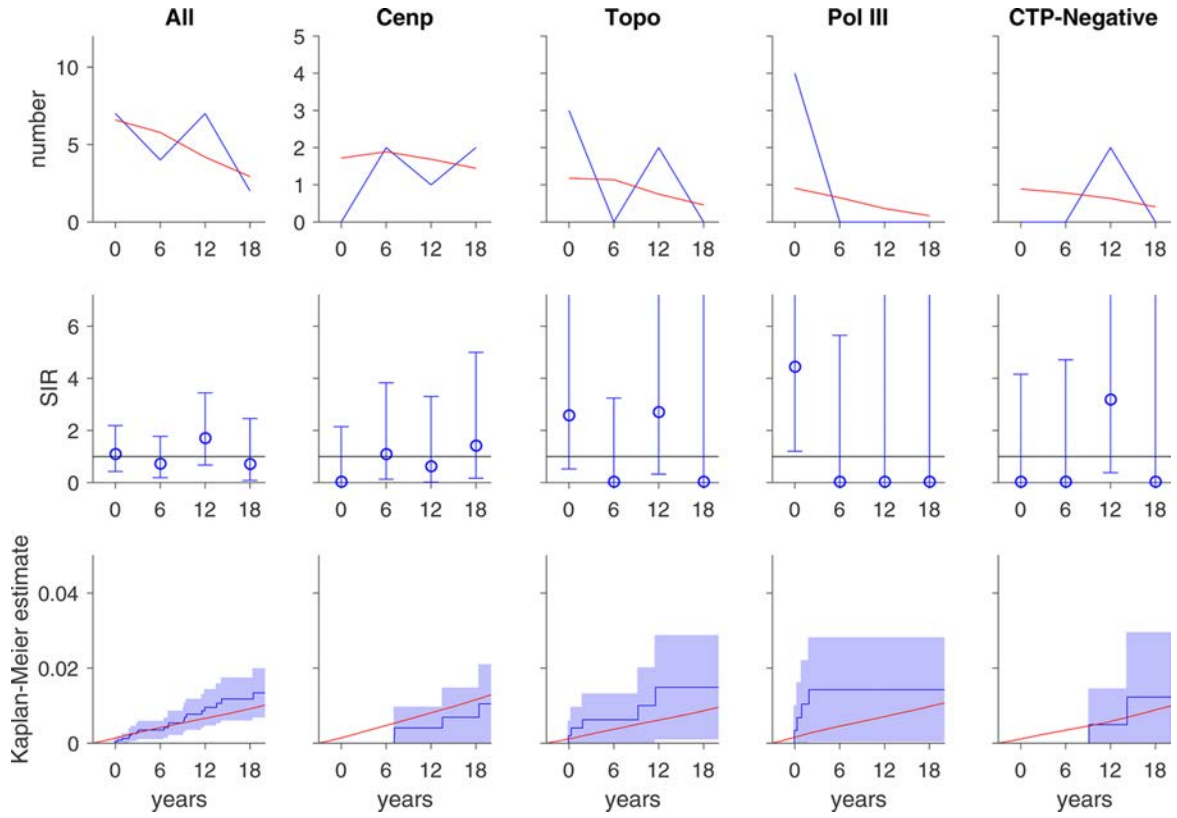


Figure 3 Risk of lung cancers over time. In each graph, the x-axis reflects time from scleroderma onset (defined as time zero). *Top and middle rows*, each time window represents a 6-year period (±3 years), eg, data plotted at time zero reflects lung cancer risk within ±3 years of scleroderma onset. *Top row*, the observed number of lung cancer cases (blue) is presented in comparison with the number of lung cancer cases that are expected based on Surveillance, Epidemiology and End Results (SEER) data (red). *Middle row*, the ratio between the observed and expected lung cancer cases is presented as a standardised incidence ratio (SIR) along with its 95% CI. Values of 1 denote a lung cancer risk equivalent to that of the background population. *Bottom row*, the cumulative incidence of lung cancer among patients with scleroderma (solid blue line) starting at 3 years before scleroderma onset is presented with 95% CIs (shaded blue region). Red lines represent the expected cumulative incidence of lung cancer based on SEER data for the general population. Patients with scleroderma with RNA polymerase (pol) III antibodies may have an increased risk of lung cancer at the time of scleroderma onset. cenp, centromere; CTP, centromere, topoisomerase-1 and RNA polymerase III; topo, topoisomerase-1.

the SEER registry, limiting our ability to fully adjust for this risk factor. Lastly, although our prior biologic studies suggest that certain subsets of patients with scleroderma may have cancer-induced autoimmunity, we acknowledge that these data do not prove causality. The relationship between cancer and autoimmunity in scleroderma is likely complex and bidirectional, with many potential links between the two diseases including immunosuppressive therapies or damage from the disease triggering malignancy, or a shared genetic or environmental exposure.

These data suggest that segregation by clinical features and autoantibody response identify scleroderma subgroups with distinct risks of both overall cancer, and specific types of cancer. Application of these simple filters may be useful in designing studies that define guidelines for cancer detection in patients with scleroderma. Investigating the mechanistic basis for differences in cancer risk across scleroderma subgroups is likely to enhance our understanding of scleroderma, autoimmunity and cancer immunity.

Acknowledgements The authors would like to thank Adrianne Woods and Margaret Sampedro for their excellent support in database management and quality control.

Funding This study was supported by the NIH (K23-AR061439, P30-AR053503, P30-AR070254, R01- DE12354-15A1, T32-AR048522), the Donald B and Dorothy L Stabler Foundation, the Jerome L Greene Foundation, the Chresanthe Stauralakis Memorial Discovery Fund, the Martha McCrory Professorship and the Scleroderma Research Foundation.

Competing interests TI, LC-R, AR and AAS have recently submitted a patent application entitled 'Materials and Methods for Assessing Cancer Risk and Treating Cancer'.

Patient consent Not required.

Ethics approval Johns Hopkins Medical Institutions IRB.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There are no additional unpublished data from this study at this time.

REFERENCES

1 Abu-Shakra M, Guillemin F, Lee P. Cancer in systemic sclerosis. *Arthritis Rheum* 1993;36:460–4.
2 Derk CT, Rasheed M, Artlett CM, *et al*. A cohort study of cancer incidence in systemic sclerosis. *J Rheumatol* 2006;33:1113–6.
3 Hill CL, Nguyen AM, Roder D, *et al*. Risk of cancer in patients with scleroderma: a population based cohort study. *Ann Rheum Dis* 2003;62:728–31.
4 Kang KY, Yim HW, Kim IJ, *et al*. Incidence of cancer among patients with systemic sclerosis in Korea: results from a single centre. *Scand J Rheumatol* 2009;38:299–303.
5 Kuo CF, Luo SF, Yu KH, *et al*. Cancer risk among patients with systemic sclerosis: a nationwide population study in Taiwan. *Scand J Rheumatol* 2012;41:44–9.
6 Olesen AB, Svaerke C, Farkas DK, *et al*. Systemic sclerosis and the risk of cancer: a nationwide population-based cohort study. *Br J Dermatol* 2010;163:800–6.
7 Onishi A, Sugiyama D, Kumagai S, *et al*. Cancer incidence in systemic sclerosis: meta-analysis of population-based cohort studies. *Arthritis Rheum* 2013;65:1913–21.

8 Rosenthal AK, McLaughlin JK, Gridley G, *et al*. Incidence of cancer among patients with systemic sclerosis. *Cancer* 1995;76:910–4.
9 Rosenthal AK, McLaughlin JK, Linet MS, *et al*. Scleroderma and malignancy: an epidemiological study. *Ann Rheum Dis* 1993;52:531–3.
10 Siau K, Laversuch CJ, Creamer P, *et al*. Malignancy in scleroderma patients from south west England: a population-based cohort study. *Rheumatol Int* 2011;31:641–5.
11 Bonifazi M, Tramacere I, Pomponio G, *et al*. Systemic sclerosis (scleroderma) and cancer risk: systematic review and meta-analysis of observational studies. *Rheumatology* 2013;52:143–54.
12 Shah AA, Rosen A, Hummers L, *et al*. Close temporal relationship between onset of cancer and scleroderma in patients with RNA polymerase I/III antibodies. *Arthritis Rheum* 2010;62:2787–95.
13 Joseph CG, Darrah E, Shah AA, *et al*. Association of the autoimmune disease scleroderma with an immunologic response to cancer. *Science* 2014;343:152–7.
14 Shah AA, Casciola-Rosen L, Rosen A. Review: cancer-induced autoimmunity in the rheumatic diseases. *Arthritis Rheumatol* 2015;67:317–26.
15 Moinzadeh P, Fonseca C, Hellmich M, *et al*. Association of anti-RNA polymerase III autoantibodies and cancer in scleroderma. *Arthritis Res Ther* 2014;16:R53.
16 Nikpour M, Hissaria P, Byron J, *et al*. Prevalence, correlates and clinical usefulness of antibodies to RNA polymerase III in systemic sclerosis: a cross-sectional analysis of data from an Australian cohort. *Arthritis Res Ther* 2011;13:R211.
17 Shah AA, Hummers LK, Casciola-Rosen L, *et al*. Examination of autoantibody status and clinical features associated with cancer risk and cancer-associated scleroderma. *Arthritis Rheumatol* 2015;67:1053–61.
18 Airo' P, Ceribelli A, Cavazzana I, *et al*. Malignancies in Italian patients with systemic sclerosis positive for anti-RNA polymerase III antibodies. *J Rheumatol* 2011;38:1329–34.
19 Saigusa R, Asano Y, Nakamura K, *et al*. Association of anti-RNA polymerase III antibody and malignancy in Japanese patients with systemic sclerosis. *J Dermatol* 2015;42:524–7.
20 Shah AA, Xu G, Rosen A, *et al*. Brief report: Anti-RNPC-3 antibodies as a marker of cancer-associated scleroderma. *Arthritis Rheumatol* 2017;69:1306–12.
21 Xu GJ, Shah AA, Li MZ, *et al*. Systematic autoantigen analysis identifies a distinct subtype of scleroderma with coincident cancer. *Proc Natl Acad Sci U S A* 2016;113:E7526–E34.
22 Hasegawa M, Sato S, Sakai H, *et al*. Systemic sclerosis revealing T-cell lymphoma. *Dermatology* 1999;198:75–8.
23 Juarez M, Marshall R, Denton C, *et al*. Paraneoplastic scleroderma secondary to hairy cell leukaemia successfully treated with cladribine. *Rheumatology* 2008;47:1734–5.
24 Bruni C, Lages A, Patel H, *et al*. Resolution of paraneoplastic PM/Scl-positive systemic sclerosis after curative resection of a pancreatic tumour. *Rheumatology* 2017;56:317–8.
25 Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980;23:581–90.
26 van den Hoogen F, Khanna D, Fransen J, *et al*. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013;65:2737–47.
27 LeRoy EC, Black C, Fleischmajer R, *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;15:202–5.
28 Surveillance, Epidemiology, and End Results (SEER) Program. SEER*Stat Database: Incidence - SEER 9 Regs Research Data, Nov 2016 Sub (1973- 2014) <Katrina/Rita Population Adjustment> - Linked To County Attributes - Total U.S., 1969-2015 Counties, National Cancer Institute, DCCPS, Surveillance Research Program, released April 2017, based on the November 2016 submission. www.seer.cancer.gov
29 Liddell FD. Simple exact analysis of the standardised mortality ratio. *J Epidemiol Community Health* 1984;38:85–8.
30 Chatterjee S, Dombi GW, Severson RK, *et al*. Risk of malignancy in scleroderma: a population-based cohort study. *Arthritis Rheum* 2005;52:2415–24.
31 Lazzaroni MG, Cavazzana I, Colombo E, *et al*. Malignancies in patients with Anti-RNA Polymerase III antibodies and systemic sclerosis: analysis of the EULAR scleroderma trials and research cohort and possible recommendations for screening. *J Rheumatol* 2017;44:639–47.
32 Atalay C, Atalay G, Yilmaz KB, *et al*. The role of anti-CENP-B and anti-SS-B antibodies in breast cancer. *Neoplasma* 2005;52:32–5.
33 Atalay C, Dogan L, Atalay G. Anti-CENP-B antibodies are associated with prolonged survival in breast cancer. *Future Oncol* 2010;6:471–7.
34 Fritzler MJ, Rattner JB, Luft LM, *et al*. Historical perspectives on the discovery and elucidation of autoantibodies to centromere proteins (CENP) and the emerging importance of antibodies to CENP-F. *Autoimmun Rev* 2011;10:194–200.
35 Hansen JE, Chan G, Liu Y, *et al*. Targeting cancer with a lupus autoantibody. *Sci Transl Med* 2012;4:157ra142.
36 Bernatsky S, Ramsey-Goldman R, Labrecque J, *et al*. Cancer risk in systemic lupus: an updated international multi-centre cohort study. *J Autoimmun* 2013;42:130–5.

EXTENDED REPORT

Racial/ethnic variation and risk factors for allopurinol-associated severe cutaneous adverse reactions: a cohort study

Sarah F Keller,[1] Na Lu,[1] Kimberly G Blumenthal,[1] Sharan K Rai,[1] Chio Yokose,[1] Jee Woong J Choi,[1] Seoyoung C Kim,[2,3] Yuqing Zhang,[1] Hyon K Choi[1]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212905).

[1]Division of Rheumatology, Allergy and Immunology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts, USA
[2]Division of Pharmacoepidemiology and Pharmacoeconomics, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts, USA
[3]Division of Rheumatology, Immunology and Allergy, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts, USA

Correspondence to
Dr Hyon K Choi, Division of Rheumatology, Allergy, and Immunology Department of Medicine, Massachusetts General Hospital, Boston MA 02114, USA;
hchoi@partners.org

SFK and NL contributed equally.

Received 26 December 2017
Revised 23 March 2018
Accepted 26 March 2018
Published Online First 13 April 2018

ABSTRACT

Objectives To examine associations of race/ethnicity and purported risk factors with hospitalised allopurinol-associated severe cutaneous adverse reactions (AASCARs).

Methods We used US Medicaid data to identify incident allopurinol users between 1999 and 2012. We examined the risk of hospitalised AASCARs according to race/ethnicity and purported key risk factors and calculated relative risks (RR).

Results Among 400 401 allopurinol initiators, we documented 203 hospitalised AASCAR cases (1 in 1972 initiators). The average AASCAR hospitalisation was 9.6 days and 43 individuals (21%) died. The multivariable-adjusted RRs for AASCARs among blacks, Asians and Native Hawaiians/Pacific Islanders compared with whites or Hispanics were 3.00 (95% CI 2.18 to 4.14), 3.03 (95% CI 1.72 to 5.34) and 6.68 (95% CI 4.37 to 10.22), respectively. Female sex, older age (≥60 years), chronic kidney disease and initial allopurinol dose (>100 mg/day) were independently associated with a 2.5-fold, 1.7-fold, 2.3-fold and 1.9-fold higher risk of AASCAR, respectively. In our combined demographic analysis, older women (≥60 years) of a high-risk race/ethnicity (blacks, Asians or Native Hawaiians/Pacific Islanders) had over a 12-fold higher risk of hospitalised AASCARs than younger men of a low-risk race/ethnicity (whites or Hispanics) (multivariable-adjusted RR, 12.25; 95% CI 6.46 to 23.25).

Conclusions This racially diverse (yet mostly white) cohort study indicates that the risk of hospitalised AASCAR is rare overall, although blacks, Asians and Native Hawaiians/Pacific-Islanders have a substantially higher risk of hospitalised AASCARs, particularly among older women. These data also support the practice of initiating allopurinol at a low dose (eg, ≤100 mg/day).

INTRODUCTION

Allopurinol is the predominant, first-line urate-lowering drug (ULD) worldwide for the treatment of gout.[1–4] Although allopurinol is generally well tolerated, an uncommon yet feared adverse reaction is severe allopurinol hypersensitivity syndrome (AHS), manifesting as severe cutaneous adverse reactions such as Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN). Severe cutaneous adverse reactions due to AHS frequently involve major organs, with long-term sequelae such as corneal damage and renal insufficiency, and can be fatal in up to 32% of cases.[5 6] A previous study found allopurinol to be the most common cause of both SJS and TEN in Europe and Israel.[7]

Understanding the risk factors for allopurinol-associated severe cutaneous adverse rea (AASCARs) is essential to mitigate the risk of this severe adverse drug reaction; however, relevant data are scarce. Beyond the established risk of AASCARs associated with chronic kidney disease (CKD),[8 9] the *HLA-B*5801* allele has also been found to portend a substantially higher risk of severe cutaneous adverse reactions.[10–16] A meta-analysis reported that the risk of developing severe cutaneous adverse reactions was 97 times higher among allopurinol users with the *HLA-B*5801* allele compared with those without the allele.[17] Furthermore, the allele frequency of *HLA-B*5801* varies substantially among different races/ethnicities. For example, the allele frequency in the USA has been estimated to be 7.4%, 4%, 1%, and 1% among Asians, blacks, whites and Hispanics, respectively.[18] These varying frequencies of *HLA-B*5801* could lead to substantial variations in the risk of AASCARs across different races and ethnicities; however, relevant data are scarce. Furthermore, female sex, older age, asymptomatic hyperuricaemia and diuretic use have been purported to increase the risk of AASCARs.[5 8 9 19] Such information, together with knowledge of other independent risk factors, can help identify high-risk patients to target with potential preventive measures. For example, a recent prospective study showed that screening for the *HLA-B*5801* allele in Taiwanese patients (who have an *HLA-B*5801* carriage rate of 20%), coupled with the use of an alternative ULD for those deemed to be carriers, substantially reduced the incidence of AASCARs.[20]

We examined the risk of AASCARs according to race/ethnicity and other candidate risk factors[5 8 9 19] in a large, racially diverse population and evaluated their potential independent associations.

METHODS

Source population

Our source population was the Medicaid Analytic eXtract database, an administrative data system containing all billing claims for Medicaid enrollees in 47 US states and the District of Columbia from 1 January 1999 to 31 December 2012. The database contains clinical, demographic and death status information for beneficiaries as well as Medicaid claims for covered healthcare services including pharmacy benefits

To cite: Keller SF, Lu N, Blumenthal KG, *et al*. *Ann Rheum Dis* 2018;**77**:1188–1194.

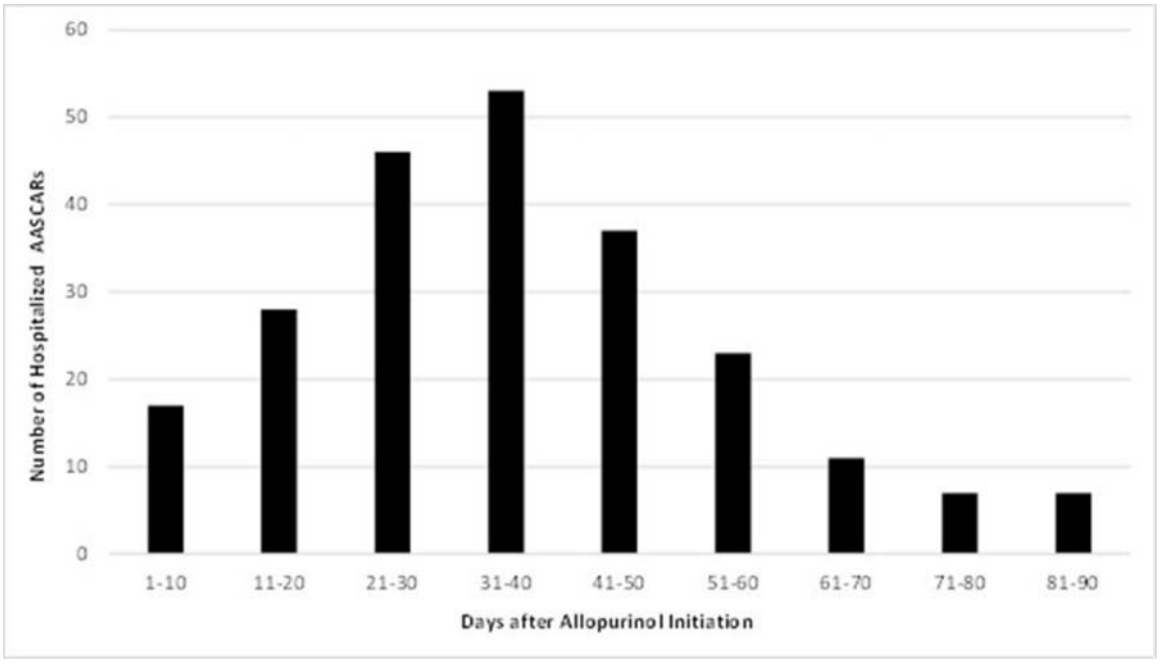


Figure 1 Frequency of hospitalised allopurinol-associated severe cutaneous adverse reactions (AASCARs).

and hospitalisations from the time of a person's Medicaid eligibility until death or the end of Medicaid eligibility. As about 17% of Medicaid beneficiaries are also enrolled in Medicare,[21] Medicare data were obtained to ensure complete data capture in dually eligible beneficiaries.

Study population and design

We conducted a cohort study among adults (ie, 18–90 years of age) who had at least 180 days of Medicaid eligibility and at least one outpatient or inpatient claim present before the first prescription of allopurinol. We identified new allopurinol users starting from 1 January 1999, excluding individuals who had a history of severe cutaneous adverse reactions prior to allopurinol initiation. We followed patients from allopurinol initiation until: (1) hospitalisation for a severe cutaneous adverse reaction, (2) the end of Medicaid eligibility, (3) the end of the study period or (4) death, whichever came first.

Assessment of endpoints

The primary endpoint of interest was incident cases of hospitalised AASCARs with a principal hospital discharge diagnosis of a relevant International Classification of Diseases, Ninth Revision,

Table 1 Risk of hospitalised allopurinol-associated severe cutaneous adverse reactions (AASCARs) according to race/ethnicity and other risk factors

Variable	Allopurinol initiators N (%)	Hospitalised AASCARs N	Risk of hospitalised AASCARs (/1000 persons)	Age-adjusted, sex-adjusted relative risk	Multivariable-adjusted relative risk*
All	400 401 (100)	203	0.51 (0.45 to 0.59)	–	–
Race/ethnicity					
White/Hispanic	248 501 (62)	64	0.26 (0.20 to 0.33)	1.0	1.0
Black	111 619 (28)	91	0.82 (0.66 to 1.00)	3.02 (2.20 to 4.17)	3.00 (2.18 to 4.14)
Asian	21 442 (5)	15	0.70 (0.39 to 1.15)	2.94 (1.67 to 5.17)	3.03 (1.72 to 5.34)
Native Hawaiian/Pacific Islander	18 839 (5)	33	1.75 (1.21 to 2.46)	6.54 (4.28 to 10.00)	6.68 (4.37 to 10.22)
Sex					
Male	213 041 (53)	61	0.29 (0.22 to 0.37)	1.0	1.0
Female	187 360 (47)	142	0.76 (0.64 to 0.89)	2.37 (1.74 to 3.21)	2.49 (1.83 to 3.38)
Age					
<60 years	208 151 (52)	70	0.34 (0.26 to 0.42)	1.0	1.0
≥60 years	192 250 (48)	133	0.71 (0.60 to 0.84)	1.73 (1.29 to 2.33)	1.66 (1.23 to 2.24)
Chronic kidney disease					
No	381 561 (95)	184	0.48 (0.42 to 0.56)	1.0	1.0
Yes	18 840 (5)	19	1.01 (0.63 to 1.54)	2.11 (1.32 to 3.39)	2.33 (1.44 to 3.77)
Initial allopurinol dose (>100 mg/day)					
No	157 138 (39)	58	0.37 (0.28 to 0.47)	1.0	1.0
Yes	243 263 (61)	145	0.60 (0.51 to 0.70)	1.74 (1.28 to 2.36)	1.85 (1.36 to 2.51)

*Mutually adjusted for the variables in this table.

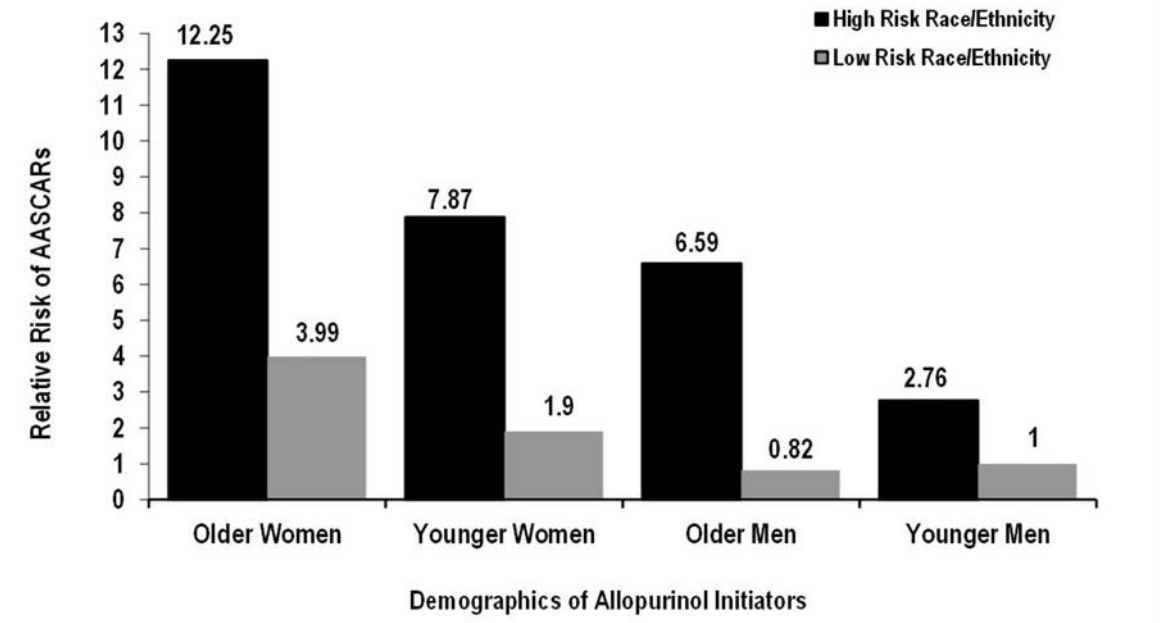


Figure 2 Relative risk of hospitalised allopurinol-associated severe cutaneous adverse reactions (AASCARs) according to demographic factors. Older age defined as ≥60 years; high-risk race/ethnicity=blacks, Asians or Native Hawaiians/Pacific Islanders; low-risk race/ethnicity=whites or Hispanics.

Clinical Modification (ICD-9-CM) code, occurring within the first 3 months after filling the first prescription for allopurinol and followed by discontinuation of the drug after the episode.[5] The employed ICD-9-CM codes for the definition consisted of those used by a recent claims database study (ie, dermatitis due to drugs and medicines (693.0); erythema multiforme, SJS and TEN (695.1); unspecified erythematous conditions (695.9); and other specified erythematous conditions (695.89)).[5] The 3-month time window was employed because AHS predominantly occurs within the first 3 months of drug exposure (figure 1).[5 12 22] The aforementioned study evaluated the accuracy of this definition and found that all 33 cases of AASCARs meeting the definition were confirmed by a medical record review conducted by experienced dermatologists.[5] Our secondary definition of hospitalised severe cutaneous adverse reactions restricted the principal hospital discharge diagnosis to the ICD-9-CM code 695.1x but did not require any other conditions, as was adopted by an earlier claims database study.[6] This definition was found to have a positive predictive value of >90% for hospitalised severe cutaneous adverse reactions.[6 23–25] Finally, we examined the risk of AASCAR mortality, defined as death within 2 months of the AASCAR hospitalisation date.[5]

Assessment of race/ethnicity and covariates

Our primary risk factor of interest was race/ethnicity, consisting of white, black, Asian, Native Hawaiian or other Pacific Islander and Hispanic or Latino as reported by patients in the Medicaid database. Patients for whom information on race or ethnicity was unclassifiable or missing (eg, 'more than one race' or 'other/unknown') were excluded from these analyses. Covariates of interest consisted of purported risk factors, including demographic factors (ie, age and sex), presence of CKD (ICD-9-CM codes 580–586), presence of gout (ICD-9-CM code 274), use of diuretics and initial allopurinol dose (>100 mg/day vs ≤100 mg/day)).[5 8 9 19]

Statistical analyses

We assessed the timing of AASCAR incidence after initiating allopurinol by graphically displaying the number of events every 10 days. The overall risk of hospitalised AASCARs per 1000 allopurinol initiators and corresponding 95% CI were calculated. We then estimated the risk of hospitalised AASCARs per 1000 allopurinol initiators according to race/ethnicity and other purported risk factors. We used Poisson regression models to determine the relative risk (RR) of AASCARs in relation to race/ethnicity while adjusting for covariates. Our final multivariable models included variables that were significantly associated with the risk of hospitalised AASCARs in age-adjusted and sex-adjusted analyses or affected the RR estimate for any race by at least 10%. Our reference group consisted of whites and Hispanics, given their similarly low frequencies of *HLA-B*5801* (both 1% in the US population)[18] and similar risks of AASCARs observed in the current study. We further examined the risk and RR of hospitalised AASCARs according to combined demographic profiles of age, sex and race/ethnicity. In compliance with the Healthcare Cost and Utilization Project Data Use Agreement,[26] we did not report data when the tabulated cell size was less than 11. All p values were two sided with a significance threshold of $P<0.05$. Statistical analyses were performed using SAS V.9.3.

RESULTS

Baseline characteristics

Our cohort consisted of 400 401 allopurinol initiators. The baseline characteristics of these initiators are summarised in table 1. Sixty-two per cent of allopurinol initiators were white, 53% were male and 52% were younger than 60 years of age. Five per cent of initiators had CKD at initiation and 61% were prescribed allopurinol at an initial dose greater than 100 mg/day.

Table 3 Risk race/ethnicity and risk of hospitalised allopurinol-associated severe cutaneous adverse reactions (AASCARs) according to chronic kidney disease (CKD) and initial allopurinol dose

		High-risk race/ethnicity		Low-risk race/ethnicity	
Variable	Initial allopurinol dose	Risk of hospitalised AASCARs (/1000 persons), n (95% CI)	Multivariable-adjusted relative risk*, n (95% CI)	Risk of hospitalised AASCARs (/1000 persons), n (95% CI)	Multivariable-adjusted relative risk*, n (95% CI)
CKD	>100 mg/day	1.63 (0.60 to 3.55)	9.08 (3.58 to 22.77)	1.12 (0.36 to 2.60)	5.65 (2.10 to 15.22)
	≤100 mg/day	0.78 (0.21 to 1.99)	4.10 (1.37 to 12.13)	0.72 (0.20 to 1.85)	3.62 (1.23 to 10.71)
No CKD	>100 mg/day	1.14 (0.92 to 1.39)	5.86 (3.54 to 9.68)	0.25 (0.17 to 0.34)	1.32 (0.76 to 2.34)
	≤100 mg/day	0.55 (0.38 to 0.78)	2.69 (1.51 to 4.79)	0.20 (0.12 to 0.32)	1.0 (reference)

*Adjusted for age and sex.

Risk of hospitalised AASCARs after allopurinol initiation according to race/ethnicity

Among allopurinol initiators, we documented 203 hospitalised AASCAR cases (table 1). The risk of hospitalised AASCARs was apparent within 10 days of allopurinol initiation, peaked around 1 month after initiation, and declined progressively thereafter, reaching its nadir at the end of the third month (figure 1). The average length of an AASCAR hospitalisation was 9.6 days, and 43 patients (21%) died.

The risk of hospitalised AASCARs was 1 in 3883 initiators among whites and Hispanics, whereas the risk was 1 in 1227, 1429 and 571 initiators among blacks, Asians and Native Hawaiians/Pacific Islanders, respectively (table 1). The baseline characteristics according to race/ethnicity are summarised in online supplementary table 1. The multivariable-adjusted RRs for AASCARs among blacks and Asians as compared with whites and Hispanics were 3.00 (95% CI 2.18 to 4.14) and 3.03 (95% CI 1.72 to 5.34), respectively. The corresponding multivariable-adjusted RR of hospitalised AASCARs was 6.68 (95% CI 4.37 to 10.22) among Native Hawaiian/Pacific Islanders (table 1). After adjusting for age as a continuous variable, these RRs remained similarly large (online supplementary table 2). Furthermore, after applying our secondary definition of hospitalised severe cutaneous adverse reactions,[6] these RRs remained similar (online supplementary table 3). Finally, there was no significant subgroup effect according to the presence of gout (p for interaction=0.36).

Risk of hospitalised AASCARs according to other risk factors

All covariates that were statistically significant in the age-adjusted and sex-adjusted analyses remained independently associated with the risk of hospitalised AASCARs in our multivariable analyses, including female sex (multivariable-adjusted RR, 2.49; 95% CI 1.83 to 3.38), age ≥60 years (multivariable-adjusted RR, 1.66; 95% CI 1.23 to 2.24), CKD (multivariable-adjusted RR, 2.33; 95% CI 1.44 to 3.77) and initial allopurinol dose >100 mg/day (multivariable-adjusted RR, 1.85; 95% CI 1.36 to 2.51) (table 1). In our analysis using age as a continuous variable, a 10-year increase was associated with a 29% higher risk of hospitalised AASCAR (multivariable-adjusted RR, 1.29; 95% CI 1.16 to 1.42) (online supplementary table 2). Neither a history of gout nor diuretic use was significantly associated with the risk of AASCAR in age-adjusted and sex-adjusted analyses (RRs, 1.21 (95% CI 0.91 to 1.60) and 1.38 (95% CI 0.99 to 1.92), respectively) or significantly affected the RR estimate for any race/ethnicity category.

Risk of hospitalised AASCARs according to combined demographic profiles

We further examined the risk of hospitalised AASCARs according to combined demographic profiles by classifying whites and Hispanics as low-risk race/ethnicities, and blacks, Asians and Hawaiians/Pacific Islanders as high-risk races/ethnicities (figure 2 and table 2). Older women (≥60 years) of a high-risk race/ethnicity had over a 12-fold higher risk of hospitalised AASCARs than younger men (<60 years) of a low-risk race/ethnicity (multivariable-adjusted RR, 12.25; 95% CI 6.46 to 23.25). Older men of a high-risk race/ethnicity had over a sixfold higher risk of hospitalised AASCARs than younger men of a low-risk race/ethnicity (multivariable-adjusted RR, 6.59; 95% CI 3.28 to 13.26) (figure 2 and table 2).

Risk of hospitalised AASCARs according to race/ethnicity combined with CKD status and initial allopurinol dose

We also examined the risk of hospitalised AASCARs according to race/ethnicity combined with CKD status and initial allopurinol dose (table 3). Patients with CKD of a high-risk race/ethnicity who received an initial allopurinol dose >100 mg/day had over a ninefold higher risk of hospitalised AASCARs compared with

Table 2 Risk of hospitalised allopurinol-associated severe cutaneous adverse reactions (AASCARs) according to combined demographic profiles

	High-risk race/ethnicity		Low-risk race/ethnicity	
Variable	Risk of hospitalised AASCARs (/1000 persons), n (95% CI)	Multivariable-adjusted relative risk*, n (95% CI)	Risk of hospitalised AASCARs (/1000 persons), n (95% CI)	Multivariable-adjusted relative risk*, n (95% CI)
Female				
≥60 years	1.50 (1.16 to 1.90)	12.25 (6.46 to 23.25)	0.53 (0.38 to 0.72)	3.99 (2.01 to 7.89)
<60 years	1.01 (0.70 to 1.41)	7.87 (3.95 to 15.66)	0.24 (0.13 to 0.42)	1.90 (0.82 to 4.38)
Male				
≥60 years	0.83 (0.56 to 1.18)	6.59 (3.28 to 13.26)	0.10 (0.03 to 0.23)	0.82 (0.29 to 2.37)
<60 years	0.47 (0.21 to 0.57)	2.76 (1.28 to 5.95)	0.13 (0.07 to 0.23)	1.0 (reference)

*Adjusted for chronic kidney disease and initial allopurinol dose.

non-CKD patients of a low-risk race/ethnicity who received an initial allopurinol dose ≤100 mg/day (multivariable-adjusted RR, 4.37; 95% CI 2.27 to 8.42).

Mortality of AASCARs according to race/ethnicity and other risk factors

Compared with patients of a low-risk race/ethnicity (whites/Hispanics), allopurinol initiators of a high-risk race/ethnicity had a 3.65-fold higher risk of mortality from AASCARs (multivariable-adjusted RR, 3.65; 95% CI 1.90 to 6.99) (table 4). Other significant independent risk factors for AASCAR mortality included female sex (multivariable-adjusted RR, 1.96; 95% CI 1.02 to 3.74) and age ≥60 years (multivariable-adjusted RR, 2.79; 95% CI 1.39 to 5.59) (table 4).

DISCUSSION

In the present study of 400 401 allopurinol initiators, we observed substantial variations in the incidence of hospitalised AASCARs according to race/ethnicity. Blacks, Asians and Native Hawaiians/Pacific Islanders had a 3–6 times higher risk of AASCARs compared with whites and Hispanics. These associations persisted similarly after adjusting for age, sex, presence of CKD and initial allopurinol dose >100 mg daily, all of which we also found to be independently associated with the risk of hospitalised AASCARs. However, the presence of gout or the use of diuretics were not significantly associated with this risk. In our combined demographic analysis, elderly high-risk race/ethnicity women had over a 12-fold higher risk of hospitalised AASCARs compared with young white or Hispanic men. These findings support the use of extra caution among Native Hawaiians/Pacific Islanders, Asians and blacks when considering allopurinol (including screening for *HLA-B*5801*[2]), particularly among elderly women with CKD. Importantly, a low initial allopurinol dose (eg, <100 mg/day) was the only modifiable risk factor, which is readily implementable and is also recommended by the latest rheumatology guidelines.[1 2]

The risk of AASCARs was over six times higher among Native Hawaiians/Pacific Islanders compared with whites in this study. To our knowledge, this study provides the first evidence that this racial/ethnic group has a high risk of AASCARs. This finding corroborates the allele frequency of *HLA-B*5801* (eg, 5.8%[27 28] vs <1%–1.9%[18] in US Pacific Islanders and whites, respectively). In other Pacific Island countries, the allele frequencies and prevalence of positive carriage are even higher (eg, 6%–12% and 11%–22%, respectively, in Malaysia),[18] and thus, one would expect their risk of AASCARs to be at least as high as that observed in the current study. The recommendation to screen for *HLA-B*5801*[20] or to consider the use of an alternative ULD would be applicable to Native Hawaiians/Pacific Islanders prior to initiating allopurinol therapy, particularly when additional AASCAR risk factors are present (eg, in the case of being an elderly woman with CKD).

The increased risk of AASCARs in blacks in the current study expands on the findings of the recent US Nationwide Inpatient Sample study that showed a higher risk of SJS/TEN hospitalisations in blacks compared with whites,[19] although that study was not able to definitively determine the drug responsible for the increased risk. To that end, the current study identifies allopurinol as the culprit drug while also confirming the previously observed association.[19] These findings are also reflective of the allele frequencies of *HLA-B*5801* (eg, 2.6%–6.4% vs <1%–1.9% in US blacks and whites, respectively[18]). Furthermore, the allele frequencies among blacks in Africa are known to be even higher (eg, 7%–10% in Kenya and 8% in South Africa), suggesting a higher risk of AASCARs among those populations as well.[18]

The higher risk of AASCARs in Asians in the current study is consistent with the high incidence rate of AASCARs in Taiwan,[5] the increased risk of this adverse reaction among Chinese descendants compared with European descendants[8] and the high carriage prevalence of *HLA-B*5801* in Asian countries (eg, 20% in Taiwan[20]). Accordingly, the Taiwanese Food and Drug Administration has recently adopted an alternative first-line ULD for patients with CKD.[5] Furthermore, a recent prospective study screened the *HLA-B*5801* allele among 2926 Taiwanese allopurinol initiators and was able to reduce the incidence of

Table 4 Mortality of allopurinol-associated severe cutaneous adverse reactions (AASCARs) according to race/ethnicity and purported risk factors

Variable	Allopurinol initiators N (%)	Mortality risk of hospitalised AASCARs (/1000 persons), n (95% CI)	Age-adjusted, sex-adjusted relative risk, n (95% CI)	Multivariable-adjusted relative risk*, n (95% CI)
All	400 401 (100)	0.11 (0.08 to 0.14)	–	–
Race/ethnicity				
White/Hispanic	248 501 (62)	0.05 (0.03 to 0.09)	1.0	1.0
Black/Asian/Native Hawaiian/Pacific Islander	151 900 (38)	0.20 (0.14 to 0.28)	3.65 (1.90 to 6.99)	3.65 (1.90 to 6.99)
Sex				
Male	213 041 (53)	0.07 (0.04 to 0.11)	1.0	1.0
Female	187 360 (47)	0.16 (0.11 to 0.22)	1.93 (1.01 to 3.70)	1.96 (1.02 to 3.74)
Age				
<60 years	208 151 (52)	0.05 (0.03 to 0.09)	1.0	1.0
≥60 years	192 250 (48)	0.17 (0.12 to 0.23)	2.75 (1.37 to 5.53)	2.79 (1.39 to 5.59)
Chronic kidney disease				
No	381 561 (95)	0.10 (0.07 to 0.14)	1.0	1.0
Yes	18 840 (5)	0.22 (0.07 to 0.52)	2.16 (0.77 to 6.04)	2.16 (0.77 to 6.09)
Initial allopurinol dose (>100 mg/day)				
No	157 138 (39)	0.09 (0.05 to 0.15)	1.0	1.0
Yes	243 263 (61)	0.12 (0.08 to 0.17)	1.47 (0.77 to 2.78)	1.57 (0.83 to 2.98)

*Mutually adjusted for the variables in this table.

AASCARs from seven expected cases to zero (P=0.003), demonstrating the effectiveness of such a practice.[20] In contrast, despite the sufficient sample size of Hispanic allopurinol initiators at our study baseline, there was an obviously low risk of AASCARs, similar to that seen in whites (online supplementary table 4). These data were similar to the recent US Nationwide Inpatient Sample study[19] and are concordant with the low frequency of *HLA-B*5801* reported among Hispanics in the USA and among Mexicans (ie, ~1%).[18]

We also found that female sex was associated with a 2.5 times higher risk of AASCARs than male sex, even after adjusting for other risk factors. The aforementioned Taiwanese study also found a 45% higher risk of AHS among Chinese women compared with Chinese men.[5] The mechanism behind the difference in sex remains speculative, including the potential role of female hormones, which calls for further study. Older age was also independently associated with an increased risk of AASCARs, which was also consistent with the Taiwanese study findings among Chinese patients.[5] While this could reflect an ageing and vulnerable immune system or a slower metabolic rate (which would predispose older individuals to develop this type of severe hypersensitivity reaction), further mechanistic clarification is needed. Finally, our analysis confirmed a strong independent association with CKD and the initial allopurinol dose of >100 mg/day. This provides support for the latest rheumatology guideline recommendations to initiate allopurinol at a dose ≤100 mg daily.[1 2]

The main strength of this study is the use of 400 401 allopurinol initiators in a nationwide database, which provides information relevant to AASCAR outcomes. The racial and ethnic diversity of the US Medicaid study population made it possible to directly compare different racial/ethnic groups, which is not feasible in homogenous populations. Similar to the aforementioned study,[5] our study examined all allopurinol initiators regardless of gout status. As such, proportions of males and those with CKD tended to be lower than those of a typical gout cohort. While our analysis did not suggest a significant subgroup effect by the presence of gout, larger studies conducted specifically among gout patients would be valuable. We used pharmacy claims data, which are considered to be one of the best data sources for drug exposure. Also, because our administrative censoring for AASCARs was at the end of 3 months, potential issues associated with discontinuation of the medication are expected to be minimal. Because the Medicaid database is an administrative database, a certain degree of diagnostic code misclassification is inevitable. However, as mentioned, a recent study found our endpoint definition to have a high level of accuracy according to dermatologists' medical record review.[5] As we further narrowed our primary endpoint definition to a primary discharge diagnosis of hospitalised cases, we expect the specificity to be even higher. Moreover, the high AASCAR-associated mortality rate as well as the long hospital stay corroborate its validity, and our secondary definition of AASCARs[6] led to similar results. As our case definition did not include potential outpatient cases of AASCARs, the risk estimates for AASCARs in our study should be interpreted as conservative. Regardless, this would not have affected the RR measures and instead further guarded against the misclassification of cases.

In conclusion, these findings from a large, racially diverse cohort indicate that Native Hawaiians/Pacific Islanders, Asians and blacks all have a substantially higher risk of hospitalised AASCARs compared with whites and Hispanics, calling for heightened vigilance when initiating allopurinol in these racial/ethnic groups. Furthermore, female sex, older age, CKD and an initial allopurinol dose >100 mg/day are all independent risk factors for hospitalised AASCARs and should also be considered when initiating allopurinol to help prevent this severe and potentially fatal adverse reaction.

Contributors All authors participated in the conception, design and analyses of the study. SFK, NL and HKC drafted the manuscript and are guarantors. All authors contributed to interpretation of the results. SFK, NL and HKC had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding This work was supported in part by a grant from the National Institutes of Health (R01AR065944).

Competing interests HKC has received research grants from Pfizer and AstraZeneca to Massachusetts General Hospital for unrelated studies and served as a consultant for Takeda Pharmaceuticals, Selecta, Horizon and Ironwood. SCK has received research grants to the Brigham and Women's Hospital from Pfizer, AstraZeneca, Bristol-Myers Squibb and Roche for unrelated studies.

Patient consent Not required.

Ethics approval This study was exempted by the Partners Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional unpublished data are available.

REFERENCES

1 Richette P, Doherty M, Pascual E, *et al*. 2016 updated EULAR evidence-based recommendations for the management of gout. *Ann Rheum Dis* 2017;76:29–42.
2 Khanna D, Fitzgerald JD, Khanna PP, *et al*. 2012 American College of Rheumatology guidelines for management of gout. Part 1: systematic nonpharmacologic and pharmacologic therapeutic approaches to hyperuricemia. *Arthritis Care Res* 2012;64:1431–46.
3 Jutkowitz E, Choi HK, Pizzi LT, *et al*. Cost-effectiveness of allopurinol and febuxostat for the management of gout. *Ann Intern Med* 2014;161:617–26.
4 Stern RJ. Reducing life-threatening allopurinol hypersensitivity. *JAMA Intern Med* 2015;175:1558.
5 Yang CY, Chen CH, Deng ST, *et al*. Allopurinol use and risk of fatal hypersensitivity reactions: a nationwide population-based study in Taiwan. *JAMA Intern Med* 2015;175:1550–7.
6 Kim SC, Newcomb C, Margolis D, *et al*. Severe cutaneous reactions requiring hospitalization in allopurinol initiators: a population-based cohort study. *Arthritis Care Res* 2013;65:578–84.
7 Halevy S, Ghislain PD, Mockenhaupt M, *et al*. Allopurinol is the most common cause of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe and Israel. *J Am Acad Dermatol* 2008;58:25–32.
8 Stamp LK, Taylor WJ, Jones PB, *et al*. Starting dose is a risk factor for allopurinol hypersensitivity syndrome: a proposed safe starting dose of allopurinol. *Arthritis Rheum* 2012;64:2529–36.
9 Hande KR, Noone RM, Stone WJ, *et al*. Severe allopurinol toxicity. Description and guidelines for prevention in patients with renal insufficiency. *Am J Med* 1984;76:47–56.
10 Kang HR, Jee YK, Kim YS, *et al*. Positive and negative associations of HLA class I alleles with allopurinol-induced SCARs in Koreans. *Pharmacogenet Genomics* 2011;21:303–7.
11 Jung JW, Song WJ, Kim YS, *et al*. HLA-B58 can help the clinical decision on starting allopurinol in patients with chronic renal insufficiency. *Nephrol Dial Transplant* 2011;26:3567–72.
12 Hung SI, Chung WH, Liou LB, *et al*. HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. *Proc Natl Acad Sci U S A* 2005;102:4134–9.
13 Kaniwa N, Saito Y, Aihara M, *et al*. HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. *Pharmacogenomics* 2008;9:1617–22.
14 Tassaneeyakul W, Jantararoungtong T, Chen P, *et al*. Strong association between HLA-B*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population. *Pharmacogenet Genomics* 2009;19:704–9.
15 Lonjou C, Borot N, Sekula P, *et al*. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics* 2008;18:99–107.
16 Zineh I, Mummaneni P, Lyndly J, *et al*. Allopurinol pharmacogenetics: assessment of potential clinical usefulness. *Pharmacogenomics* 2011;12:1741–9.

17 Somkrua R, Eickman EE, Saokaew S, *et al*. Association of HLA-B*5801 allele and allopurinol-induced Stevens Johnson syndrome and toxic epidermal necrolysis: a systematic review and meta-analysis. *BMC Med Genet* 2011;12:118.
18 The Allele frequency net database. http://www.allelefrequencies.net/
19 Lu N, Rai SK, Terkeltaub R, *et al*. Racial disparities in the risk of Stevens-Johnson Syndrome and toxic epidermal necrolysis as urate-lowering drug adverse events in the United States. *Semin Arthritis Rheum* 2016;46:253–8.
20 Ko TM, Tsai CY, Chen SY, *et al*. Use of HLA-B*58:01 genotyping to prevent allopurinol induced severe cutaneous adverse reactions in Taiwan: national prospective cohort study. *BMJ* 2015;351:h4848.
21 Clark WD, Hulbert MM. Research issues: dually eligible medicare and medicaid beneficiaries, challenges and opportunities. *Health Care Financ Rev* 1998;20:1–10.
22 Chung WH, Chang WC, Stocker SL, *et al*. Insights into the poor prognosis of allopurinol-induced severe cutaneous adverse reactions: the impact of renal insufficiency, high plasma levels of oxypurinol and granulysin. *Ann Rheum Dis* 2015;74:2157–64.
23 Strom BL, Carson JL, Halpern AC, *et al*. A population-based study of Stevens-Johnson syndrome. Incidence and antecedent drug exposures. *Arch Dermatol* 1991;127:831–8.
24 Chan HL, Stern RS, Arndt KA, *et al*. The incidence of erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis. A population-based study with particular reference to reactions caused by drugs among outpatients. *Arch Dermatol* 1990;126:43–7.
25 Strom BL, Carson JL, Halpern AC, *et al*. Using a claims database to investigate drug-induced Stevens-Johnson syndrome. *Stat Med* 1991;10:565–76.
26 Nationwide data use agreement. https://www.hcup-us.ahrq.gov/team/NationwideDUA.jsp
27 Maiers M, Gragert L, Klitz W. High-resolution HLA alleles and haplotypes in the United States population. *Hum Immunol* 2007;68:779–88.
28 High-Resolution HLA alleles and haplotypes in the US population. https://bioinformatics.bethematchclinical.org/hla-resources/haplotype-frequencies/high-resolution-hla-alleles-and-haplotypes-in-the-us-population/

EXTENDED REPORT

Identification of calcium pyrophosphate deposition disease (CPPD) by ultrasound: reliability of the OMERACT definitions in an extended set of joints—an international multiobserver study by the OMERACT Calcium Pyrophosphate Deposition Disease Ultrasound Subtask Force

Georgios Filippou,[1] Carlo Alberto Scirè,[1] Antonella Adinolfi,[2] Nemanja S Damjanov,[3,4] Greta Carrara,[4] George A W Bruyn,[5] Tomas Cazenave,[6] Maria Antonietta D'Agostino,[7] Andrea Delle Sedie,[8] Valentina Di Sabatino,[2] Mario Enrique Diaz Cortes,[9] Emilio Filippucci,[10] Frederique Gandjbakhch,[11] Marwin Gutierrez,[12] Daryl K Maccarter,[13] Mihaela Micu,[14] Ingrid Möller Parera,[15] Gaël Mouterde,[16] Mohamed Atia Mortada,[17] Esperanza Naredo,[18] Carlos Pineda,[12] Francesco Porta,[19] Anthony M Reginato,[20] Iulia Satulu,[21] Wolfgang A Schmidt,[22] Teodora Serban,[23] Lene Terslev,[24] Violeta Vlad,[25] Florentin Ananu Vreju,[26] Pascal Zufferey,[27] Panagiotis Bozios,[28] Carmela Toscano,[2] Valentina Picerno,[29] Annamaria Iagnocco[23]

Handling editor Josef S Smolen

For numbered affiliations see end of article.

Correspondence to
Dr Georgios Filippou, Department of Medical Sciences, Section of Rheumatology, University of Ferrara and Azienda Ospedaliero-Universitaria Sant'Anna di Cona, Ferrara 44124, Italy; gf.filippou@gmail.com

Received 16 October 2017
Accepted 23 February 2018
Published Online First 13 March 2018

To cite: Filippou G, Scirè CA, Adinolfi A, *et al*. *Ann Rheum Dis* 2018;**77**:1195–1200.

ABSTRACT

Objectives To assess the reliability of the OMERACT ultrasound (US) definitions for the identification of calcium pyrophosphate deposition disease (CPPD) at the metacarpal-phalangeal, triangular fibrocartilage of the wrist (TFC), acromioclavicular (AC) and hip joints.

Methods A web-based exercise and subsequent patient-based exercise were carried out. A panel of 30 OMERACT members, participated at the web-based exercise by evaluating twice a set of US images for the presence/absence of CPPD. Afterwards, 19 members of the panel met in Siena, Italy, for the patient-based exercise. During the exercise, all sonographers examined twice eight patients for the presence/absence of CPPD at the same joints. Intraoberserver and interobserver kappa values were calculated for both exercises.

Results The web-based exercise yielded high kappa values both in intraobserver and interobserver evaluation for all sites, while in the patient-based exercise, inter-reader agreement was acceptable for the TFC and the AC. TFC reached high interobserver and intraobserver k values in both exercises, ranging from 0.75 to 0.87 (good to excellent agreement). AC reached moderate kappa values, from 0.51 to 0.85 (moderate to excellent agreement) and can readily be used for US CPPD identification.

Conclusions Based on the results of our exercise, the OMERACT US definitions for the identification of CPPD demonstrated to be reliable when applied to the TFC and AC. Other sites reached good kappa values in the web-based exercise but failed to achieve good reproducibility at the patient-based exercise, meaning the scanning method must be further refined.

INTRODUCTION

Calcium pyrophosphate deposition disease (CPPD) is one of the most common arthropathies in the elderly, yet there is no specific treatment for this disease.[1] Prevalence rates range from 4% to over 50%[2–5] and increase with the age of the patient and the diagnostic method. The most recent EULAR recommendations on the diagnosis and terminology of CPPD endorsed ultrasound (US) as a promising diagnostic imaging modality for CPPD despite the use of synovial fluid analysis as the gold standard.[6]

Recently, an OMERACT special interest group for US in CPPD has been created with the aim to assess the potential utility of US in the diagnosis of CPPD and created for the first time definitions for US identification of calcium pyrophosphate (CPP) crystals in joints and soft tissues.[7] Furthermore, the reliability of these definitions has been assessed in some sites (knee fibrocartilage and hyaline cartilage, patellar tendons, quadriceps tendons, Achilles tendons, triangular fibrocartilage of the wrist (TFC), synovial fluid of knee and wrist) demonstrating a good reliability in the menisci and hyaline cartilage of the knee and poor reliability at the other sites, specially at tendons and synovial fluid.[7] However, previous imaging studies have demonstrated that CPP crystal deposition could also be found in other sites such as shoulders, hips or metacarpal-phalangeal joints (MCPs).[8–11]

The aim of this study was to assess the reliability of US by using the new OMERACT definitions for US identification of CPPD at the wrist (TFC), MCPs, acromioclavicular (AC) and hip joints and provide a comprehensive atlas of images of CPPD identified by US applying the new OMERACT US definitions for CPPD.

PATIENTS AND METHODS

Background and study design

The OMERACT US CPPD task force was created and held its first meeting during the American College of Rheumatology (ACR) congress in 2014. Since then, the group has produced a systematic literature review and meta-analysis[12] on the use of US in the identification of CPPD and has created the US definitions for the identification of CPPD.[7] First, reliability assessment of the definitions was then carried out in 2015 in Siena involving the fibrocartilage of the knee and wrist (TFC), the hyaline cartilage of the knee, the quadriceps/patellar/Achilles tendons and the synovial fluid of the knee and wrist. In the OMERACT US meeting group held during the EULAR congress in London (2016), the members of the group agreed that it would be very important for the validation process to extend the reliability tests in other joints, CPPD being a systemic disease, and to refine the scanning technique pertaining to difficult sites. A new workshop was organised in order to assess the reliability in the following sites: TFC, meniscus of the AC joint, hyaline cartilage of the MCP joints, labrum (HL) and hyaline cartilage (HCH) of the hip joints.

Following the OMERACT methodology,[13] a web-based and a patient-based exercise was performed with the aim of testing the reliability of US in the detection of CPP deposits at difficult to evaluate joints.

Reporting of the results in this manuscript followed previously published guidelines.[14] The study was notified and approved by the local ethics committee. All patients gave informed consent before participation in the workshop.

First step: the web-based exercise

Thirty rheumatologists from 14 different countries covering three continents (one from Colombia, one from Denmark, one from Egypt, three from France, one from Germany, nine from Italy, one from The Netherlands, two from Mexico, four from Romania, one from Serbia, two from Spain, one from Sweden, one from Switzerland and two from USA), experts in US and crystal-induced arthritis and members of the OMERACT US CPPD task force participated in the web-based exercise.

All participants were asked to send five images to the organisers of the workshop of the anatomical sites under examination (TFC, AC, MCP, HL and HCH) in order to prepare the web-based exercise. A set of 65 US images, equally distributed between the five different sites (13 images for each site), was then prepared based on the quality of the images (some of the scanned images were excluded), on the uniformity of the setting (trying to avoid excessive differences of the setting of the machines) and on the sites proposed (not all participants sent images of all sites). The sample was estimated to be the minimum size to accurately estimate kappa values significantly greater than 0.4, setting alpha at 0.05 and beta at 0.10.

Each participant rated the images according to a dichotomous score (presence/absence) by applying the definitions previously published.[7] The US definition was available above every single image in order to avoid any misinterpretations.

Two weeks after the first assessment, all participants rated the same images again to assess the intraobserver reliability.

The web-based agreement exercise was carried out on a web-based platform (RedCap) that did not allow the submission of the survey in case of missing data. Only the facilitator and the epidemiologists of the study had access to the online data and were responsible for the upload and preparation of the Delphi rounds and the web-based exercise.

Second step: patient-based exercise

The patient-based exercise was held in Siena, Italy, in January 2017. Eight isolated stations were created with eight US machines (three Esaote, four GE and one Samsung) equipped with high frequency linear probes. The settings of the machines were created to better enhance calcific depositions and were tested and approved by the experts before the workshop. Each sonographer could modify only the basic functions (depth, gain, time gain control and frequency) in order to obtain the best possible image for CPP identification according to the patient's physical characteristics.

Eight volunteer patients, five affected with CPPD (three men and two women; mean age 69.4±8.9 years) and three with osteoarthritis (two women and one man; mean age 58±8.2 years), as defined by synovial fluid analysis performed within 1 month before the study for routine clinical practice, participated in the patient-based study. Nineteen out of 30 ultrasonographers participated in this phase. Eight US stations were created for the workshop, four patients were sited and examined for the II and III MCP and the TFC bilaterally while in the remaining four stations the patients were examined for the hip and the AC joint in the supine position. Each sonographer examined all the patients and rated the presence/absence of CPP deposits. Four rheumatologists, experts in US and members of the local organising committee assisted the ultrasonographers during the whole procedure by collecting the datasheets, organising and timing the shifts at each station.

The US exam was performed according to a standardised sequence that was decided for each site, the day before the exercise during a briefing following the most recent guidelines of the EULAR.[15] Briefly, the MCP joints' hyaline cartilage (HC) was examined on the dorsal aspect of the hands with the fingers in maximum flexion to reveal a large portion of HC. Longitudinal and transverse scans were used. The AC joint was scanned on the

Table 1 Web-based exercise results

Section	Mean prevalence	Mean observed agreement	Mean kappa
Inter-reader reliability			
1) All	50.2	0.83	0.66
2) Fibrocartilage	54.7	0.84	0.68
3) Hyaline cartilage	41.0	0.82	0.63
4) Hand	38.8	0.80	0.58
5) Wrist fibrocartilage	56.7	0.87	0.75
6) Acromioclavicular joint	53.8	0.87	0.75
7) Hip*	50.3	0.80	0.61
7a) Hip labrum	53.6	0.78	0.56
7b) Hip cartilage	44.0	0.85	0.70
Intra-reader reliability			
1) All	49.7	0.91	0.81
2) Fibrocartilage	53.9	0.91	0.80
3) Hyaline cartilage	40.8	0.92	0.81
4) Hand	38.1	0.89	0.76
5) Wrist fibrocartilage	56.5	0.92	0.82
6) Acromioclavicular joint	53.5	0.93	0.85
7) Hip*	49.0	0.90	0.79
7a) Hip labrum	51.7	0.87	0.73
7b) Hip cartilage	44.4	0.95	0.89

Strength of agreement: <0.20: poor; 0.21–0.40: fair; 0.41–0.60: moderate; 0.61–0.80: substantial; 0.81–1.00: excellent.
*Hip is the kappa value including both sites of evaluation of the hip (hip labrum and hip cartilage).

Table 2 Patient-based exercise results

Section	Mean prevalence	Mean observed agreement	Mean kappa	PABAK
Inter-reader reliability				
1) All	48.2	0.71	0.43	0.42
2) Fibrocartilage (all sites)	72.7	0.75	0.39	0.51
3) Hyaline cartilage (all sites)	23.7	0.67	0.09	0.34
4) Hand	22.6	0.69	0.12	0.38
5) Wrist fibrocartilage	95.1	0.91	0.01	0.82
6) Acromioclavicular joint	61.1	0.75	0.51	0.51
7) Hip†	43.7	0.61	0.23	0.23
7a) Hip labrum	61.8	0.60	0.16	0.19
7b) Hip cartilage	25.7	0.63	0.04	0.26
Intra-reader reliability				
1) All	48.3	0.85	0.69	0.71
2) Fibrocartilage (all sites)	73.1	0.85	0.57	0.71
3) Hyaline cartilage (all sites)	23.4	0.86	0.53	0.73
4) Hand	23.0	0.84	0.48	0.69
5) Wrist fibrocartilage	95.1	0.93	0.66	0.87
6) Acromioclavicular joint	62.5	0.88	0.68	0.76
7) Hip*	42.9	0.82	0.58	0.66
7a) Hip labrum	61.7	0.73	0.32	0.47
7b) Hip cartilage	23.9	0.91	0.67	0.83

Strength of agreement: <0.20: poor; 0.21–0.40: fair; 0.41–0.60: moderate; 0.61–0.80: substantial; 0.81–1.00: excellent.
*Hip is the kappa value including both sites of evaluation of the hip (hip labrum and hip cartilage).
PABAK, prevalence-adjusted bias-adjusted kappa.

longitudinal plane by placing the probe on the anterior aspect of the joint and sliding posteriorly making the most of the available acoustic window. Transverse scans were optional. The hip HC and HL were assessed only on the anterior aspect of the joint to reduce the discomfort of the patient as much as possible. Both structures were assessed mainly on the longitudinal axis, and transverse scans were optional. Also in this case, the sonographers were asked to use the entire available acoustic window and scan the largest possible portion of the structures under examination. Specifically, with regards to better identifying CPP deposits in the TFC, exam was done by sliding the probe over the structure, without lifting it, from the dorsal to the palmar aspect in longitudinal scanning and from proximal to distal for the transverse scanning. Dynamic scanning was also used by moving the wrist on the coronal plane and/or by pressing with the probe on the TFC when necessary to identify any pitfalls.

Each sonographer had 8 min to assess each joint of interest. After time expiration, the sonographer moved to the next station until every sonographer examined all patients. Power Doppler (PD) examination was not necessary for CPPD identification, but PD exam was allowed on sonographers' judgement to better identify anatomical landmarks (vessels) or avoid pitfalls/artefacts (posterior enhancement of vessels that could mimic CPPD). Each sonographer rated the images according to a dichotomous score (presence/absence) by applying the previously published OMERACT definitions.[7] The definitions were printed and provided to each sonographer before the exercise to avoid misinterpretations.

The procedure was repeated twice with the same patients the same day (morning and afternoon) to assess the intraobserver reliability.

Atlas of CPPD images

During the patient-based exercise (both the current one and the previously published[7]), sonographers were asked to save a representative image of each structure they examined. Three members of the panel reviewed all the stored images and choose four images that they considered to be the most representative images based on the OMERACT US definitions for CPPD at each site in order to create a US atlas.

Statistical analysis

Intraobserver and interobserver reliability were calculated using the kappa coefficient. Intraobserver reliability was assessed by Cohen's kappa. Interobserver reliability was studied by calculating the mean kappa on all pairs (ie, Light's kappa).[16] Kappa coefficients were interpreted according to Landis and Koch.[17] Kappa values of 0–0.20 were considered poor, 0.20–0.40 fair, 0.40–0.60 moderate, 0.60–0.80 good and 0.80–1 excellent. The percentage of observed agreement (ie, percentage of observations that obtained the same score), prevalence of the observed lesions and prevalence-adjusted bias-adjusted kappa were also calculated were also calculated.[18] Analyses were performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Web-based interobserver and intraobserver reliability exercise

All participants successfully completed both rounds of the web-based exercise. Inter-reader reliability, including both rounds, ranged from 0.56 (moderate agreement) reached for the HL to 0.75 (good agreement) achieved for the TFC and AC joint. Intraobserver reliability was higher in all sites varying from a minimum value of 0.73 (good agreement) for the HL to a maximum value of 0.85 (very good agreement) for the AC joint. Detailed results of the patient-based exercise are shown in table 1.

Patient-based interobserver and intraobserver reliability

The patient-based exercise was successfully completed in two rounds of approximately 3 hours each: one in the morning and one in the afternoon of the same day. Interobserver reliability, including both rounds, ranged from 0.19 (poor agreement) for the HL to 0.82 (excellent agreement) for the TFC. Intraobserver reliability was higher in all sites and varied from 0.47 (moderate agreement) for the HL to 0.87 (excellent agreement) for the TFC. Detailed results of the patient-based exercise are presented in table 2.

US atlas of CPPD images

A US atlas of representative CPPD images of the various joints according to the new OMERACT definitions was created from selected images. More than 5500 files were reviewed, and the most representative images have been included in the atlas as shown in figure 1. The atlas also includes sites assessed in the previous exercise[7] in order to provide a more comprehensive pictorial document that can be used as reference for the evaluation of CPPD by US.

DISCUSSION

CPPD is considered to be one of the most frequent arthropathies of the elderly[2 11]; however, its true prevalence and incidence rates remain uncertain, and its impact on the health and disability of the persons affected is unknown. One of the major reasons for

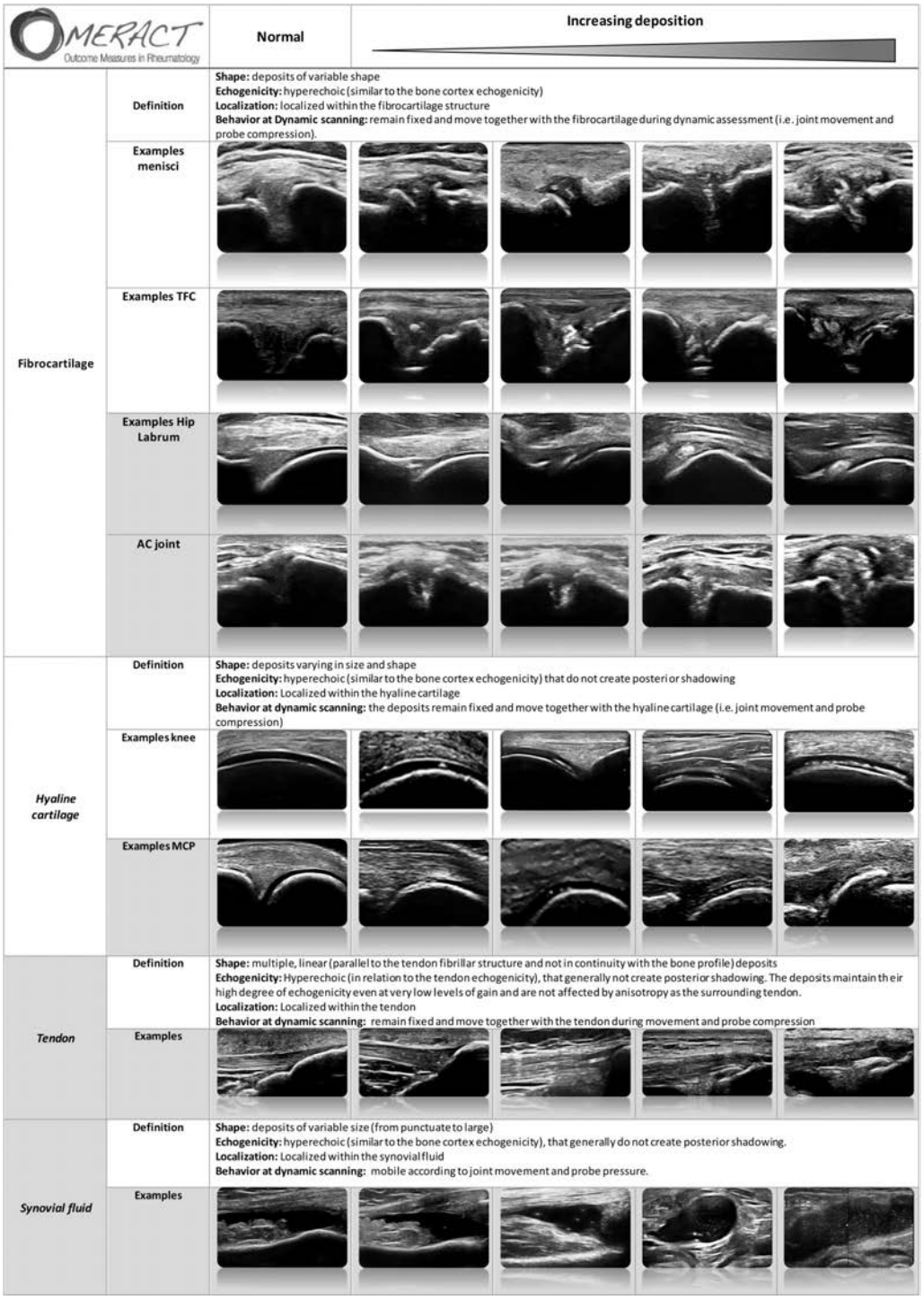


Figure 1 Atlas of images of CPPD in the sites assessed by the OMERACT US CPPD group. The sites in white background achieved good reliability values during the exercises, while the sites in grey background did not. AC, acromioclavicular; CPPD, calcium pyrophosphate deposition disease; MCP, metacarpal-phalangeal joint; TFC, triangular fibrocartilage of the wrist; US, ultrasound.

this discrepancy is that the only available methods for diagnosing CPPD until recently has been synovial fluid analysis and/or X-rays, which are invasive modalities and cannot be applied to large-scale epidemiological studies. Considering this aspect, one could also stipulate that the prevalence of CPPD is also underestimated because only patients with symptoms undergo these two diagnostic exams and a large proportion of our elderly population present the asymptomatic form and are never diagnosed. Furthermore, X-rays have been demonstrated to have a low sensitivity for the identification of CPP deposition,[5] while synovial fluid analysis is not always performed, and CPP crystal are often missed contributing to the underdiagnosis.

Over the last decade, an increasing number of researchers have focused on the use of US in the identification of CPPD, highlighting the potential utility of this technique. The OMERACT US group acknowledged the growing evidence and interest of this application of US and founded the special interest group on CPPD with the objective to assess and standardise the potential use of the technique, following the OMERACT methodology. After creating for the first time the definitions for US

identification of CPPD in various sites,[7] the OMERACT CPPD subtask force gathered in Siena for the first reliability CPPD US exercise[7] in 2015. In that occasion, due to time limitations for the relatively large number of sonographers and patients, it was chosen to assess the hyaline cartilage of the knee, the menisci, the tendons of the knee, the TFC and the synovial fluid. In that first exercise, the results showed good reliability for the menisci and the hyaline cartilage of the knee, but kappa values were low for the tendons, synovial fluid and for the wrist (TFC).

It was a common feeling at that time that high kappa values would be very difficult to reach for the tendons and the synovial fluid because of the big variety of conditions that could resemble CPP deposition according to the definition (ie, enthesophytes for the tendons, gout aggregates in tendons and synovial fluid and bubble airs in synovial fluid). However, there was enough evidence to convince the group that scanning the fibrocartilage and the cartilage should be sufficient to identify CPPD also in early stages and before the other diagnostic methods.[12 19–21] Furthermore, the main sites of involvement in CPPD appear to be the joints, rich in fibrocartilage and hyaline cartilage such as knee, hip and wrist[7 9 10] as suggested by the pathogenetic mechanisms and the role of chondrocytes in the formation of CPP deposition.[22 23]

Given this, the group agreed that it was important to assess the reliability and the potential of US in a larger number of joints and mainly to test again the wrist joint (TFC) as it seems to be one of the most frequently involved sites.[8] During the second reliability exercise, a long briefing was held the day before the patient-based exercise in order to better define the scanning protocol and to test again the definitions on static images. The new EULAR standardised procedures for US[15] were followed and considered sufficient for most of the joints plus the specific scanning of the triangular fibrocartilage (including dynamic scanning) as explained in the methods.

The results of the static exercise (both inter-reader and intra-reader) and the kappa values of the intra-reader agreement of the patient based exercise were generally good, but the inter-reader agreement of the patient-based exercise yielded good kappa values only for the TFC of the wrist (0.82: excellent) followed by the AC joint (0.52: moderate) and with other sites ranging from poor to fair, though not acceptable. That means that definitions were understood and applied, but the scanning technique probably needs to be further refined as differences in the kappa values can be only ascribed to different evaluation of the site under investigation by the sonographer at the time of examination.

However, as the most involved single sites in CPPD seem to be the wrist and the knee (TFC, menisci and HC of the knee), a most extensive evaluation and new exercises aimed to improve the kappa values in other sites could be superfluous if the diagnostic accuracy of the definitions is not good enough to allow their application in the clinical practice and/or for research purposes. For these reasons, the OMERACT US CPPD group agreed to move to the assessment of the diagnostic accuracy of the definitions at the knee before considering again the possibility to refine the scanning technique at sites that do not reach high reliability values.

This study was carried out by a group of rheumatology experts in musculoskeletal ultrasound (MSUS), but not all of them are experts in CPPD imaging. The members of the group adhere liberally to the initiative from the pool of sonographers of the OMERACT US group because of the interest on the disease even if they had not research experience previously on CPPD but only the skills that they developed with the daily clinical practice. The achievement of good kappa values between experts in CPPD and expert sonographers without particular interest before this study on CPPD suggests that the results could be reproducible also in the real world between expert sonographers. Furthermore, at the briefing session, the group decided to adopt the EULAR recommended scanning procedures[15] that are freely available for every sonographer. Finally, in the *US atlas of CPPD images* included in this paper, the reader may find a comprehensive review of the US aspect of CPPD deposition in the different sites investigated during the two workshops even if they did not reach high reliability values. The images included in the CPPD US atlas portray some of the typical aspects of CPPD deposition according to the OMERACT definitions from a single CPP aggregate to diffuse deposition in order to provide a pictorial sample that can be used by both clinicians and researchers as a guide for diagnostic and research purposes.

In conclusion, US identification of CPPD is one of the most challenging applications of US in rheumatology. However, the lack of a harmless and non-invasive technique for the diagnosis of CPPD before the advent of US makes the research on US even more important. US could allow large epidemiological studies, follow-up of patients and, possibly, efficacy studies of drugs regarding either the inflammatory aspects of the disease or the joint damage/deposition extension due to CPPD.

Author affiliations
[1]Department of Medical Sciences, Section of Rheumatology, University of Ferrara and Azienda Ospedaliero-Universitaria Sant'Anna di Cona, Ferrara, Italy
[2]University of Siena, Siena, Italy
[3]University of Belgrade, Belgrade, Serbia
[4]SIR Epidemiology Unit, Milan, Italy
[5]Department of Rheumatology, MC Groep, Lelystad, The Netherlands
[6]Instituto de Rehabilitación, Buenos Aires, Argentina
[7]Université Versailles Saint-Quentin en Yvelines, Paris, France
[8]University of Pisa, Pisa, Italy
[9]Rheumatology Unit, University Hospital Fundación Santa Fe de Bogota, Bogota, Colombia
[10]Università Politecnica delle Marche, Jesi, Italy
[11]Department of Rheumatology, APHP, CHU Pitie-Salpetriere, Paris, France
[12]Instituto Nacional de Rehabilitación, Mexico City, Mexico
[13]North Valley Hospital, Whitefish, Montana, USA
[14]Rehabilitation Clinical Hospital, Cluj-Napoca, Romania
[15]Instituto Poal, University of Barcelona, Barcelona, Spain
[16]Rheumatology department, Lapeyronie Hospital & EA 2415, Montpellier, France
[17]Department of Rheumatology and Rehabilitation, Zagazig University, Zagazig, Egypt
[18]Department of Rheumatology, Joint and Bone Research Unit, Hospital Universitario Fundación Jiménez Díaz and Autónoma University, Madrid, Spain
[19]ASL 3, Pistoia, Italy
[20]Division of Rheumatology, The Warren Alpert School of Medicine of Brown University, Providence, Rhode Island, USA
[21]Department of Rheumatology, Internal Medicine Clinic, Kalmar County Hospital, Kalmar, Sweden
[22]Immanuel Krankenhaus Berlin, Berlin, Germany
[23]Rheumatology Unit, Università di Torino, Torino, Italy
[24]Center for Rheumatology and Spine Diseases, Rigshospitalet, Copenhagen, Denmark
[25]Sf. Maria HospitaL, Bucharest, Romania
[26]Rheumatology Department, University of Medicine and Pharmacy Craiova, Craiova, Romania
[27]Lausanne University Hospital, Lausanne, Switzerland
[28]Department of Rheumatology, University of Ioannina, Ioannina, Greece
[29]Rheumatology Department of Lucania, "San Carlo" Hospital of Potenza and "Madonna delle Grazie" Hospital of Matera, Potenza, Italy

Acknowledgements The authors greatly acknowledge the contribution of General Electric Healthcare 27 Italy and Samsung Electronics Italia s.p.A for providing the US equipment for free for this study.

Contributors Planning of the work: GF, CAS, AA, GC and AI. Conducting of the work: all authors. Reporting of the work: GF, CAS, NSD, GAWB, MAD, EF, IMP, EN, CP, AMR, LT and AI.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

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 Comitato Etico di Area Vasta Sud-Est (siena).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Sivera F, Andrés M, Pascual E. Current advances in therapies for calcium pyrophosphate crystal arthritis. *Curr Opin Rheumatol* 2016;28:140–4.
2 Ciancio G, Bortoluzzi A, Govoni M. Epidemiology of gout and chondrocalcinosis. *Reumatismo* 2012;63:207–20.
3 Salaffi F, De Angelis R, Grassi W, *et al*. Prevalence of musculoskeletal conditions in an Italian population sample: results of a regional community-based study. I. The MAPPING study. *Clin Exp Rheumatol* 2005;23:819–28.
4 Musacchio E, Ramonda R, Perissinotto E, *et al*. The impact of knee and hip chondrocalcinosis on disability in older people: the ProVA Study from northeastern Italy. *Ann Rheum Dis* 2011;70:1937–43.
5 Filippou G, Adinolfi A, Cimmino MA, *et al*. Diagnostic accuracy of ultrasound, conventional radiography and synovial fluid analysis in the diagnosis of calcium pyrophosphate dihydrate crystal deposition disease. *Clin Exp Rheumatol* 2016;34.
6 Zhang W, Doherty M, Bardin T, *et al*. European league against rheumatism recommendations for calcium pyrophosphate deposition. Part I: terminology and diagnosis. *Ann Rheum Dis* 2011;70:563–70.
7 Filippou G, Scirè CA, Damjanov N, *et al*. Definition and reliability assessment of elementary ultrasonographic findings in calcium pyrophosphate deposition disease: a study by the OMERACT calcium pyrophosphate deposition disease ultrasound subtask force. *J Rheumatol* 2017;44:1744–9.
8 Filippou G, Filippucci E, Tardella M, *et al*. Extent and distribution of CPP deposits in patients affected by calcium pyrophosphate dihydrate deposition disease: an ultrasonographic study. *Ann Rheum Dis* 2013;72:1836–9.
9 Abhishek A, Doherty S, Maciewicz R, *et al*. Chondrocalcinosis is common in the absence of knee involvement. *Arthritis Res Ther* 2012;14:R205.
10 Abhishek A, Doherty M. Update on calcium pyrophosphate deposition. *Clin Exp Rheumatol* 2016;34:32.
11 Abhishek A. Calcium pyrophosphate deposition disease: a review of epidemiologic findings. *Curr Opin Rheumatol* 2016;28:133–9.
12 Filippou G, Adinolfi A, Iagnocco A, *et al*. Ultrasound in the diagnosis of calcium pyrophosphate dihydrate deposition disease. A systematic literature review and a meta-analysis. *Osteoarthritis Cartilage* 2016;24:973–81.
13 Boers M, Kirwan JR, Gossec L, *et al*. How to choose core outcome measurement sets for clinical trials: OMERACT 11 approves filter 2.0. *J Rheumatol* 2014;41:1025–30.
14 Kottner J, Audigé L, Brorson S, *et al*. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. *J Clin Epidemiol* 2011;64:96–106.
15 Möller I, Janta I, Backhaus M, *et al*. The 2017 EULAR standardised procedures for ultrasound imaging in rheumatology. *Ann Rheum Dis* 2017;76:1974–9.
16 Light RJ. Measures of response agreement for qualitative data: some generalizations and alternatives. *Psychol Bull* 1971;76:365–77.
17 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159–74.
18 Byrt T, Bishop J, Carlin JB. Bias, prevalence and kappa. *J Clin Epidemiol* 1993;46:423–9.
19 Filippucci E, Di Geso L, Girolimetti R, *et al*. Ultrasound in crystal-related arthritis. *Clin Exp Rheumatol* 2014;32:S42–7.
20 Filippucci E, Scirè CA, Delle Sedie A, *et al*. Ultrasound imaging for the rheumatologist. XXV. Sonographic assessment of the knee in patients with gout and calcium pyrophosphate deposition disease. *Clin Exp Rheumatol* 2010;28:2–5.
21 Filippou G, Frediani B, Gallo A, *et al*. A "new" technique for the diagnosis of chondrocalcinosis of the knee: sensitivity and specificity of high-frequency ultrasonography. *Ann Rheum Dis* 2007;66:1126–8.
22 Abhishek A, Doherty M. Pathophysiology of articular chondrocalcinosis--role of ANKH. *Nat Rev Rheumatol* 2011;7:96–104.
23 Ryan L, McCarty D. Calcium pyrophosphate crystal deposition disease, pseudogout and articular chondrocalcinosis. In: *Arthritis and allied conditions. A textbook of Rheumatology*. Philadelphia, USA: Lea & Febiger, 1997:2013–25.

EXTENDED REPORT

Low miR200b-5p levels in minor salivary glands: a novel molecular marker predicting lymphoma development in patients with Sjögren's syndrome

Efstathia K Kapsogeorgou,[1,2] Aristea Papageorgiou,[1,2] Athanase D Protogerou,[1,2] Michael Voulgarelis,[1,2] Athanasios G Tzioufas[1,2]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212639).

[1]Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
[2]Academic Joint Rheumatology Program, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Correspondence to
Professor Athanasios G Tzioufas, Department of Pathophysiology, School of Medicine, National University of Athens, Athens 11527, Greece; agtzi@med.uoa.gr

Received 2 November 2017
Revised 21 April 2018
Accepted 24 April 2018
Published Online First 19 May 2018

ABSTRACT

Objectives Development of non-Hodgkin's lymphoma (NHL) is the major adverse outcome of Sjögren's syndrome (SS) affecting both morbidity and mortality. Preliminary evidence suggested that, although not deregulated compared with sicca controls, miR200b-5p levels are decreased in the minor salivary glands (MSGs) of SS patients with NHL. The aim of the current study was to evaluate the MSG expression of miR200b-5p in SS-associated NHLs and its potential predictive value for the identification of patients with SS susceptible to develop NHL.

Methods miR200b-5p expression was investigated in MSG tissues of patients with SS who were at: (A) low risk and did not develop NHL during follow-up (n=27; median follow-up time on biopsy performance, range: 8.9 years, 1.33–14 years), (B) high-risk and diagnosed with NHL during follow-up (prelymphoma; n=17; median follow-up to until lymphoma diagnosis, range: 3.67 years, 0.42–8.5 years) and (C) had NHL (n=35), as well as non-SS sialadenitis controls (sarcoidosis and hepatitis C virus (HCV) infection, four each). The differential miR200b-5p expression, correlations with disease features and its discriminative/predictive value, was evaluated by appropriate statistical approaches.

Results The MSG levels of miR200b-5p were significantly downregulated in patients with SS who will develop or have NHL and strongly discriminated ($p<0.0001$) them from those without lymphoma or non-SS sialadenitis. Furthermore, they were reduced long before clinical onset of lymphoma, did not significantly change on transition to lymphoma and, importantly, were proved strong independent predictors of patients who will develop NHL ($p<0.0001$).

Conclusions These findings support that miR200b-5p levels in MSGs represent a novel predictive and possibly pathogenetic mechanism-related factor for the development of SS-associated NHL, since its expression is impaired years before lymphoma clinical onset.

INTRODUCTION

Primary Sjögren's syndrome (SS) is an autoimmune disease with a diverse clinical picture ranging from benign, mild exocrinopathy to severe, systemic, disorder with high prevalence (5%–10%) of B cell non-Hodgkin's lymphoma (NHL).[1 2] NHL is the major adverse outcome of the disease, affecting both morbidity and mortality.[3–5] Several clinical, laboratory and histological features, including high EULAR SS disease activity index (ESSDAI) score, salivary gland enlargement (SGE), purpura, vasculitis, leucopenia, cryoglobulinaemia, hypocomplementaemia, rheumatoid factor, formation of germinal centres (GCs) in the histopathological lesion and infiltration by certain cell types, such as macrophages, have been associated with the development of lymphoma in SS.[3 4 6–11]

The miR200 micro-RNA (miRNA) family, consisting of miR200a, miR200b, miR429, miR141 and miR200c miRNAs, possesses a central role in oncogenesis, tumour metastasis and drug resistance. The miR200b-3p and miR200b-5p miRNAs are considered powerful regulators of epithelial-to-mesenchymal transition (EMT) and as such they have been implicated in the oncogenesis of solid tumours.[12–17] Recently, in the context of investigating the expression of several miRNAs that are predicted to target the Ro/SSA and La/SSB autoantigens, we examined the expression of miR200b-3p and miR200b-5p in the minor salivary glands (MSGs) of SS patients and sicca controls.[18] Although their expression in the MSGs of patients with SS was not deregulated compared with sicca controls, miR200b-5p levels were significantly reduced in four SS patients with mucosa-associated lymphoid tissue (MALT) lymphomas compared with those without.[18]

Prompted by this finding, we sought to: (A) validate the hypothesis that miR200b-5p levels are decreased in the MSGs of patients with SS-associated NHL by studying its expression in an adequate population of SS patients with or without NHL, as well as in sialadenitis controls; (B) evaluate its predictive value by investigating its expression in MSG samples from both low-risk patients with SS who did not develop lymphoma during follow-up and high-risk patients who developed SS-associated NHL in the future; (C) test its independent predictive utility over that of previously identified adverse predictive factors for the development of SS-associated NHL.

MATERIALS AND METHODS

Patients

MSG samples obtained from 79 patients with primary SS and 8 non-SS sialadenitis associated with sarcoidosis and HCV infection (four each) were studied after informed consent. Patients with SS were diagnosed according to the American–European classification criteria.[19] The patients with SS included 27 low risk who did not develop

To cite: Kapsogeorgou EK, Papageorgiou A, Protogerou AD, *et al*. *Ann Rheum Dis* 2018;**77**:1201–1208.

lymphoma during follow-up (without lymphoma (SSwo); median follow-up time on biopsy performance, range: 8.9 years, 1.33–14 years), 17 high risk who were diagnosed with SS-associated NHL during follow-up (prelymphoma (SSpL); median follow-up time to NHL diagnosis, range: 3.67 years, 0.42–8.5 years) and 35 with SS-associated lymphoma at the time of biopsy (lymphoma (SSL)). The low-risk group consisted of SS patients with low probability to develop lymphoma,[11 20] including 17 without risk factors for lymphoma development, 5 with low serum C4 levels, four with SGE and one with both low serum C4 levels and SGE (median follow-up time of the patients expressing adverse predictive factors: 8.47 years, range: 4.91–12.91 years). The prelymphoma SS group included 14 MALTs, 2 nodal marginal zone lymphomas (NMZLs) and 1 diffuse large B cell lymphoma (DLCBL). The SS-associated NHLs consisted of 28 MALTs, 2 NMZLs, 2 DLCBLs, 1 B cell bronchial associated lymphoid tissue (BALT), 1 lymphoplasmacytic (LP) and 1 small lymphocytic (SLL) lymphoma. In 14 cases (11 MALTs, 2 NMZLs and 1 DLCBL), the SSpL and SSL samples were paired sequential specimens from the same patient (before and on lymphoma onset). All prelymphoma and lymphoma SS samples that had available MSG specimens from a total of 84 SS patients with NHL who were followed up during 1993–2016 in the Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Greece, were studied.

The medical records were retrospectively evaluated for various clinical, laboratory and histological parameters of SS and lymphoma that are described in table 1. The characteristics of the patients are summarised in table 2, whereas lymphoma features in table 3 and online supplementary table S1. Three SS patients had received corticosteroids, four hydroxychloroquine, one cyclophosphamide and five B cell depletion therapy (anti-CD20) prior to biopsy performance.

Table 1 Clinical, laboratory and histological features of the patients with SS that were retrospectively recorded and their definition

Features	Defined as/documented by
SS associated	
Clinical	
EULAR SS disease activity index (ESSDAI)	31
Arthralgias, arthritis	
Raynaud's phenomenon	
Salivary gland enlargement (SGE)	
Lung involvement	Pulmonary function tests and X-ray and/or CT scans
Renal involvement	Persistent proteinuria and verification by renal biopsy
Liver involvement	Liver biopsy indicative of primary biliary cirrhosis
Palpable purpura	
Vasculitis	
Peripheral neuropathy	Nerve conduction studies
Laboratory	
Anti-Ro/SSA and/or anti-La/SSB autoantibodies	
Rheumatoid factor	
Complement C3 and C4 levels	
Hypocomplementaemia	C4 <16 mg/dL and C3 <75 mg/dL
Cryoglobulinaemia	
Hypergammaglobulinaemia	Total gammaglobulins >2 g/L
Anaemia	Haemoglobin <12 g/dL (females) and 13.5 g/dL (males)
Leucopenia	White cell count <4000/mm^3
Lymphopaenia	Lymphocyte count <1000/mm^3
Neutropaenia	Neutrophil count <1500/mm^3
Histological	
Biopsy focus score	Number of lymphocytic foci/4 mm^2
Germinal centre formation	
Lymphoma associated	
Non-Hodgkin's lymphoma (NHL) subtype	
Eastern Cooperative Oncology Group performance status	32
Ann Arbor stage (I–IV)	33
Number and type of involved sites	
International Prognostic Index	0–1 points: low risk, 2 points: low-intermediate risk, 3 points: high-intermediate risk, 4–5 points: high risk[34]
Splenomegaly	
Lymphadenopathy	
Presence of B symptoms	
Serum lactate dehydrogenases	
β2-microglobulin levels	

SS, Sjögren's syndrome.

Evaluation of miR200b-5p levels by quantitative PCR (qPCR)

MSG tissues were obtained during diagnostic biopsy, fresh-frozen in RNAlater stabilisation reagent (Qiagen, Venlo, The Netherlands) according to manufacturer's instructions and stored at −80°C until RNA isolation. Total RNA including small RNA molecules, such as miRNAs, was isolated from an MSG lobe using the mirVana PARIS kit (Ambion, Applied Biosystems, Carlsbad, California, USA) according to the manufacturer's instructions. The time of storage has not been found to significantly affect the quantity or quality of the isolated RNA (data not shown). Subsequently, 0.25 μg RNA were reverse-transcribed by TaqMan miRNA reverse transcription kit and miRNA-specific primers (TaqMan-MicroRNA Assays, Applied Biosystems), followed by miRNA-specific amplification by qPCR using primers from TaqMan-MicroRNA Assays and TaqMan Universal PCR Master Mix, no AmpErase UNG (Applied Biosystems). RNU48 small nucleolar RNA (snoRNA) was selected for normalisation after NormFinder analysis.[21] Three snoRNAs, namely U6, RNU44 and RNU48, have been examined in preliminary experiments, including samples from sicca controls, low-risk SS patients without lymphoma and patients with SS-NHL (six in each group) for identifying the most appropriate reference. In these experiments, a common reverse transcription using Megaplex RT Primers, Human Pool B V.3.0 (Applied Biosystems) was performed in order to exclude variations due to distinct RT reactions. NormFinder analysis[21] revealed that RNU48 had the higher stability value (0.006) compared with RNU44 (stability value: 0.008) or U6 miRNA (stability value: 0.016). The relative quantification of miRNA expression was performed by the $2^{-\Delta\Delta CT}$ method using non-malignant parotid gland tissue from a patient subjected to parotidectomy as calibrator. The relative occurrence of various cell types, including epithelial cells, B cells, T cells and macrophages, in MSG samples was evaluated by qPCR for keratin-8 (KRT8), CD19, CD3D and CD68, respectively, using TaqMan expression assays. Samples were processed randomly and without grouping. All samples were run in duplicates. Additionally, in a proportion of patients (six from each SS subgroup and four sialadenitis controls; randomly selected) the infiltration by $CD20^+$ B cells, $CD3^+$ T cells and $CD68^+$ macrophages

Table 2 Characteristics of the patients studied

Features	Non-SS	Patients with SS		
	Sialadenitis (n=8)	SSwo (n=27)	SSpL (n=17)	SSL (n=35)
General				
Age (years), median (range)	61.5 (53–74)	55 (30–76)	35 (24–75)	43.5 (26–79)
Men/women	2/6	0/27	1/16	4/31
Duration (years) of sicca symptoms, median (range)	0.65 (0.1–5.0)	3 (0.5–10.0)	5.7 (1.0–13.0)	8.0 (0.5–36.0)
Histological (MSG biopsy)				
Biopsy focus score (number of lymphocytic foci/4mm^2), median (range)	0.42 (0.0–4.4)	1.45 (1.0–4.0)	4.44 (1.0–11.5)	5.14 (1.0–9.23)
Germinal center formation, n (%)	0 (0)	3 (11.1)	8 (47.1)	16 (45.7)
Clinical				
Arthralgias, n (%)	0 (0)	8 (29.6)	5 (29.41)	12 (34.3)
Arthritis, n (%)	0 (0)	3 (11.1)	1 (5.9)	3 (8.6)
SG enlargement, n (%)	0 (0)	5 (18.5)	9 (52.9)	26 (74.3)
Raynaud's phenomenon, n (%)	0 (0)	6 (22.2)	5 (29.4)	6 (17.1)
Parenchymal organ involvement, n (%)	0 (0)	1 (3.7)	1 (5.9)	4 (11.4)
Lung involvement, n (%)	0 (0)	1 (3.7)	1 (5.9)	4 (11.4)
Renal involvement, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
Liver involvement, n (%)	0 (0)	0 (0)	0 (0)	0 (0
Indicative of vasculitic involvement, n (%)	0 (0)	0 (0)	7 (41.2)	18 (51.4)
Palpable purpura, n (%)	0 (0)	0 (0)	7 (41.2)	18 (51.4)
Vasculitis (%), n (%)	0 (0)	0 (0)	3 (17.6)	4 (11.4)
Glomerulonephritis, n (%)	0 (0)	0 (0)	1 (5.9)	4 (11.4)
Peripheral neuropathy, n (%)	0 (0)	0 (0)	2 (11.8)	6 (17.1)
ESSDAI score, median (range)	NA	1 (0–4)	9 (1–14)	10 (4–19)
Laboratory				
Anti-Ro/SSA and/or La/SSB positive, n (%)	0 (0)	17 (62.96)	11 (64.7)	28 (80)
Anti-Ro/SSA positive, n (%)	0 (0)	17 (63.0)	11 (64.7)	28 (80)
Anti-La (SSB) positive, n (%)	0 (0)	3 (11.1)	8 (47.1)	20 (57.1)
Rheumatoid factor positive, n (%)	1 (12.5)	9 (33.3)	12 (70.6)	28 (80)
C3 levels, median (range)	84 (79–118)	105 (74–139)	87.5 (65–191)	98.2 (36–191)
C4 levels, median (range)	24.5 (13–36.0)	22.0 (4.0–47.3)	10.4 (1.0–28.0)	10.4 (1.0–30.0)
C4 hypocomplementaemia, n (%)	1 (12.5)	7 (25.9)	10 (58.8)	24 (68.6)
Cryoglobulinaemia, n (%)	0 (0)	0 (0)	4 (23.5)	11 (31.4)
Hypergammaglobulinaemia, n (%)	0 (0)	2 (7.4)	10 (58.8)	22 (62.9)
Leucopenia, n (%)	0 (0)	2 (7.4)	1 (5.9)	4 (11.4)
Treatment, n (%)				
Corticosteroids	0 (0)	0 (0)	2 (11.8)	3 (8.5)
Hydroxychloroquine	0 (0)	2 (7.4)	2 (11.8)	2 (5.7)
Cyclophosphamide	0 (0)	0 (0)	1 (5.9)	1 (2.9)
Rituximab	0 (0)	0 (0)	1 (5.9)	5 (14.3)

ESSDAI, EULAR SS disease activity index; MSG, minor salivary gland; NA, not applicable; SG, salivary gland; SS, Sjögren's syndrome; SSL, SS-associated lymphoma; SSpL, pre-lymphoma; SSwo, without lymphoma.

(number of infiltrating cells per mm^2 tissue area), as well as its correlation with miR200b-5p levels were analysed immunohistochemically using ImageJ software, as previously.[7 22]

Statistical analyses

The differential expression of miR200b-5p levels among the subgroups of patients with SS and sialadenitis controls was evaluated by the non-parametric Tukey's multiple comparison test. Significant differences in miR200b-5p levels between patients expressing or not various clinical, histological and serological markers were analysed by the non-parametric Mann-Whitney U test, whereas associations with patient features and overtime changes by Spearman's rank correlation and Wilcoxon's matched pairs tests, respectively. Holm-Bonferroni sequential correction was applied for correcting for multiple comparisons.[23]

Analyses regarding the diagnostic/discriminative utility of miR200b-5p were performed in two levels: (A) all patients with SS who did not have lymphoma at the time of biopsy (SSwo and SSpL) were compared with lymphoma patients (SSL) and (B) comparisons among the three SS subgroups. The 14 patients that were common in the prelymphoma and lymphoma SS group were excluded from lymphoma group, thus remaining 21 SSL patients in the analyses. The diagnostic/discriminative ability of miR200b-5p levels was evaluated by receiver operating characteristic (ROC) curve analysis. Categorical variables were compared by the Pearson χ^2 or the Fisher's exact

Table 3 Features of the patients with SS-associated NHLs (SSL)

Features	Patients with SSL (n=35)
Type	
MALT, n (%)	28 (80)
NMZL, n (%)	2 (5.7)
LP, n (%)	1 (2.8)
DLCBL, n (%)	2 (5.7)
SLL, n (%)	1 (2.8)
BALT, n (%)	1 (2.8)
Involved organs	
Nodal, n (%)	8 (22.9)
Extranodal	
MSG, n (%)	26 (74.3)
Parotid gland (PG), n (%)	7 (20.0)
Both MSG and PG, n (%)	4 (11.4)
Stomach, n (%)	3 (8.5)
Lung, n (%)	2 (5.7)
Nodal and extranodal, n (%)	4 (11.4)
Bone marrow infiltration, n (%)	10 (28.6)
Splenomegaly, n (%)	4 (11.4)
Ann Arbor staging	
I, n (%)	17 (48.6)
II, n (%)	0 (0.0)
III, n (%)	3 (8.5)
IV, n (%)	15 (42.9)
IPI score	
0, n (%)	7 (20.0)
1, n (%)	5 (14.3)
2, n (%)	15 (42.9)
3, n (%)	7 (20.0)
4, n (%)	1 (2.8)
ECOG	
0, n (%)	30 (85.7)
1, n (%)	5 (14.3)
EFS (months), median (range)	61.7 (14–206)
OS (months), median (range)	68.4 (14–206)

BALT, B cell bronchial associated lymphoid tissue; DLCBL, diffuse large B cell lymphoma; ECOG, Eastern Cooperative Oncology Group performance status; EFS, event-free survival; IPI, International Prognostic Index; LP, lymphoplasmacytic; MALT, mucosa-associated lymphoid tissue; MSG, minor salivary gland; NHL, non-Hodgkin's lymphoma; NMZL, nodal marginal zone lymphoma; OS, overall survival; SLL, small lymphocytic lymphoma; SS, Sjögren's syndrome.

test, when appropriate. HRs are provided with a 95% CI. To identify independent risk factors for NHL development in SS, all variables associated with lymphoma with a p value less than 0.1 in univariate analysis were further evaluated by multivariate logistic or Cox regression analysis using a backward stepwise exclusion method. The predictive ability of miR200b-5p levels was evaluated by Kaplan-Meier lymphoma-free survival curves compared by the log-rank test in the prelymphoma and without lymphoma SS patients, who were split in two groups according to low or not miR200b-5p expression, as this defined by ROC discriminative value.Similar analysis was performed for low-risk and high-risk patients according to previously described models.[4 9 11] Descriptive analyses of all data were performed.

GraphPad Prism-5 (GraphPad Software, San Diego, California, USA) and SPSS V.17 software were used. Statistical significance was defined as a p value of less than 0.05 for all comparisons; p values were two tailed. Only the statistically significant differences are reported. Using two-sided 95% CI, the observed difference of the means was proved to have 96.5% power (OpenEpi, V.3, open source calculator).

RESULTS

The MSG levels of miR200b-5p are reduced in patients with SS who have or will develop NHL and discriminate them from those who will not

The miR200b-5p levels were significantly lower in the MSGs of high-risk patients with SS who were diagnosed with NHL during follow-up (SSpL; mean relative expression±SD: 0.31±0.33) and lymphoma SS patients (SSL; 0.21±0.25) compared with the low risk that did not develop lymphoma during follow-up (SSwo; 0.72±0.37; $p \leq 0.01$ and $p \leq 0.0001$ for prelymphoma and lymphoma, respectively) or non-SS sialadenitis controls (0.95±0.84, $p \leq 0.01$ and $p \leq 0.001$, respectively) (figure 1A). Low miR200b-5p levels were also detected in patients with SSL who had not lymphoma in MSGs. Interestingly, low miR200b-5p levels (0.17) were also detected in an HBV-patient that had MALT lymphoma (patient excluded from the analysis). Additionally, miR200b-5p levels were not found to significantly change on transition to lymphoma, as indicated by the analysis of 14 sequential paired samples from SS patients before and on lymphoma diagnosis (figure 1B). The significantly lower expression of miR200b-5p in the MSGs of SS patients with NHL long before lymphoma diagnosis indicates that it is possibly implicated in the pathogenesis of SS-associated lymphoma. miR200b-5p levels correlate with clinical, laboratory and histologic factors of adverse outcome and/or NHL development, as well as NHL prognosis.

The low levels of miR200b-5p were associated with several clinical, laboratory and histological features indicative of adverse outcome and lymphoma development that are summarised in table 4. Hence, miR200b-5p levels were negatively correlated with ESSDAI, whereas they were positively correlated with serum C4 levels (table 4). Significantly lower miR200b-5p levels were detected in SS patients with SGE, purpura, peripheral neuropathy, cryoglobulinaemia, hypergammaglobulinaemia and rheumatoid factor compared with those without (table 4). Despite the lower age of SSpL and SSL patients compared with SSwo (table 2), miR200b-5p levels were not correlated with patient age ($p=0.1$), whereas they were negatively correlated with biopsy focus score (table 4); however, this did not affect their higher expression in SSwo, compared with SSpL and SSL patients (supplementary table S3 and supplementary figure S2). Furthermore, it was negatively correlated with CD3D and CD68 mRNA expression in MSGs ($r=-0.5613$, $p<0.0001$ and $r=-0.5048$, $p<0.0001$, respectively), which are indicative of T-lymphocyte and macrophage infiltration, respectively, but not CD19 (B cell; $r=-0.3072$, $p=0.068$) or KRT8 (epithelial cell; $r=0.1536$, $p=0.23$) expression. Subsequent immunohistochemical evaluation of the number of infiltrating $CD3^+$ T cells, $CD20^+$ B cells and $CD68^+$ macrophages per tissue area (mm^2) in randomly selected samples from each group of patients with SS (six patients/group), as well as sialadenitis controls (n=4) revealed that the levels of miR200b-5p in MSGs were negatively correlated with all these types of infiltrating cell populations (table 4).

The levels of miR200b-5p in MSGs were not found to associate with the type, stage or number of involved sites of NHL, as well as event-free or overall-free survival, whereas there was a trend to associate with high-intermediate/high international prognostic index (IPI: 3–4) (mean±SD: 0.24±0.28 vs 0.10±0.06 in patients with low/low-intermediate IPI (0–2),

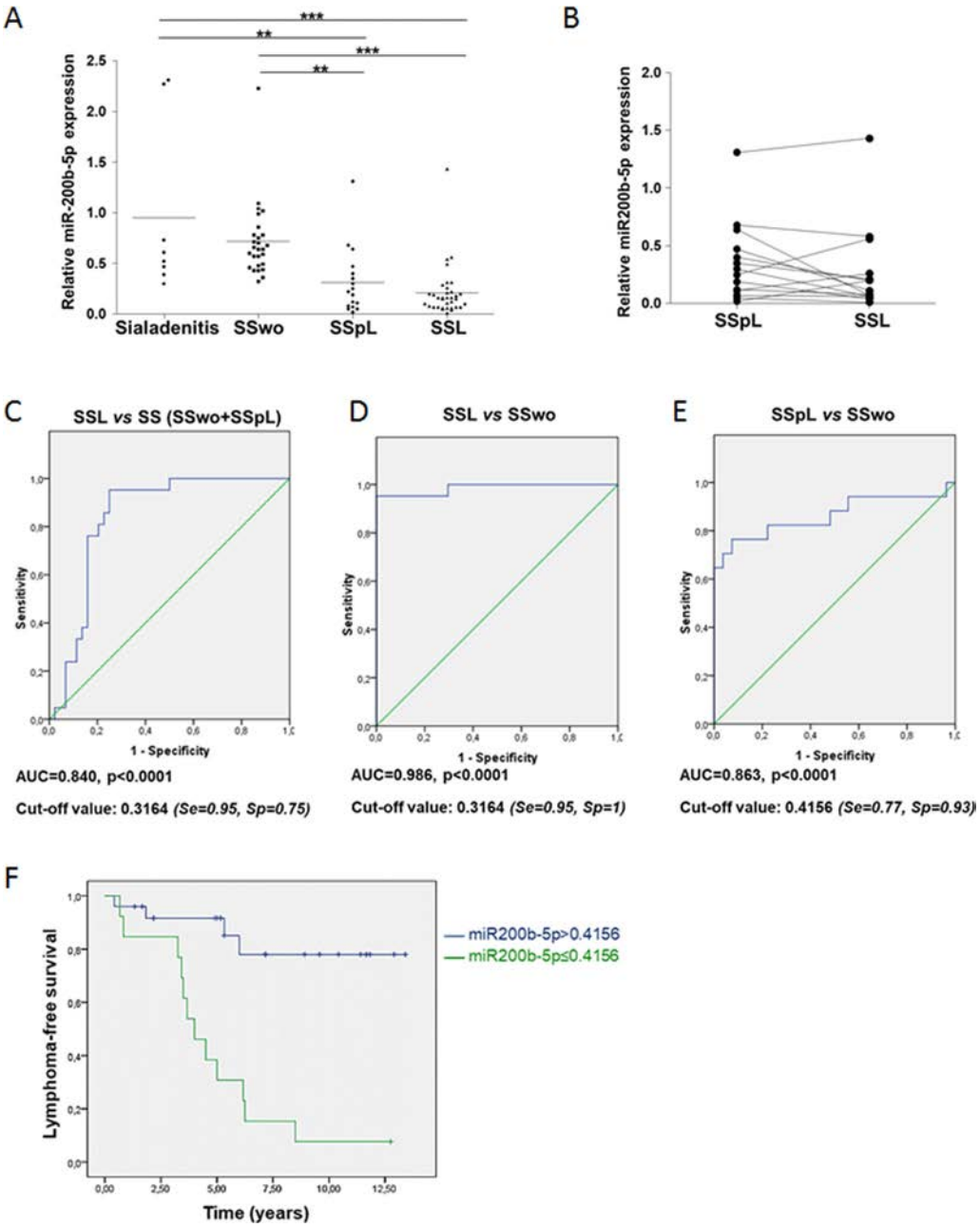


Figure 1 MSG miR-200b-5p levels are downregulated in prelymphoma and lymphoma SS patients, discriminate them from SS patients without lymphoma and predict lymphoma development. (A) Dot plot displaying the expression of miR-200b-5p in the MSG tissues of non-SS sialadenitis controls (sialadenitis), patients with SS who did not develop NHL during follow-up (SSwo), patients with SS who were diagnosed with NHL in the future during follow-up (pre lymphoma; SSpL) and SS patients with NHL (SSL). Comparisons were performed by Tukey's multiple comparison analysis. P values are designated by asterisks (**p<0.01, ***p<0.001), whereas horizontal bars represent the mean value of the group. Only statistically significant associations are indicated. (B) Wilcoxon's matched-pairs analyses of miR-200b-5p expression in sequential MSG-samples from 14 patients with SSpL that transitioned to NHL (SSL) did not reveal any significant changes in miR200b-5p expression before and on lymphoma transition. (C–E) ROC curve analyses of the ability of miR200b-5p to discriminate/diagnose patients with lymphoma SS (SSL) from those without (SSwo and SSpL) at the time of biopsy (C), that were low risk and did not develop lymphoma during follow-up (SSwo) (D), as well as patients with SSpL from those who did not develop lymphoma during follow-up (SSwo) (E). (F) Kaplan-Meier lymphoma-free curves for patients with low miR-200b-5p levels (≤0.4156) (high-risk group; green line) and patients with miR-200b-5p levels (>0.4156) (low-risk group; blue line). AUC, area under the curve; MSG, minor salivary gland; NHL, non-Hodgkin's lymphoma; ROC, receiver operating characteristic; SE, sensitivity; Sp, specificity; SS, Sjögren's syndrome.

p=0.07). miR200b-5p levels in MSGs discriminate patients with SS who have versus those who do not have NHL, as well as those who will develop NHL versus those who will not.

ROC curve analysis revealed that miR200b-5p strongly discriminates prelymphoma and lymphoma SS patients from those without lymphoma. Thus, the lymphoma SS patients were discriminated from SS patients without lymphoma at the time of biopsy (SSwo and SSpL) with AUC and cut-off values 0.840 (p<0.0001) and 0.3164 (sensitivity=0.952, specificity=0.750), respectively. More importantly, prelymphoma and lymphoma SS patients were discriminated from those that did not develop lymphoma during follow-up (SSwo) with AUC values 0.863 and

Table 4 Correlations between miR200b-5p levels and disease parameters associated with severe systemic disease, SS-associated NHL development or lymphoma prognosis, as analysed by Mann-Whitney and Spearman's rank correlation tests corrected for multiple comparisons by Holm-Bonferroni sequential correction

Features	Correlation (r)	P values	miR200b-5p levels (mean±SD) Without	With	P values
Histological *(MSG biopsy)*					
Biopsy focus score (number of lymphocytic foci/4 mm^2)	−0.6012	<0.0001			
CD3$^+$-T cells (number/mm^2 of tissue)	−0.7535	0.002			
CD20$^+$-B cells (number/mm^2 of tissue)	−0.7182	0.008			
CD68$^+$-macrophages (number/mm^2 of tissue)	−0.7808	0.002			
Clinical					
SG enlargement			0.54±0.34	0.30±0.40	0.003
Palpable purpura			0.49±0.41	0.27±0.32	0.033
Peripheral neuropathy			0.45±0.39	0.11±0.09	0.023
NHL non-Hodgkin's lymphoma			0.72±0.37	0.21±0.25	≤0.0001
Laboratory					
Serum C4 levels (mg/dL)	0.4562	<0.0001			
Cryoglobulinaemia			0.48±0.41	0.15±0.11	0.006
Hypergammaglobulinaemia (total gammaglobulins >2g/L)			0.54±0.41	0.29±0.34	0.033
Rheumatoid factor			0.53±0.31	0.35±0.39	0.033
			Low	High	
Indices	−0.5736	<0.0001			
ESSDAI score (value)					
ESSDAI score (low (≤5) versus high (≥6))			0.62±0.39	0.25±0.30	<0.0001

ESSDAI, EULAR SS disease activity index, NHL, non-Hodgkin's lymphoma; SS, Sjögren's syndrome.

0.986 (p<0.0001), respectively, and cut-off values 0.4156 (sensitivity=0.765, specificity=0.926) and 0.3164 (sensitivity=0.952, specificity=1), respectively (figure 1C–E).

Kaplan-Meier analysis of patients split into two groups according to miR200b-5p expression levels of 0.4156 (as defined by the specificity–sensitivity analysis) revealed that patients with miR200b-5p levels ≤0.4156 had a 4.8-fold (HR: 4.81, 95% CI 3.15 to 6.47, p<0.0001) higher risk to develop lymphoma compared with patients with miR200b-5p levels >0.4156 (figure 1F).

miR200b-5p levels in MSGs predict SS who will develop NHL versus those who will not, independently from other known predicting factors

The known outcome of the NHL development in the patients with SS who did not have lymphoma at the time of MSG biopsy, involving the low-risk ones who did not develop NHL during follow-up and those who evolved to an SS-associated NHL, enabled the evaluation of the utility of miR200b-5p levels in the prediction of lymphoma development. Cox regression analysis was employed to evaluate the value of miR200b-5p in the prediction of lymphoma development along with other disease parameters that were associated with lymphoma development in univariate analysis. These included high ESSDAI (defined as score ≥5, p<0.0001), SGE (p=0.002), purpura (p=0.0001), vasculitis (p=0.024), anaemia (p=0.058), splenomegaly (p=0.024), lymphadenopathy (p=0.005), C4-hypocomplementaemia (p=0.005), RF (p=0.001), GCs (p=0.007), cryoglobulinaemia (p=0.001) and hypergammaglobulinaemia (p<0.0001).

Multivariate analysis confirmed that miR200b-5p was independently associated with development of lymphoma (HR per 1-unit change: 0.10, 95% CI 0.01 to 0.87, p=0.012) along with high ESSDAI (p=0.024), SGE (p=0.012), purpura (p=0.057), vasculitis (p=0.043), splenomegaly (p=0.047), cryoglobulinaemia (p=0.032) and hypergammaglobulinaemia (p=0.055).

Possible role of miR200b-5p levels in monitoring of therapeutic response

Preliminary observations from nine patients with SSL before and after treatment suggest that MSG levels of miR200b-5p may apply in the prediction of therapeutic response. Thus, miR200b-5p levels remained stable or decreased (0.22 to 0.07) in refractory to treatment MALT (n=5) and DLCBL (n=1) lymphomas, respectively, reduced in two MALT-SSLs who relapsed (0.54 and 0.31 to 0.2 and 0.09, respectively) and increased in a MALT-SSL who reached complete remission (0.07 to 0.47).

DISCUSSION

This study supports that the MSG expression levels of miR200b-5p constitute a novel, strong, predictive biomarker for NHL development in SS, since its expression is impaired years before lymphoma clinical onset. Indeed, reduced MSG expression of miR200b-5p characterised patients with SS who have or will develop NHL. Low levels of miR200b-5p were correlated with disease parameters, previously associated with adverse outcome and NHL development. Importantly, miR200b-5p levels strongly discriminated the three groups of patients with SS, namely low-risk, prelymphoma and lymphoma, with high sensitivity and specificity and independently predicted lymphoma development. Furthermore, miR-200b-5p levels in MSGs were downregulated long before the clinical onset of lymphoma, supporting its potential as a predictive biomarker. In addition, our preliminary observations imply that miR200b-5p may also be significant for the therapeutic monitoring of patients with NHL, signifying the need for appropriate prospective studies.

The mechanisms underlying the reduced miR200b-5p expression in MSGs and its role in SS-related lymphomagenesis have not been delineated. The reduced levels of miR200b-5p long before lymphoma development suggest that it is implicated in SS lymphomagenesis, although the pathways that regulate remain unknown and are currently under investigation. Despite

the well-established role of the reduced miR200b-3p expression in EMT and associated oncogenesis, tumour metastasis and invasion,[12–17] little are known for miR200b-5p, possibly because it represents the star strand, which is generally considered to degrade during miRNA biogenesis.[24] Recently, it has been reported that miR200b-5p controls the non-canonical EMT in synergy with miR200b-3p by targeting PRKCA and PIP4K2A molecules in the RHOGDI pathway.[25] Interestingly, miR200b miRNAs have been almost exclusively associated with solid tumours. A recent study suggests that the elevated miR200b expression and subsequent inhibition of zinc finger E-box-binding homeobox 1 transcription factor and increased BCL6 protein expression is associated with the better prognosis of the *Helicobacter pylori*-positive gastric diffuse large B cell lymphomas compared with *H. pylori*-negative ones.[26] To this end, it would be interesting to investigate whether miR200b-5p levels are downregulated in other lymphomas that are not associated with SS.

Despite the clinical progress, the mechanisms underlying SS-related lymphomagenesis have not been delineated. It is considered as a multistep antigen-driven process taking place in the inflammatory MSG lesions that arises from the chronic continuous and/or inappropriate B cell stimulation, which increases the risk of chromosomal translocations, activation of proto-oncogenes and inactivation of tumour-suppression genes resulting in malignant transformation.[27 28] Most likely, the reduced miR200b-5p expression in the MSGs of patients with SS that will develop or have lymphoma does not involve B cells or other types of infiltrating lymphocytes, since we have been previously unable to detect its expression in the peripheral blood B cells.[18 29] Interestingly, miR200b-5p levels were negatively correlated with biopsy focus score and infiltration by T cells, B cells and macrophages, suggesting that the reduction of miR200b-5p in prelymphoma and lymphoma SS patients may result from 'dilution effect' due to increased infiltration by inflammatory cells. However, this reduced expression could be coincidental and reflective of reduced expression in epithelial cells, which are a possible source of miR200b-5p, in severe lesions. This is supported by the higher expression of miR200b-5p in low-risk patients compared with those with prelymphoma and lymphoma and similar MSG infiltration and the lack of correlation with KRT8 expression, which is possibly reflective of downregulated epithelial expression of miR200-5p in MSGs of patients with SS who will develop or have lymphoma and not alteration in epithelial cell number. In contrast to peripheral blood mononuclear cells, cultured salivary gland epithelial cells express miR200b-5p.[18 29] Epithelial cells are key regulators of SS autoimmune responses, whereas preliminary data suggest that they can drive B cell activation and differentiation.[30] Thus, it would be tempting to hypothesise that the reduction of miR200b-5p in MSG epithelia promotes the efficient interaction with B cells, which eventually leads in lymphomagenesis. In this context, the detection of low miR200b-5p levels in NHLs without MSG involvement is not paradoxical. The cellular types expressing miR200b-5p in the MSGs of patients with SS, the effect of its downregulation in their phenotype and the mechanisms underlying its reduction is of high importance for the discovery of novel therapeutic targets and is currently investigated in our lab.

In summary, the expression levels of miR200b-5p in MSG tissues constitute a novel, strong, disease mechanism-related, predictive biomarker for NHL development in SS. Their predictive value will be further validated during the HarmonicSS project (European Union Grant-731944) that includes 21 European patient SS cohorts.

Acknowledgements The authors gratefully acknowledge Haralampos M Moutsopoulos, MD, FACP, FRCP(hc), Master ACR, for his contribution in patient selection and manuscript preparation and editing.

Contributors EKK designed the study, performed the experiments, analysed the data and wrote the paper. AP evaluated the clinical data of patients with non-Hodgkin's lymphoma (NHL) patients, ADP performed statistical analyses, MV evaluated the clinical data of patients with NHL and contributed in data analysis and manuscript preparation and AGT designed the study and wrote the paper. AGT and EKK had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding The research has been financed by grants of the Research Grant from the Greek Rheumatology Society and Professional Association of Rheumatologists and the European-funded multicentric protocol 'HARMONIzation and integrative analysis of regional, national and international Cohorts on primary Sjögren's Syndrome (pSS) towards improved stratification, treatment and health policy making' (HARMONICSS; H2020-SC1-2016; grant agreement no: 731944).

Competing interests AGT has received research grants from Novartis, Pfizer, UCB, AbbVie and GSK pharmaceutical companies, through the National and Kapodistrian University of Athens, outside the submitted work.

Patient consent Not required.

Ethics approval Ethics Committee of School of Medicine, National and Kapodistrian University of Athens, Greece (protocol no: 1516023881).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There are no additional unpublished data related to the study.

REFERENCES

1 Kassan SS, Thomas TL, Moutsopoulos HM, *et al*. Increased risk of lymphoma in sicca syndrome. *Ann Intern Med* 1978;89:888–92.
2 Voulgarelis M, Dafni UG, Isenberg DA, *et al*. Malignant lymphoma in primary Sjögren's syndrome: a multicenter, retrospective, clinical study by the European Concerted Action on Sjögren's Syndrome. *Arthritis Rheum* 1999;42:1765–72.
3 Skopouli FN, Dafni U, Ioannidis JP, *et al*. Clinical evolution, and morbidity and mortality of primary Sjögren's syndrome. *Semin Arthritis Rheum* 2000;29:296–304.
4 Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjögren's syndrome. *Arthritis Rheum* 2002;46:741–7.
5 Theander E, Manthorpe R, Jacobsson LT. Mortality and causes of death in primary Sjögren's syndrome: a prospective cohort study. *Arthritis Rheum* 2004;50:1262–9.
6 Brito-Zerón P, Ramos-Casals M, Bove A, *et al*. Predicting adverse outcomes in primary Sjogren's syndrome: identification of prognostic factors. *Rheumatology* 2007;46:1359–62.
7 Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. *J Autoimmun* 2010;34:400–7.
8 Theander E, Vasaitis L, Baecklund E, *et al*. Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjögren's syndrome. *Ann Rheum Dis* 2011;70:1363–8.
9 Nocturne G, Virone A, Ng WF, Wf N, *et al*. Rheumatoid Factor and Disease Activity Are Independent Predictors of Lymphoma in Primary Sjögren's Syndrome. *Arthritis Rheumatol* 2016;68:977–85.
10 Papageorgiou A, Ziogas DC, Mavragani CP, *et al*. Predicting the outcome of Sjogren's syndrome-associated non-hodgkin's lymphoma patients. *PLoS One* 2015;10:e0116189.
11 Baimpa E, Dahabreh IJ, Voulgarelis M, *et al*. Hematologic manifestations and predictors of lymphoma development in primary Sjögren syndrome: clinical and pathophysiologic aspects. *Medicine* 2009;88:284–93.
12 Humphries B, Yang C. The microRNA-200 family: small molecules with novel roles in cancer development, progression and therapy. *Oncotarget* 2015;6:6472–98.
13 Senfter D, Madlener S, Krupitza G, *et al*. The microRNA-200 family: still much to discover. *Biomol Concepts* 2016;7(5-6):311–9.
14 Ragusa M, Majorana A, Banelli B, *et al*. MIR152, MIR200B, and MIR338, human positional and functional neuroblastoma candidates, are involved in neuroblast differentiation and apoptosis. *J Mol Med* 2010;88:1041–53.
15 Lee TS, Jeon HW, Kim YB, *et al*. Aberrant microRNA expression in endometrial carcinoma using formalin-fixed paraffin-embedded (FFPE) tissues. *PLoS One* 2013;8:e81421.
16 Chen MF, Zeng F, Qi L, *et al*. Transforming growth factor-β1 induces epithelial-mesenchymal transition and increased expression of matrix metalloproteinase-16 via miR-200b downregulation in bladder cancer cells. *Mol Med Rep* 2014;10:1549–54.

17 Cheng YX, Chen GT, Chen C, *et al*. MicroRNA-200b inhibits epithelial-mesenchymal transition and migration of cervical cancer cells by directly targeting RhoE. *Mol Med Rep* 2016;13:3139–46.
18 Gourzi VC, Kapsogeorgou EK, Kyriakidis NC, *et al*. Study of microRNAs (miRNAs) that are predicted to target the autoantigens Ro/SSA and La/SSB in primary Sjögren's Syndrome. *Clin Exp Immunol* 2015;182:14–22.
19 Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554–8.
20 Fragkioudaki S, Mavragani CP, Moutsopoulos HM. Predicting the risk for lymphoma development in Sjogren syndrome: An easy tool for clinical use. *Medicine* 2016;95:e3766.
21 Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res* 2004;64:5245–50.
22 Kapsogeorgou EK, Christodoulou MI, Panagiotakos DB, *et al*. Minor salivary gland inflammatory lesions in Sjögren syndrome: do they evolve? *J Rheumatol* 2013;40:1566–71.
23 Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat* 1979;6:65–70.
24 Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215–33.
25 Rhodes LV, Martin EC, Segar HC, *et al*. Dual regulation by microRNA-200b-3p and microRNA-200b-5p in the inhibition of epithelial-to-mesenchymal transition in triple-negative breast cancer. *Oncotarget* 2015;6:16638–52.
26 Huang WT, Kuo SH, Cheng AL, *et al*. Inhibition of ZEB1 by miR-200 characterizes Helicobacter pylori-positive gastric diffuse large B-cell lymphoma with a less aggressive behavior. *Mod Pathol* 2014;27:1116–25.
27 Papageorgiou A, Voulgarelis M, Tzioufas AG. Clinical picture, outcome and predictive factors of lymphoma in Sjβgren syndrome. *Autoimmun Rev* 2015;14:641–9.
28 Bombardieri M, Pitzalis C. Ectopic lymphoid neogenesis and lymphoid chemokines in Sjogren's syndrome: at the interplay between chronic inflammation, autoimmunity and lymphomagenesis. *Curr Pharm Biotechnol* 2012;13:1989–96.
29 Kapsogeorgou EK, Gourzi VC, Manoussakis MN, *et al*. Cellular microRNAs (miRNAs) and Sjögren's syndrome: candidate regulators of autoimmune response and autoantigen expression. *J Autoimmun* 2011;37:129–35.
30 Kapsogeorgou EK, Tzioufas AG. Glandular epithelium: Innocent bystander or leading actor. In: Roberto Gerli EB, Alunno A, eds. *Sjogren's Syndrome: Novel Insights in Pathogenic, Clinical and Therapeutic Aspects: Academic Press*, 2016:189–98.
31 Seror R, Ravaud P, Bowman SJ, *et al*. EULAR Sjogren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. *Ann Rheum Dis* 2010;69:1103–9.
32 Oken MM, Creech RH, Tormey DC, *et al*. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;5:649–56.
33 Carbone PP, Kaplan HS, Musshoff K, *et al*. Report of the Committee on Hodgkin's Disease Staging Classification. *Cancer Res* 1971;31:1860–1.
34 International Non-Hodgkin's Lymphoma Prognostic Factors P. A predictive model for aggressive non-Hodgkin's lymphoma. *The New England journal of medicine* 1993;329:987–94.

EXTENDED REPORT

Methyl-CpG-binding protein 2 mediates antifibrotic effects in scleroderma fibroblasts

Ye He,[1,2] Pei-Suen Tsou,[1] Dinesh Khanna,[1] Amr H Sawalha[1,3]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2018-213022).

[1]Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA
[2]Department of Dermatology, The Second Xiangya Hospital, Central South University, Changsha, China
[3]Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA

Correspondence to
Dr Amr H Sawalha, Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI 48109, USA; asawalha@umich.edu

Received 15 January 2018
Revised 19 April 2018
Accepted 21 April 2018
Published Online First 14 May 2018

To cite: He Y, Tsou P-S, Khanna D, *et al*. *Ann Rheum Dis* 2018;**77**:1209–1219.

ABSTRACT

Objective Emerging evidence supports a role for epigenetic regulation in the pathogenesis of scleroderma (SSc). We aimed to assess the role of methyl-CpG-binding protein 2 (MeCP2), a key epigenetic regulator, in fibroblast activation and fibrosis in SSc.

Methods Dermal fibroblasts were isolated from patients with diffuse cutaneous SSc (dcSSc) and from healthy controls. MeCP2 expression was measured by qPCR and western blot. Myofibroblast differentiation was evaluated by gel contraction assay in vitro. Fibroblast proliferation was analysed by ki67 immunofluorescence staining. A wound healing assay in vitro was used to determine fibroblast migration rates. RNA-seq was performed with and without MeCP2 knockdown in dcSSc to identify MeCP2-regulated genes. The expression of MeCP2 and its targets were modulated by siRNA or plasmid. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) using anti-MeCP2 antibody was performed to assess MeCP2 binding sites within MeCP2-regulated genes.

Results Elevated expression of MeCP2 was detected in dcSSc fibroblasts compared with normal fibroblasts. Overexpressing MeCP2 in normal fibroblasts suppressed myofibroblast differentiation, fibroblast proliferation and fibroblast migration. RNA-seq in MeCP2-deficient dcSSc fibroblasts identified MeCP2-regulated genes involved in fibrosis, including *PLAU*, *NID2* and *ADA*. Plasminogen activator urokinase (PLAU) overexpression in dcSSc fibroblasts reduced myofibroblast differentiation and fibroblast migration, while nidogen-2 (NID2) knockdown promoted myofibroblast differentiation and fibroblast migration. Adenosine deaminase (ADA) depletion in dcSSc fibroblasts inhibited cell migration rates. Taken together, antifibrotic effects of MeCP2 were mediated, at least partly, through modulating PLAU, NID2 and ADA. ChIP-seq further showed that MeCP2 directly binds regulatory sequences in *NID2* and *PLAU* gene loci.

Conclusions This study demonstrates a novel role for MeCP2 in skin fibrosis and identifies MeCP2-regulated genes associated with fibroblast migration, myofibroblast differentiation and extracellular matrix degradation, which can be potentially targeted for therapy in SSc.

INTRODUCTION

Systemic sclerosis (scleroderma, SSc) is a rare autoimmune connective tissue disease characterised by complex interplays between vascular dysfunction, immunological abnormalities and fibrosis.[1 2] Depending on the pattern of skin involvement, SSc is classified into two major subtypes: limited cutaneous SSc and diffuse cutaneous SSc (dcSSc).[3] Patients with dcSSc tend to have severe internal organ involvement, rapid disease development and worse prognosis.[4] SSc has the highest mortality among rheumatic diseases as a consequence of progressive tissue fibrosis; however, effective antifibrotic therapies to prevent rapid disease progression or revert existing fibrosis are currently limited.[5]

Fibrosis, secondary to excessive extracellular matrix (ECM) components accumulation, replaces normal tissue architecture with dense and stiff connective tissue, then leads to organ failure in patients with SSc.[6] Sustained fibroblast activation and differentiation into myofibroblasts, marked by increased α-smooth muscle actin (α-SMA) expression, greatly contributes to amplified fibrotic responses and pathological fibrosis. SSc fibroblasts explanted from SSc lesional skin biopsies demonstrate a persistent 'SSc phenotype' during their serial passage in vitro.[1 6 7] They are characterised by an autocrine transforming growth factor (TGF)-β signalling loop to maintain the SSc phenotype.[8] This provides the platform for our studies of SSc fibroblasts in vitro.

Recent attention has been focused on epigenetic mechanisms mediating fibroblast activation and fibrosis in SSc.[9 10] Methyl-CpG-binding protein 2 (MeCP2), an epigenetic regulator binding to methylated DNA, was originally considered to repress transcription via its interactions with HDAC-Sin3 complex, but was later shown to function mainly as an activator by recruiting the transcription factor CREB1 at activated promoters.[11] Thus, there are considerable complexities in the possible mechanisms by which MeCP2 might regulate gene expression. Emerging studies revealed that MeCP2 was involved in liver fibrosis[12 13] and pulmonary fibrosis.[14] In addition, *MECP2* has been identified as a susceptibility gene associated with dcSSc,[15] confirming our earlier observations identifying *MECP2* as a genetic risk locus in autoimmunity.[16–19]

In the current study, we hypothesise that MeCP2 is involved in fibrosis in SSc by regulating fibrotic genes and altering fibroblast functions. To test this hypothesis, we examined the expression of MeCP2 in fibroblasts isolated from healthy controls or patients with dcSSc, and then probed the effects of MeCP2 on myofibroblast differentiation, fibroblast proliferation and migration. Unbiased RNA-seq and ChIP-seq were used to identify genome-wide transcriptional targets of MeCP2, followed by bioinformatics analyses and functional validations of identified targets. Several novel MeCP2-regulated genes altering fibrotic properties and fibroblast functions in SSc were identified.

METHODS

Patients and controls

Patients with dcSSc and healthy controls were included in this study. All patients fulfilled the American College of Rheumatology/European League Against Rheumatism criteria for SSc.[20] The median age of patients with dcSSc is 54 years (range 25–81 years, n=24, including 19 women and 5 men), with median disease duration of 3 years. The median age of healthy individuals is 53.5 years (range 22–72 years, n=25, including 21 women and 4 men). All subjects included in this study signed a written informed consent approved by the institutional review board of the University of Michigan.

Cell culture

Two 4-mm-punch biopsies from clinically affected forearm skin were obtained from each patient, and the same area was biopsied in the controls. Dermal fibroblasts were isolated as previously described[21] and were validated by immunofluorescence analyses with fibroblast markers (online supplementary figure S1). Briefly, following skin sample homogenisation, dermal fibroblasts were grown in 2.05 mM L-glutamine Roswell Park Memorial Institute medium (Hyclone), with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin (Thermo Fisher Scientific) in humidified atmosphere of 5% CO_2 at 37°C. Cells between the third and sixth passage were used in all experiments.

RNA extraction and quantitative real-time (RT)-PCR

Total RNA from passage 3–6 dermal fibroblasts was isolated by the Direct-zol RNA MiniPrep (Zymo Research). cDNA was prepared using verso cDNA Synthesis Kit (Thermo Fisher Scientific). RT-PCR used for quantification of the mRNA expression of genes was performed using their primers and SYBR Green PCR Master Mix Reagent (Thermo Fisher Scientific) on ViiA V.7 Real-Time PCR System, using β-actin as an internal standard for normalisation. Duplicate measurements were performed for each sample. Primer sequences used in this study are available on request. Primers were purchased from Sigma or QIAGEN.

Western blot

Total cell extracts were prepared by scraping passage 3–6 fibroblasts into lysis buffer followed by centrifugation and protein measurement in supernatant using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). 10 µg protein per sample was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. MeCP2 proteins were probed by anti-MeCP2 antibody (Cell Signaling). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β-actin was detected by anti-GAPDH antibody (Cell Signaling) or anti-β-actin antibody (Sigma Aldrich) as loading controls. Band quantification was calculated using GelQuant.NET (BiochemLab Solutions).

Gene overexpression experiments

A 0.05 µg *MECP2* vector or 0.1 µg *PLAU* vector (OriGene; same amount of pCMV6-XL5 vector was used as control) was transfected into passage 3–6 normal or dcSSc fibroblasts using lipofectamine 2000 (Invitrogen) for 48 (for *PLAU*) or 72 hours (for *MECP2*) according to the manufacturer's protocols. Culture media were changed after 24 hours of transfection. Subsequent analyses for qPCR, gel contraction assay, wound healing migration assay or proliferation assay were performed.

Gene knockdown experiments

Passage 3–6 dcSSc dermal fibroblasts were transfected with MeCP2 small interference RNA (siRNA) or non-targeting siRNA according the manufacturer's protocols (Dharmacon). DcSSc fibroblasts were seeded at 70% confluence to 6-well or 12-well culture plates, then transfected with 150 nM (final concentration) non-targeting or MeCP2 siRNA with Transit-TKO transfection reagent (Mirus Bio) and incubated for 48 hours. Similarly, knockdown condition was optimised for AXL (75 nM), ANPEP (25 nM), NID2 (125 nM), adenosine deaminase (ADA) (100 nM), TNFA1P1 (50 nM) and NTN4 (150 nM) in passage 5–6 dcSSc fibroblasts (siRNA were all purchased from Dharmacon). All siRNA targets sequences are available on request.

Wound healing scratch assay

Scratch assay was performed to measure the effect of MeCP2 on dermal fibroblast migration.[22] Fibroblasts passage 3-6 were seeded in 12-well plates to 70% confluence followed by siRNA knockdown or overexpression experiments described earlier. When they reached 95% confluence (48 hours post transfection), cells were scraped with a plastic 200 µL pipette tip then washed with phosphate-buffered saline (PBS) twice. Cells were then grown with 0.1% FBS cell culture medium to prevent cell proliferation. The created gaps were photographed by EVOS XL Core Cell Imaging System at 0, 24 and 48 hours after scraping. Wound repair ability was used to evaluate wound closure rate. Wound repair=100 (A – B)/A, where A is the average wound surface area at 0 hour and B is the average wound surface area at 24 hours or 48 hours. Wound surface area was measured by Image J.[23]

Collagen gel contraction assay

Passage 3–6 fibroblasts transfected with empty vectors/control siRNAs, or *MECP2* plasmid, *PLAU* plasmid, or *NID2* siRNAs for 48 hours, were suspended in culture media at 2×10^6 cells/mL. Collagen solution was made according to the cell contraction assay manual (Cell Biolabs), then a mixture of cells and collagen solution was prepared to include two parts of cell suspension and eight parts of cold collagen working solution. 0.5 mL/well of cell-collagen mixture was added into a 24-well plate. After collagen polymerisation, 1.0 mL culture medium was added atop each collagen gel lattice. Cultures were incubated for 2 days, then collagen gels were gently released from culture dishes to initiate contraction. Collagen gel contraction was monitored for 2 days. Surface area of contracted gels was measured using Image J.[23] The ratio of gel surface area at 48 hours divided by gel surface area at 0 hour reflects fibroblast contractile ability.

Immunofluorescence staining

Ki67 staining was used to determine the effect of MeCP2 on fibroblast proliferation. Passage 3–6 transfected fibroblasts were fixed with 4% formaldehyde for 30 min at room temperature, washed twice with PBS, permeabilised with ice cold methanol for 5 min at 4°C. Blocking was performed with 400 µL/well of 5% FBS plus 5% goat serum for 30 min at 37°C, followed by incubation with 200 µL/well of anti-ki67 at a concentration of 1:1000 (Abcam) or IgG for 1 hour 15 min at 37°C, then washed twice with PBS. Incubation with 200 µL/well anti-rabbit fluorescent conjugated secondary antibody (5 µg/mL) for 1 hour at room temperature in the dark was then performed. Fibroblasts were washed twice with PBS and coverslipped with mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI). Ki67 and DAPI immunofluorescence images were captured using an

Olympus BX51\DP72 microscope. Ki67+ cells and total cells were counted by Image J.[23] The ratio of ki67+ cell number to total cell number was used for comparing proliferation rates of fibroblasts with different treatments. Immunofluorescence staining of fibroblast markers protocol is documented in online supplementary text.

RNA sequencing

Total RNAs from passage 4–6 dcSSc fibroblasts transfected with MeCP2 siRNA or control siRNA were extracted using Direct-zol RNA MiniPrep Kit (Zymo Research), and then were DNAse-treated using TURBO DNA-free Kit (Invitrogen) (all RIN values were >9.5). In total, 300 ng RNA were used to construct stranded mRNA-seq libraries with TruSeq Stranded mRNA Library Prep Kit (Illumina), and then underwent 100 bp, single-end reads sequencing on Illumina Hi-Seq 2500 platform (~22M reads/sample, 10 samples per lane).

RNA-seq data analysis

Sequence reads were cleaned using trimmomatic (V.0.36),[24] and then mapped to human reference genome GRCh38.p7 with STAR (V.2.5.2b).[25] Raw read counts were obtained using featurecounts from Subread package (V.1.5.0p3),[26] and annotated by human gencode V.25 with only uniquely aligned reads. Data normalisation and differential expression analysis (negative binomial Wald test) between MeCP2 knockdown and control groups were performed using DESeq2 (V.1.14.1)[27] within R (V.3.3.2) with adjusted p value (Benjamini-Hochberg multiple test correction) threshold of 0.05. Default independent filtering was performed by DESeq2 package using the mean of normalised counts as a filter criterion.[28] Genes not passing the filter threshold were assigned 'NA' as adjusted p values and were not included in subsequent analyses. Literature mining was used to identify genes associated with SSc (eg, genes associated with ECM remodelling, cell proliferation and migration).

Chromatin immunoprecipitation and sequencing

Chromatin immunoprecipitation (ChIP) assay was performed by iDeal ChIP-seq kit for Transcription Factors (Diagenode), according to the manufacturer's instruction. Briefly, passage 4–6 dermal fibroblasts from patients with dcSSc or healthy controls were fixed with 1% formaldehyde for 10 min. Glycine was added to quench fixation. Cells were lysed and then sonicated on Misonix ultrasonic liquid processor S-4000 for 15 cycles (30 s on/30 s off). MeCP2 antibody, previously used for ChIP-seq in olfactory epithelial tissue[29] or rabbit IgG and pre-washed protein A-agarose beads, were mixed and then incubated for 4 hours at 4°C on a rotator. Then 250 μL sheared chromatin was incubated with antibody-beads mixed solution at 4°C overnight under constant rotation. Also, 2.5 μL sheared chromatin was put aside as Input. Immunoprecipitated chromatin was eluted from beads using an elution buffer at room temperature with 30 min rotation. Immunoprecipitated chromatin and input were incubated for 4 hours at 65°C to decrosslink chromatin, then DNA was purified using magnetic beads included with the kits. MeCP2 ChIP-seq and input DNA libraries were prepared by the Apollo 324 Next Generation Sample Preparation System with WaferGen reagents, then PCR amplified, cleaned up and sequenced with 50 bp, single-end reads on Illumina Hi-Seq 2500 platform (20 samples pooled together in three lanes).

ChIP-seq data analysis

Raw sequence reads were aligned to hg38 genome using Bowtie (V.2.2.4).[30] Uniquely mapped reads were selected by filtering out alignments with mapping quality <23 and were used in subsequent analyses. Peak calling was performed using MACS (V.2.1.1),[31] by default threshold (q-value <0.01). Peaks overlapping DAC Blacklisted Regions[32] were removed. Peaks for nearest genes were annotated using Homer (V.4.9.1).[33] Bigwig format pile-up files were generated from MACS outputs and visualised for signals on UCSC genome browser.

Statistical analysis

All data were derived from at least two independent experiments. The results were presented as mean±SD. Statistical analysis was performed using GraphPad Prism V.7.03 (GraphPad Software). A Student's t-test was used to evaluate two-group comparisons, with statistical significance set at $p<0.05$.

RESULTS

Elevated MeCP2 expression in dcSSc fibroblasts

We investigated MeCP2 expression in normal fibroblasts and dcSSc fibroblasts using qPCR and western blotting. MeCP2 expression was increased by 1.75-fold in dcSSc fibroblasts compared with normal fibroblasts at protein levels (figure 1A,B). However, no change was observed at the mRNA level for total *MECP2* (figure 1C), *MECP2A* or *MECP2B* (online supplementary figure S2), suggesting that dysregulation of MeCP2 in dcSSc occurs at the post-transcriptional level. To assess effects of increased MeCP2 in dcSSc fibroblasts on skin fibrosis, we performed gain or loss of MeCP2 experiments. We first optimised conditions for overexpression or knockdown of MeCP2 in normal fibroblasts or dcSSc fibroblasts, respectively. As shown in figure 1D, transfection of 0.05 μg *MECP2* plasmid into normal fibroblasts for 72 hours led to overwhelming increase of *MECP2* mRNA and 1.4-fold increase of MeCP2 protein level compared with negative controls. A 48-hour transfection of 150 nM *MECP2* siRNA into dcSSc fibroblasts caused approximately 72% mRNA knockdown compared with non-targeting siRNA (figure 1E, lower panel). We further confirmed diminished MeCP2 expression at the protein level (figure 1E, upper panel). In subsequent experiments, we used 48-hour transfection of 150 nM *MECP2* siRNA to knockdown MeCP2 in dcSSc fibroblasts and 72-hour transfection of 0.05 μg *MECP2* plasmid to overexpress MeCP2 in normal and dcSSc fibroblasts.

Reduced myofibroblast-like phenotype by MeCP2 overexpression

To investigate if increased expression of MeCP2 alters myofibroblast differentiation and fibrogenic properties in normal dermal fibroblasts, we overexpressed MeCP2 in normal dermal fibroblasts and measured expression levels of several well-characterised fibrogenic genes. MeCP2 overexpression significantly attenuated pro-fibrotic *α-SMA* and collagen type I alpha 1 chain (*COL1A1*) mRNA expression in normal dermal fibroblasts, whereas antifibrotic peroxisome proliferator-activated receptor gamma (*PPAR-γ*) was upregulated, and no effect was observed on *TGF-β* (figure 2A, upper panel). To confirm the results in dcSSc, we transfected dcSSc fibroblasts with *MECP2* plasmid or *MECP2* siRNA. A similar mRNA expression pattern was observed in MeCP2-overexpressing dcSSc fibroblasts (figure 2A, lower panel), indicating that reduced myofibroblast-like phenotype characteristics induced by MeCP2 overexpression is independent of stimuli secreted by dcSSc fibroblasts. Unexpectedly,

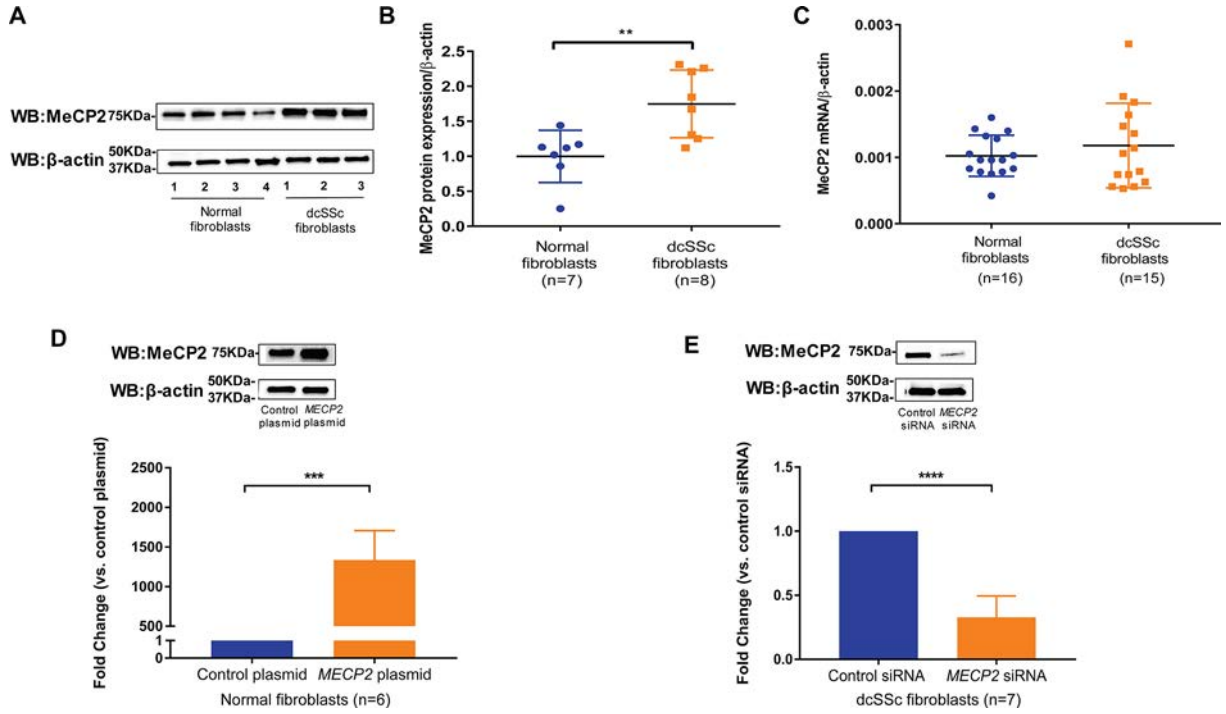


Figure 1 Methyl-CpG-binding protein 2 (MeCP2) was increased in diffuse cutaneous systemic sclerosis (dcSSc) fibroblasts compared with normal fibroblasts. (A) Representative blots of MeCP2 expression in normal fibroblasts and dcSSc fibroblasts. (B) Protein expression of MeCP2 was significantly increased in dcSSc fibroblasts by 1.75-fold (p=0.0051). Values are the mean and SD from seven healthy controls and eight dcSSc patient fibroblasts. (C) Expression of *MECP2* mRNA between normal fibroblasts (n=16) and dcSSc fibroblasts (n=15) was not significantly different. (D) 0.05 µg *MECP2* plasmid transfection into normal fibroblasts for 72 hours successfully upregulated MeCP2 expression at both mRNA and protein levels compared with control plasmid. (E)150 nM *MECP2* siRNA transfection in dcSSc fibroblasts for 48 hours resulted in an average of 72% mRNA knockdown. Diminished MeCP2 expression at the protein level was confirmed. Results are expressed as mean±SD. *p<0.05, ***p<0.005, ****p<0.001.

mRNA expression of *COL1A1*, *α-SMA* and *PPAR-γ* was not significantly altered by MeCP2 knockdown in dcSSc fibroblasts (data not shown), implying that other pro-fibrotic co-regulators exist for COL1A1, α-SMA and PPAR-γ.

To provide more evidence that MeCP2 suppresses myofibroblast differentiation and functionally decreases contractile properties, we performed a collagen gel contraction assay. MeCP2 overexpression significantly reduced myofibroblast-mediated contraction of collagen gels compared with the control group. Representative images and quantified surface area of contracted gels are shown in figure 2B.

MeCP2 overexpression inhibits fibroblast proliferation and migration

We observed that MeCP2 overexpression in normal fibroblasts reduced cell proliferation rates compared with mock-transfected cells (figure 2C, upper-left panel). To validate and quantify this observation, proliferation analysis was performed using ki67 labelling (figure 2C, right panel). MeCP2-transfected fibroblasts showed a significantly reduced ki67-positive cell ratio compared with negative controls confirming decreased fibroblast proliferation with MeCP2 overexpression (figure 2C, lower-left panel).

Next, we conducted a wound scratch assay in vitro to determine if MeCP2 affects fibroblast migration. After 24 and 48 hours transfection, relatively less wound closure was seen in MeCP2 transfected normal fibroblasts compared with negative controls (figure 2D, left). Quantification of wound repair demonstrated that MeCP2 overexpressing fibroblasts had lower wound repair ability (figure 2D, right).

Transcriptome analysis of MeCP2-deficient dermal fibroblasts

The data above suggest that MeCP2 overexpression exerted antifibrotic effects in normal dermal fibroblasts by inhibiting myofibroblast differentiation, fibroblast proliferation and fibroblast migration. To uncover antifibrotic mechanisms of MeCP2 in dcSSc fibroblasts, we examined genome-wide transcriptional changes in dcSSc fibroblasts with and without MeCP2 knockdown using RNA-seq. We identified 51 differentially expressed genes (DEGs), in addition to *MECP2* downregulation as expected, with MeCP2 knockdown in dcSSc fibroblasts (n=5, fold change ≥1.2 or ≤0.8, adjusted p value<0.05) (figure 3A). Of those, 46 genes were downregulated with MeCP2 knockdown, indicating that MeCP2 primarily acts as a transcription activator in SSc fibroblasts, echoing the findings in other tissues indicating that MeCP2 activates the majority of genes it regulates.[11]

To identify MeCP2-regulated genes relevant to SSc, we first looked at functional annotations of all 51 DEGs using DAVID V.6.7,[34] then performed literature search for all DEGs using PubMed. Genes relevant to at least one of the following four categories were selected: genes that affect fibrotic signalling pathways, genes that affect ECM remodelling, genes that affect cell proliferation or migration and genes that have deregulated expression in fibrosis-involved diseases. Eventually, we identified 14 out of 51 DEGs to be relevant or potentially relevant to SSc, among which 5 downregulated genes (*NTN4*, *SCL6A8*, *PRELP*, *ITGB1*, *NID2*) were shown to be associated with ECM remodelling, 7 genes (*TNFAIP1*, *AXL*, *C10or54*, *ITGB1*, *NID2*, *ADA*, *PLAU*) were involved in cell adhesion/migration and 3 genes (*ADA*, *PLAU* and *GDF11*) were related to cell proliferation

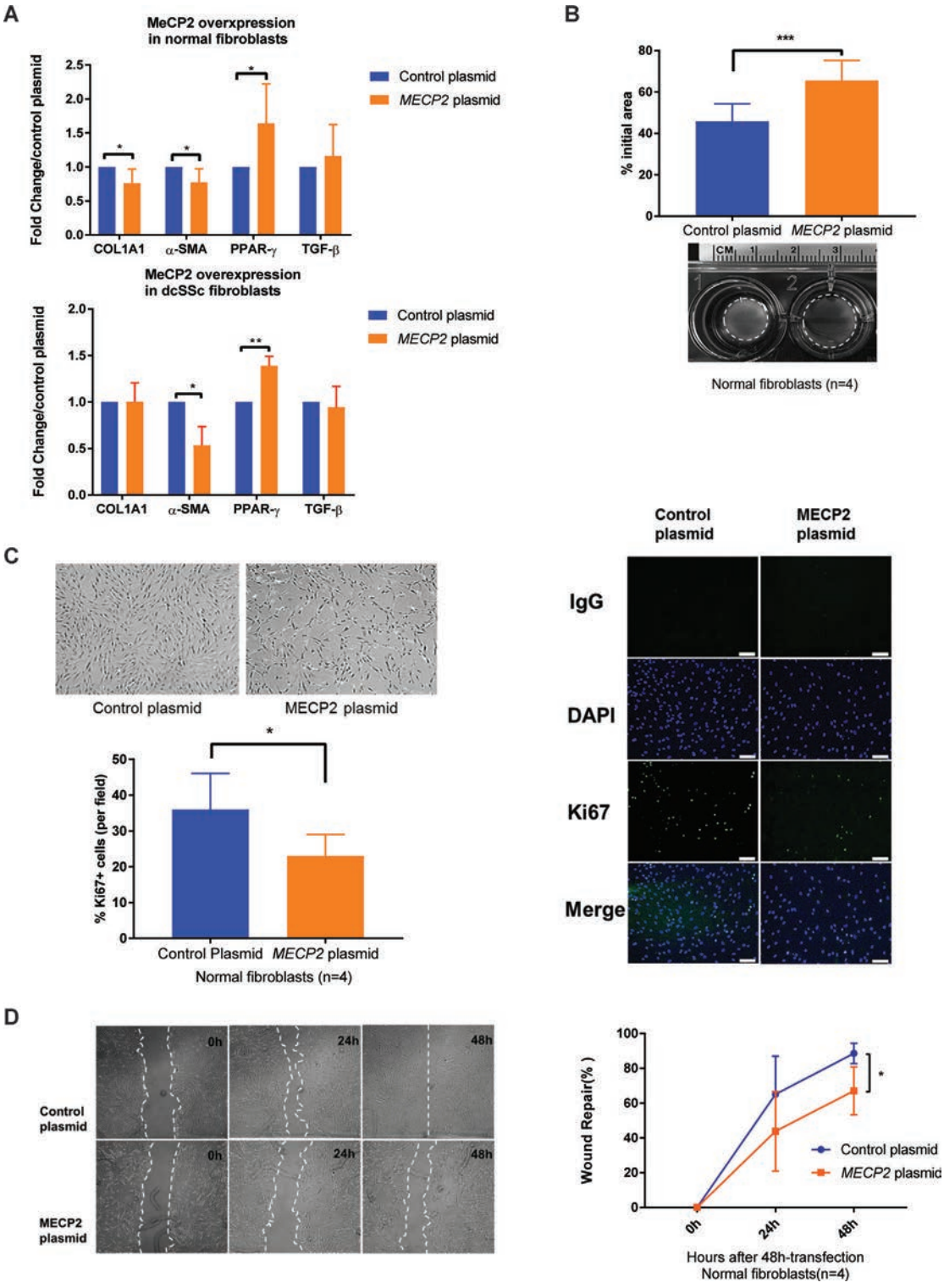


Figure 2 Overexpression of methyl-CpG-binding protein 2 (MeCP2) in fibroblasts inhibited myofibroblast differentiation, cell proliferation and migration rates. (A) Upper panel: overexpressing MeCP2 in normal fibroblasts reduced collagen type I alpha 1 chain (*COL1A1*) and α-smooth muscle actin (*α-SMA*) mRNA expression (0.76±0.19-fold and 0.78±0.25-fold, respectively), while induced 1.50±0.23-fold increase in peroxisome proliferator-activated receptor gamma (*PPAR-γ*) mRNA expression (n=6, p<0.05). Lower panel: overexpressing MeCP2 in diffuse cutaneous systemic sclerosis (dcSSc) fibroblasts reduced *α-SMA* mRNA expression (0.53±0.28-fold, n=4, p=0.02), while induced 1.39±0.22-fold increase in *PPAR-γ* mRNA expression (n=4, p<0.01). Transforming growth factor (*TGF-β*) expression was not altered by MeCP2 overexpression. (B) *MECP2*-transfectd fibroblasts exhibited weaker contractile ability than empty vector transfected fibroblasts at 48 hours after gel stress was lifted (n=4, p<0.005). Representative images of contracted collagen gels are shown along with the quantification. (C) *MECP2*-transfected fibroblasts were noted to have decreased proliferation rates than negative controls. Quantification of ki67+ fibroblasts indicated that fibroblasts transfected with *MECP2* plasmid exhibited attenuated proliferative capacity (n=4, p<0.01). Scale bar=100 μm. (D) MeCP2 overexpressing normal fibroblasts showed decreased cell migration rates at 0, 24 and 48 hours post scratch as quantified by wound repair (n=4, p<0.05). Results are expressed as mean ±SD. *p<0.05, **p<0.01, ***p<0.005. DAPI, 4′,6-diamidino-2-phenylindole.

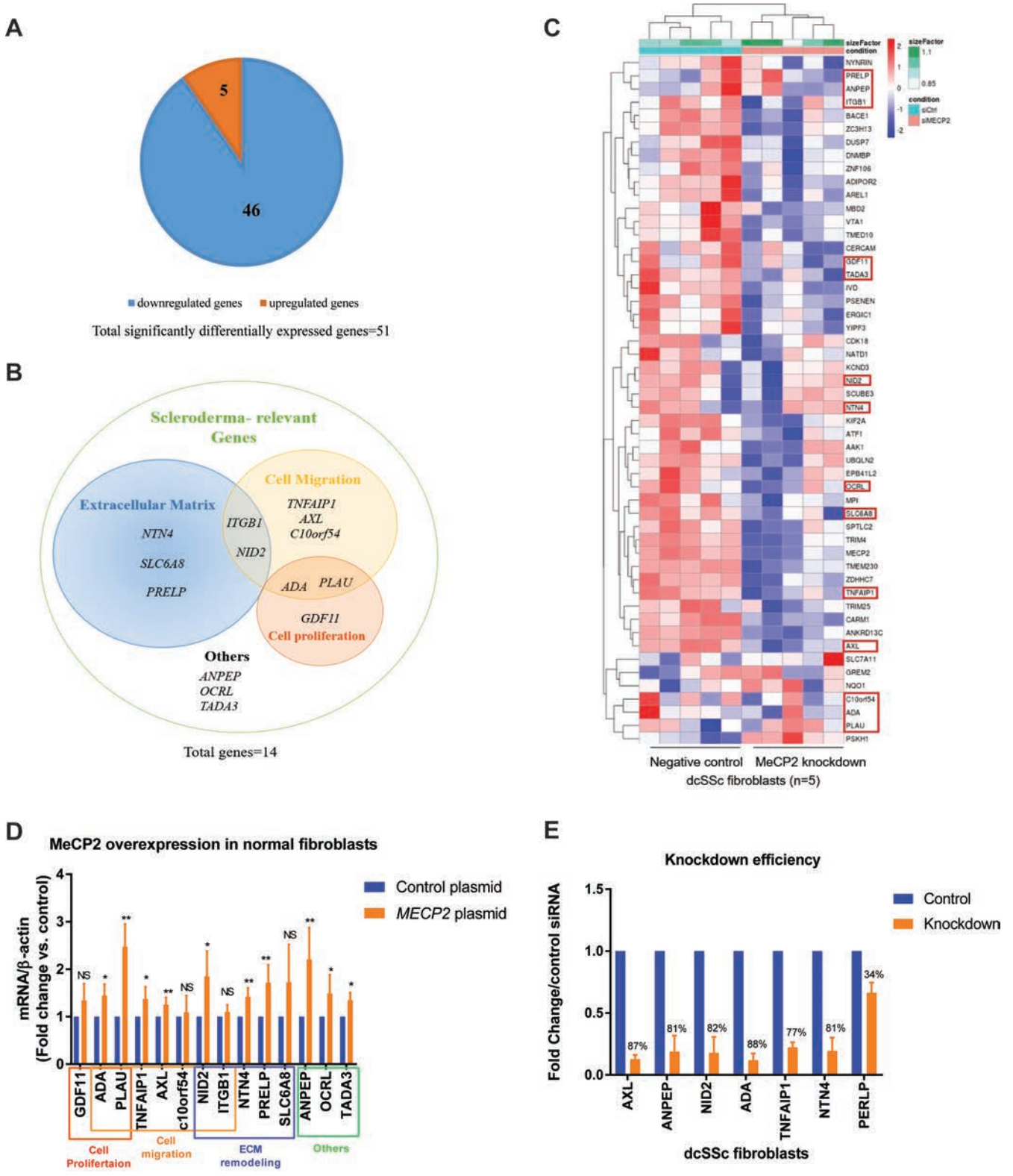


Figure 3 Methyl-CpG-binding protein 2 (MeCP2) depletion followed by RNA-seq identified targets important for extracellular matrix (ECM) remodelling, cell migration and cell proliferation. (A) In total, 51 genes (in addition to *MECP2*) were significantly differentially expressed by MeCP2 knockdown in dcSSc fibroblasts (46 downregulated and 5 upregulated). (B) Venn diagram depicting functional categories of 14 systemic sclerosis (SSc)-relevant genes identified by literature search. (C) All differentially expressed genes are shown in the heatmap, red circled genes are scleroderma-relevant genes. (D) mRNA expression of 14 SSc-relevant genes in MeCP2 overexpressing fibroblasts. Ten genes (*ADA, PLAU, TNFAIP1, AXL, NID2, NTN4, PRELP, ANPEP, OCRL, TADA3*) were significantly upregulated with MeCP2 overexpression. (E) Transfection of 75 nM *AXL* small interference RNA (siRNA), 25 nM *ANPEP* siRNA, 125 nM *NID2* siRNA, 100 nM adenosine deaminase (*ADA*) siRNA, 50 nM *TNFAIP1* siRNA and 150 nM *NTN4* siRNA in dcSSc fibroblasts for 48 hours resulted in 87%, 81%, 82%, 88%, 77% and 81% knockdown efficiency compared with same amounts of control siRNAs, respectively. PERLP knockdown could not be achieved even with 250 nM siRNA and was excluded from subsequent functional assays. Results are expressed as mean±SD. *p<0.05, **p<0.01, ***p<0.005. NID2. nidogen-2; PLAU, plasminogen activator urokinase.

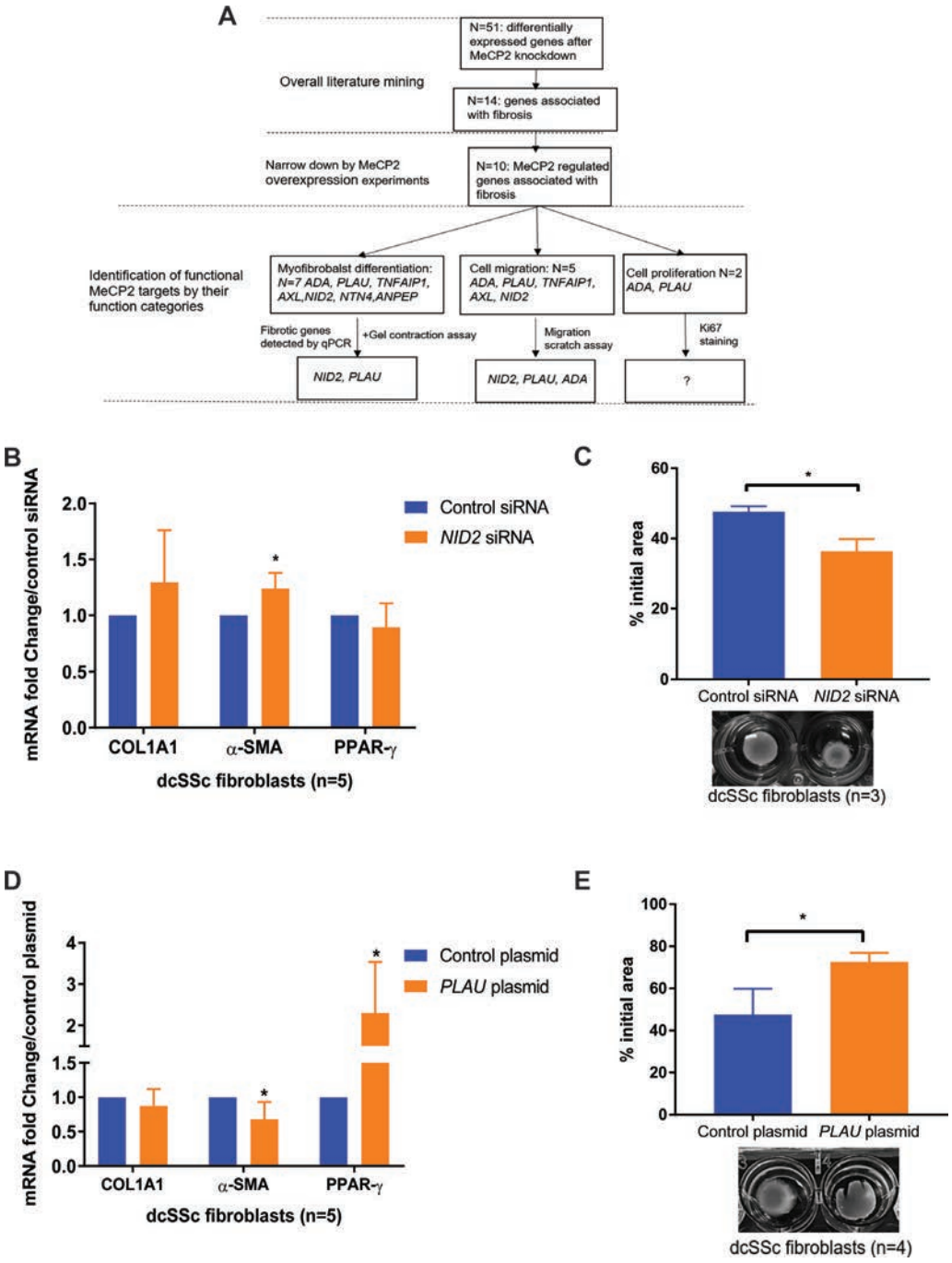


Figure 4 Nidogen-2 (NID2) and plasminogen activator urokinase (PLAU) blocked myofibroblast formation. (A) A workflow illustrating bioinformatics analyses strategies for narrowing down genes involved in systemic sclerosis (SSc). (B) NID2 knockdown significantly increased (1.24±0.14-fold) α-smooth muscle actin (α-SMA) mRNA in diffuse cutaneous SSc (dcSSc) fibroblasts (n=5, p=0.020). (C) NID2-silenced dcSSc fibroblasts exhibited stronger contractile ability than empty vector transfected fibroblasts (n=3, p=0.021). Representative images of contracted collagen gels are shown along with the quantification. (D) Significantly decreased *α-SMA* mRNA expression (fold change=0.59 ± 0.22 vs control, n=5, p=0.021) and increased peroxisome proliferator-activated receptor gamma (*PPAR-γ*) mRNA expression (fold change=2.26 ± 1.42 vs control, n=5, p=0.046) were detected in PLAU-overexpressing dcSSc fibroblasts. (E) PLAU overexpression significantly blocked myofibroblast-mediated contraction of collagen gels (n=4, p=0.029). Representative images of contracted collagen gels are shown along with the quantification. Results are expressed as mean ±SD. *p<0.05. COL1A1, collagen type I alpha 1 chain; MeCP2, methyl-CpG-binding protein 2.

(figure 3B). Of note, 13 out of the 14 identified genes were downregulated with MeCP2 knockdown, while *PLAU* was upregulated (figure 3C).

We then confirmed that 9 out of 13 downregulated fibrosis-related genes identified by RNA-seq were indeed MeCP2-regulated genes as they were consistently upregulated when MeCP2 was overexpressed in normal fibroblasts (figure 3D). *PLAU* was upregulated when MeCP2 was either knocked down or overexpressed (figure 3C, D).

From these 10 MeCP2 potential targets, we excluded *OCRL* and *TADA3* because their known functional roles (ie, apoptosis) in SSc were beyond the scope of our current studies in fibroblasts, then proceeded with the rest of the eight genes (*ADA*, *PLAU*, *TNFAIP1*, *AXL*, *NID2*, *NTN4*, *PRELP*, *ANPEP*) to further

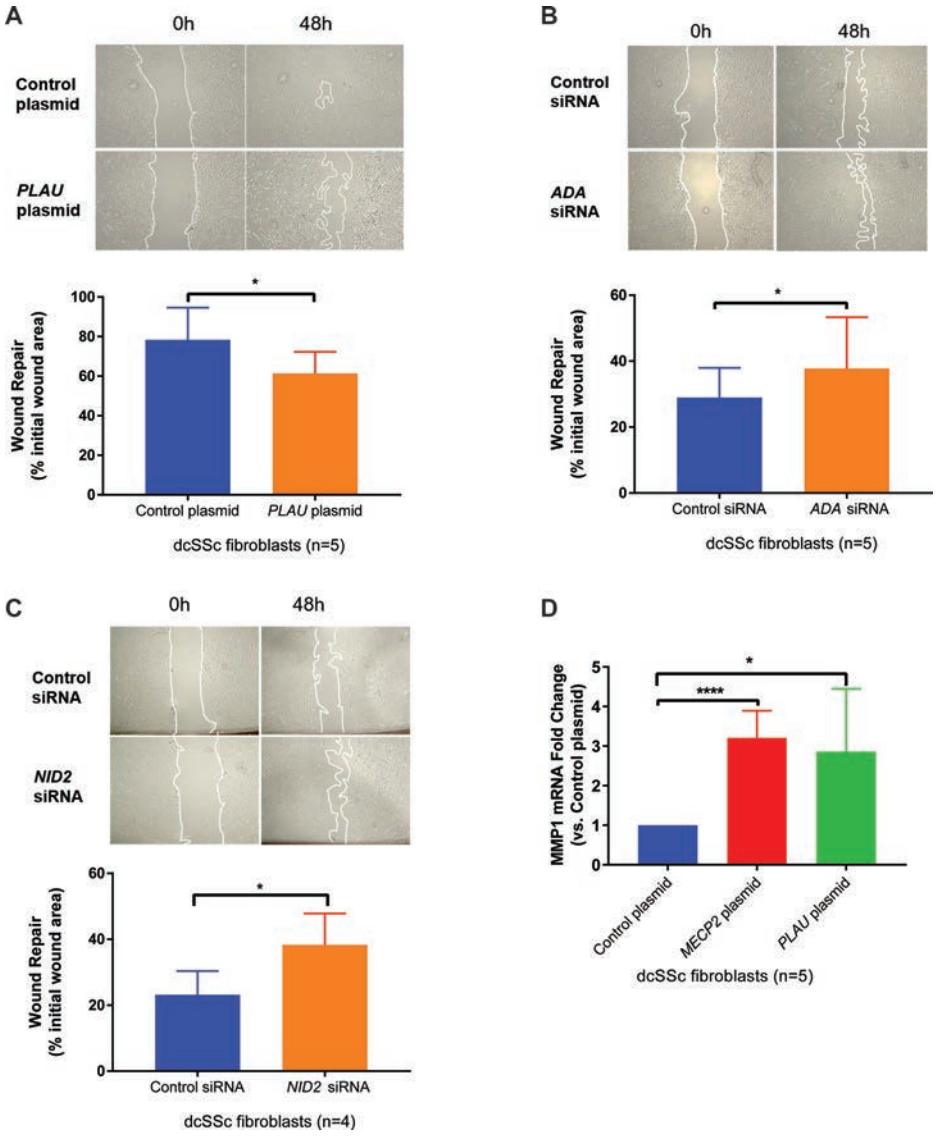


Figure 5 (A) Representative images of wound closure with plasminogen activator urokinase (PLAU) overexpression in diffuse cutaneous systemic sclerosis (dcSSc) fibroblasts at 0 and 48 hours post scratch. Quantification of wound healing migration assay suggested that PLAU overexpression delayed fibroblast migration (n=5, p=0.022). (B) Representative images of wound closure with adenosine deaminase (ADA) knockdown in dcSSc fibroblasts at 0 and 48 hours post scratch. Quantification of wound healing migration assay suggested that ADA knockdown increased fibroblast migration (n=5, p=0.044) (C) Representative images of wound closure with nidogen-2 (NID2) knockdown in dcSSc fibroblasts at 0 and 48 hours post scratch. Quantification of wound healing migration assay suggested that NID2 knockdown induced higher fibroblast migration rates (n=4, p=0.043). (D) Overexpression of methyl-CpG-binding protein 2 (MeCP2) caused 3.20±0.30-fold increase of *MMP1* mRNA expression compared with control plasmid (n=5, p<0.001). Overexpression of PLAU caused 2.86±0.71-fold increase in *MMP1* mRNA expression compared with control plasmid (n=5, p=0.030). Results are expressed as mean±SD. *p<0.05, ****p<0.001.

elucidate their roles in fibroblast functions according to their functional categories.

Identification of MeCP2 target genes that play functional roles in myofibroblast differentiation, fibroblast migration and proliferation

We performed knockdown experiments for seven selected potential MeCP2 targets (*ADA*, *TNFAIP1*, *AXL*, *NID2*, *NTN4*, *PRELP, ANPEP*) and overexpression experiments for *PLAU* to determine their roles in dcSSc fibrosis individually. For knockdown experiments, at least an average 70% gene downregulation was reached for every potential target, with the exception of *PRELP* which we were unable to knockdown successfully and was therefore excluded from our functional studies (figure 3E).

Among all seven investigated potential MeCP2 targets, *NID2* and *PLAU* were identified as pivotal genes in MeCP2-mediated myofibroblast differentiation (figure 4A). When NID2 was knocked down in dcSSc fibroblasts, the expression of *α-SMA* mRNA was moderately but significantly increased (figure 4B). In addition, NID2 knockdown in dcSSc fibroblasts increased collagen gel contraction compared with control siRNA (figure 4C), suggesting NID2 knockdown stimulated myofibroblast differentiation. Similarly, overexpression of PLAU suppressed *α-SMA* mRNA expression, while increased *PPAR-γ* mRNA expression (figure 4D), and PLAU overexpressing fibroblasts exhibited weaker contractile ability than those transfected with empty vector (figure 4E). Taken together, our data suggest that NID2 and PLAU block myofibroblast formation, indicating

that MeCP2-mediated inhibition of myofibroblast differentiation is, at least partly, mediated through activating NID2 and PLAU.

We next show that ADA, NID2 and PLAU are capable of inhibiting fibroblast migration. As shown in figure 5A, overexpressing PLAU suppressed dcSSc fibroblasts migration rates, while silencing ADA (figure 5B) and NID2 (figure 5C) significantly enhanced dcSSc fibroblast migration rates.

We could not identify genes mediating the fibroblast proliferation effect of MeCP2 in two cell proliferation-related MeCP2 targets. Overexpressing PLAU did not change proliferation of fibroblasts (data not shown). We could not assess cell proliferation effect of ADA because fibroblasts detached from the culture chamber used for ki67 staining and died after 24-hour ADA knockdown.

Potential ECM degradation by MeCP2-mediated PLAU activation

Since *PLAU* was upregulated with MeCP2 overexpression, we hypothesised that MeCP2 prevents ECM turnover through positively regulating PLAU and MMPs in dcSSc fibroblasts. As expected, MeCP2 overexpression significantly increased *MMP1* mRNA (figure 5D). In addition, we show that PLAU overexpression in dcSSc fibroblasts was able to increase *MMP1* mRNA expression (figure 5D). Collectively, these data indicate that MeCP2 could promote ECM degradation by overexpressing PLAU and PLAU-mediated MMP1.

MeCP2 binding is enriched at *NID2* and *PLAU* in dermal fibroblasts

To determine if the identified MeCP2-regulated genes in dermal fibroblasts are directly regulated by MeCP2, we mapped genome-wide MeCP2 binding sites in a dermal fibroblast sample from a healthy control. Possible chromatin-shearing biases were controlled by Input library from the same chromatin sample used for MeCP2 bound DNA pulldown. Significant peaks can be visualized in *NID2* and *PLAU* on UCSC genome browser (figure 6), suggesting MeCP2 directly regulates NID2 and PLAU. Indeed, 6 out of 10 relevant MeCP2 target genes we identified in fibroblasts show significant MeCP2 binding enrichments (online supplementary table 1).

DISCUSSION

In this study, we clearly show MeCP2 as a novel antifibrotic epigenetic regulator in dcSSc. MeCP2, which was elevated in dcSSc fibroblasts, has inhibitory effects on myofibroblast differentiation, fibroblast migration and fibroblast proliferation. Non-biased RNA-seq was employed to evaluate transcription alterations after MeCP2 knockdown in dcSSc fibroblasts, and a set of genes dysregulated by MeCP2 and related to fibrosis were identified. RNA profiles from fibroblasts after MeCP2 overexpression and depletion indicated that MeCP2 not only modulates well-known fibrosis-related genes, like *COL1A1*, *α-SMA* and *PPAR-γ*, but also targeted additional genes participating in myofibroblast differentiation, fibroblast migration and fibroblast proliferation. NID2, PLAU and ADA were shown to be antifibrotic mediators of MeCP2 through functional studies.

In agreement with findings reported by Wang *et al*,[35] we demonstrated that MeCP2 was significantly elevated in dcSSc fibroblasts compared with normal fibroblasts. Recent observations of the role MeCP2 in fibrosis were reported in hepatic stellate cells and lung fibroblasts derived from animal models with chronic CCl4-induced liver injury[12] and bleomycin-induced pulmonary fibrosis[14] , respectively. Our studies are the first to report the role of MeCP2 in fibrosis in human dermal fibroblasts from patients with dcSSc. Interestingly, the data generated from animal models showed that MeCP2 upregulated α-SMA expression in lung fibroblasts[14] and decreased PPAR-γ in a liver fibrosis animal model.[12] Our data in human dcSSc fibroblasts indicate that MeCP2 attenuates pro-fibrotic responses, and that MeCP2 overexpression alters biological functions important in fibrosis, through antifibrotic MeCP2 target genes we identified and validated using functional studies. Therefore, our data suggest that increased MeCP2 in dcSSc fibroblasts might be a defence mechanism to counteract the pro-fibrotic nature of the disease in the early stages of dcSSc. *COL1A1*, *α-SMA* and *PPAR-γ* were responsive to MeCP2 regulation in normal fibroblasts, but they might also be co-regulated by other pro-fibrotic factors (eg, TGF-β), which exert prominent influence on dcSSc fibroblasts to maintain the 'SSc phenotype' compared with normal fibroblasts. *COL1A1* expression was attenuated with MeCP2 overexpression in normal fibroblasts but not in dcSSc fibroblasts, indicating that the effects of MeCP2 on COL1A1 might be neutralised

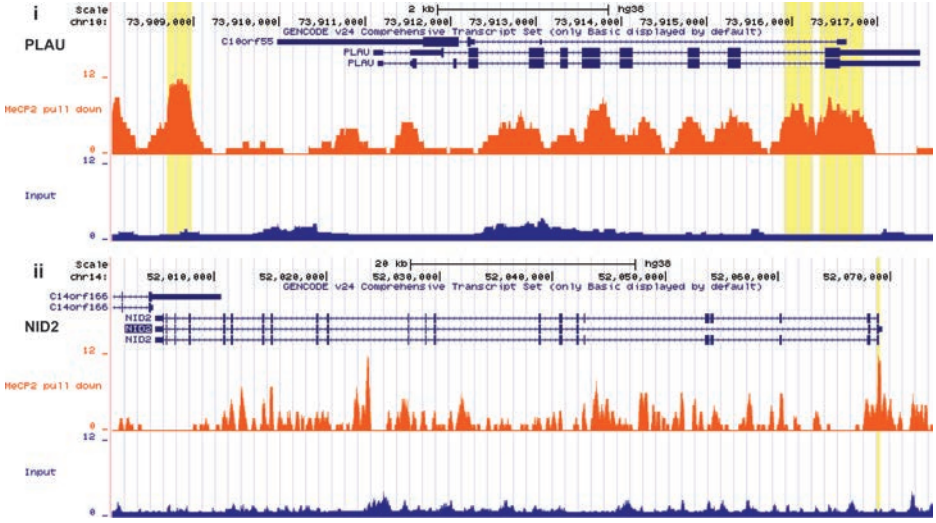


Figure 6 Genome browser tracks show MeCP2 binding peaks in normal fibroblasts (orange) in *PLAU* (i) and *NID2* (ii). Significant peak regions relative to input control (blue) are highlighted in yellow. NID2, nidogen-2; PLAU, plasminogen activator urokinase.

by other pro-fibrotic regulators amplified in dcSSc fibroblasts. A reduction in *α-SMA* and increase of *PPAR-γ* expression were observed with MeCP2 overexpression. Neither was significantly altered after MeCP2 knockdown. Plasminogen activator urokinase (PLAU), an antifibrotic enzyme dampening *α-SMA* mRNA levels and stimulating *PPAR-γ* mRNA expression in dcSSc fibroblasts, was upregulated both with MeCP2 overexpression and knockdown. Therefore, we postulate that PLAU activation in MeCP2-deficient fibroblasts may antagonise effects of MeCP2 knockdown on *α-SMA* and *PPAR-γ* gene expression.

By coupling RNA-seq with functional assays, not only were fibrotic genes like *COL1A1*, *α-SMA* and *PPAR-γ* confirmed as MeCP2-regulated genes, but also novel targets like *PLAU*, *NID2* and *ADA* were identified as potential mediators in the antifibrotic effects of MeCP2. *PLAU* encodes a secreted serine protease urokinase-type plasminogen activator (uPA) that converts plasminogen to plasmin, which is the important component of the extracellular protease system.[36] Plasmin was linked to antifibrotic properties by directly degrading ECM proteins, such as fibronectin,[37] and also activating MMPs that degrade the ECM proteins. In addition, PLAU plays a pivotal role in the cell migration and proliferation via direct binding to its receptor PLAUR or indirect binding to α−8/ β−1 integrin.[38 39] Plasminogen activator inhibitor-1, an inhibitor of uPA, mediates a variety of functions involved in fibrosis of different organs including lung, liver, kidney, as well as cardiovascular system.[40 41] In our study, PLAU overexpression reduced pro-fibrotic properties, including downregulation of *COL1A1* and *α-SMA*, upregulation of *PPAR-γ* and *MMP1*, and resulted in inhibitory effects on myofibroblast differentiation and cell migration in dcSSc fibroblasts. Urokinase is used as a thrombolytic agent and a possible therapeutic role in SSc is suggested by our findings, indicating a possible protective effect of PLAU in SSc fibrosis.

Nidogen-2 (*NID2*) encodes a secretory protein also known as osteonidogen, which is one of the key components of the basement membrane that stabilises the ECM network.[42 43] It is a cell-adhesion molecule that binds collagens I and IV and laminin and may be involved in maintaining the structure of the basement membrane.[44] In our study, NID2-depleted dcSSc fibroblasts exhibit increased *α-SMA* expression, contractility and migration ability, suggesting that NID2, a direct MeCP2 target, plays antifibrotic roles in dcSSc fibroblasts. Further experiments to understand the mechanisms of how these functions were modulated are warranted.

Adenosine deaminase (ADA) regulates levels of adenosine and 2′-deoxyadenosine in tissues and cells.[45] ADA binds to the cell surface by means of either CD26, or adenosine receptors A_1 or A_{2B}.[46] Some studies demonstrated that adenosine and its receptors may promote fibrosis in skin and liver fibrosis models, but inhibit fibrosis in cardiac tissues.[47–49] In addition, lower serum level of ADA was reported in patients with cystic fibrosis.[50] Mice lacking ADA accumulated 10-fold higher adenosine levels and underwent diffuse dermal fibrosis.[48 49] Although we did not detect a change in collagen production after *ADA* knockdown, our data suggest a complementary antifibrotic role of ADA, as *ADA* silencing in dermal dcSSc fibroblasts promoted fibroblast migration. Pegademase bovine is the enzyme replacement drug in ADA-deficient severe combined immunodeficiency disease.[51] Explorations unravelling mechanisms and the therapeutic effect of pegademase bovine in SSc would be of interest.

Limitations of our study include a focus on dermal fibroblasts isolated from patients with dcSSc and healthy controls, and therefore, whether MeCP2 dysregulation also plays a role in lung fibroblasts or other cell types involved in the pathogenesis of SSc, such as endothelial cells or immune cells, remains unknown. In addition, our studies were limited to in vitro experiments, and validation in in vivo models of fibrosis are warranted.

In summary, our results collectively imply that MeCP2 overexpression acts as protective mechanism against skin fibrosis in early dcSSc and that exploiting this mechanism might provide new avenues for therapeutic intervention in this disease. Several canonical and novel fibrotic genes regulated by MeCP2 were identified and functionally characterised. Drugs or compounds modulating MeCP2 expression or targeting these MeCP2-regulated genes might provide attractive new strategies to prevent the progression of fibrosis in scleroderma.

Correction notice This article has been corrected since it published Online First. The subtitle 'Gene knockdown experiments' has been updated.

Contributors All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health grant number R01AI097134 to Dr Sawalha. Dr Khanna is supported by NIH (National Institute of Arthritis and Musculoskeletal and Skin Diseases) grant number K24AR063120.

Competing interests None declared.

Patient consent Not required.

Ethics approval This study was approved by the institutional review board of the University of Michigan.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest* 2007;117:557–67.
2 Denton CP, Khanna D. Systemic sclerosis. *Lancet* 2017;390:1685–99.
3 Wirz EG, Jaeger VK, Allanore Y, *et al*. Incidence and predictors of cutaneous manifestations during the early course of systemic sclerosis: a 10-year longitudinal study from the EUSTAR database. *Ann Rheum Dis* 2016;75:1285–92.
4 Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med* 2009;360:1989–2003.
5 Tyndall AJ, Bannert B, Vonk M, *et al*. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis* 2010;69:1809–15.
6 Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. *Nat Rev Rheumatol* 2011;8:42–54.
7 Rosenbloom J, Castro SV, Jimenez SA. Narrative review: fibrotic diseases: cellular and molecular mechanisms and novel therapies. *Ann Intern Med* 2010;152:159–66.
8 Ihn H, Yamane K, Kubo M, *et al*. Blockade of endogenous transforming growth factor beta signaling prevents up-regulated collagen synthesis in scleroderma fibroblasts: association with increased expression of transforming growth factor beta receptors. *Arthritis Rheum* 2001;44:474–80.
9 Altorok N, Kahaleh B. Epigenetics and systemic sclerosis. *Semin Immunopathol* 2015;37:453–62.
10 Tsou PS, Sawalha AH. Unfolding the pathogenesis of scleroderma through genomics and epigenomics. *J Autoimmun* 2017;83:73–94.
11 Chahrour M, Jung SY, Shaw C, *et al*. MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* 2008;320:1224–9.
12 Mann J, Chu DC, Maxwell A, *et al*. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. *Gastroenterology* 2010;138:705–14.
13 Bian EB, Huang C, Wang H, *et al*. The role of methyl-CpG binding protein 2 in liver fibrosis. *Toxicology* 2013;309:9–14.
14 Hu B, Gharaee-Kermani M, Wu Z, *et al*. Essential role of MeCP2 in the regulation of myofibroblast differentiation during pulmonary fibrosis. *Am J Pathol* 2011;178:1500–8.
15 Carmona FD, Cénit MC, Diaz-Gallo LM, *et al*. New insight on the Xq28 association with systemic sclerosis. *Ann Rheum Dis* 2013;72:2032–8.
16 Sawalha AH, Webb R, Han S, *et al*. Common variants within MECP2 confer risk of systemic lupus erythematosus. *PLoS One* 2008;3:e1727.

17 Webb R, Wren JD, Jeffries M, *et al*. Variants within MECP2, a key transcription regulator, are associated with increased susceptibility to lupus and differential gene expression in patients with systemic lupus erythematosus. *Arthritis Rheum* 2009;60:1076–84.
18 Koelsch KA, Webb R, Jeffries M, *et al*. Functional characterization of the MECP2/IRAK1 lupus risk haplotype in human T cells and a human MECP2 transgenic mouse. *J Autoimmun* 2013;41:168–74.
19 Sawalha AH. Overexpression of methyl-CpG-binding protein 2 and autoimmunity: evidence from MECP2 duplication syndrome, lupus, MECP2 transgenic and Mecp2 deficient mice. *Lupus* 2013;22:870–2.
20 van den Hoogen F, Khanna D, Fransen J, *et al*. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis* 2013;72:1747–55.
21 Altorok N, Tsou PS, Coit P, *et al*. Genome-wide DNA methylation analysis in dermal fibroblasts from patients with diffuse and limited systemic sclerosis reveals common and subset-specific DNA methylation aberrancies. *Ann Rheum Dis* 2015;74:1612–20.
22 Liang CC, Park AY, Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc* 2007;2:329–33.
23 Rueden CT, Schindelin J, Hiner MC, *et al*. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 2017;18:529.
24 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–20.
25 Dobin A, Davis CA, Schlesinger F, *et al*. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 2013;29:15–21.
26 Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 2014;30:923–30.
27 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550.
28 Bourgon R, Gentleman R, Huber W. Independent filtering increases detection power for high-throughput experiments. *Proc Natl Acad Sci U S A* 2010;107:9546–51.
29 Rube HT, Lee W, Hejna M, *et al*. Sequence features accurately predict genome-wide MeCP2 binding in vivo. *Nat Commun* 2016;7:11025.
30 Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357–9.
31 Zhang Y, Liu T, Meyer CA, *et al*. Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 2008;9:R137.
32 ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;489:57–74.
33 Heinz S, Benner C, Spann N, *et al*. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 2010;38:576–89.
34 Huang daW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009;4:44–57.
35 Wang Y, Fan PS, Kahaleh B. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. *Arthritis Rheum* 2006;54:2271–9.
36 Myöhänen H, Vaheri A. Regulation and interactions in the activation of cell-associated plasminogen. *Cell Mol Life Sci* 2004;61:2840–58.
37 Bonnefoy A, Legrand C. Proteolysis of subendothelial adhesive glycoproteins (fibronectin, thrombospondin, and von Willebrand factor) by plasmin, leukocyte cathepsin G, and elastase. *Thromb Res* 2000;98:323–32.
38 Blasi F, Sidenius N. The urokinase receptor: focused cell surface proteolysis, cell adhesion and signaling. *FEBS Lett* 2010;584:1923–30.
39 Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol* 2010;11:23–36.
40 Liu F, Lagares D, Choi KM, *et al*. Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2015;308:L344–57.
41 Flevaris P, Vaughan D. The Role of Plasminogen Activator Inhibitor Type-1 in Fibrosis. *Semin Thromb Hemost* 2017;43:169–77.
42 Kohfeldt E, Sasaki T, Göhring W, *et al*. Nidogen-2: a new basement membrane protein with diverse binding properties. *J Mol Biol* 1998;282:99–109.
43 Miosge N, Holzhausen S, Zelent C, *et al*. Nidogen-1 and nidogen-2 are found in basement membranes during human embryonic development. *Histochem J* 2001;33:523–30.
44 Fox JW, Mayer U, Nischt R, *et al*. Recombinant nidogen consists of three globular domains and mediates binding of laminin to collagen type IV. *Embo J* 1991;10:3137–46.
45 Cristalli G, Costanzi S, Lambertucci C, *et al*. Adenosine deaminase: functional implications and different classes of inhibitors. *Med Res Rev* 2001;21:105–28.
46 Pacheco R, Martinez-Navio JM, Lejeune M, *et al*. CD26, adenosine deaminase, and adenosine receptors mediate costimulatory signals in the immunological synapse. *Proc Natl Acad Sci U S A* 2005;102:9583–8.
47 Chunn JL, Mohsenin A, Young HW, *et al*. Partially adenosine deaminase-deficient mice develop pulmonary fibrosis in association with adenosine elevations. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L579–87.
48 Cronstein BN. Adenosine receptors and fibrosis: a translational review. *F1000 Biol Rep* 2011;3:21.
49 Chan ES, Fernandez P, Merchant AA, *et al*. Adenosine A2A receptors in diffuse dermal fibrosis: pathogenic role in human dermal fibroblasts and in a murine model of scleroderma. *Arthritis Rheum* 2006;54:2632–42.
50 Farahmand F, Tajdini P, Falahi G, *et al*. Evaluation of Serum Adenosine Deaminase in Cystic Fibrosis Patients in an Iranian Referral Hospital. *Iran J Pediatr* 2016;26:e2246.
51 Booth C, Gaspar HB. Pegademase bovine (PEG-ADA) for the treatment of infants and children with severe combined immunodeficiency (SCID). *Biologics* 2009;3:349–58.

EXTENDED REPORT

In vivo visualisation of different modes of action of biological DMARDs inhibiting osteoclastic bone resorption

Yoshinobu Matsuura,[1,2,3] Junichi Kikuta,[1,3] Yuika Kishi,[1] Tetsuo Hasegawa,[1] Daisuke Okuzaki,[4] Toru Hirano,[2] Masafumi Minoshima,[5] Kazuya Kikuchi,[3,5] Atsushi Kumanogoh,[2,3] Masaru Ishii[1,3]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212880).

[1]Department of Immunology and Cell Biology, Graduate School of Medicine and Frontier Biosciences, Osaka University, Osaka, Japan
[2]Department of Respiratory Medicine and Clinical Immunology, Graduate School of Medicine, Osaka University, Osaka, Japan
[3]WPI-Immunology Frontier Research Center, Osaka University, Osaka, Japan
[4]Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
[5]Department of Material and Life Science, Graduate School of Engineering, Osaka University, Osaka, Japan

Correspondence to
Assistant Professor Junichi Kikuta and Professor Masaru Ishii, Department of Immunology and Cell Biology, Graduate School of Medicine and Frontier Biosciences, Osaka University, Osaka 565-0871, Japan; jkikuta@icb.med.osaka-u.ac.jp, mishii@icb.med.osaka-u.ac.jp

Received 19 December 2017
Accepted 10 April 2018
Published Online First 28 April 2018

To cite: Matsuura Y, Kikuta J, Kishi Y, *et al*. *Ann Rheum Dis* 2018;**77**:1220–1226.

ABSTRACT

Objectives Osteoclasts play critical roles in inflammatory bone destruction. Precursor cell migration, cell differentiation, and functional cell activation are all in play. Biological disease-modifying antirheumatic drugs (DMARDs) have been shown to significantly inhibit both bone erosion as well as synovitis, although how such agents reduce osteoclastic bone destruction*in vivo* has not been fully explained. Here, we used an intravital time-lapse imaging technique to directly visualise mature osteoclasts and their precursors, and explored how different biological DMARDs acted *in vivo*.

Methods Lipopolysaccharide (LPS) was injected into the calvarial periosteum of fluorescent reporter mice to induce inflammatory bone destruction. Time-lapse imaging was performed via intravital multiphoton microscopy 5 days after LPS injection. Biological DMARDs, including monoclonal antibodies (mAbs) against the interleukin (IL) 6 receptor (IL-6R) and tumour necrosis factor α (TNFα), or cytotoxic T-lymphocyte-associated protein 4 (CTLA4)-Ig, were intraperitoneally administered at the time of LPS injection. We determined CD80/86 expression levels in mature osteoclasts and their precursors by flow cytometry, quantitative PCR and immunohistochemistry.

Results Of the biologicals tested, anti-IL-6R and anti-TNFα mAbs affected mature osteoclasts and switched bone-resorbing osteoclasts to non-resorbing cells. CTLA4-Ig had no action on mature osteoclasts but mobilised osteoclast precursors, eliminating their firm attachment to bone surfaces. In agreement with these results, CD80/86 (the target molecules of CTLA4-Ig) were prominently expressed only in osteoclast precursor cells, being suppressed during osteoclast maturation.

Conclusions Intravital imaging revealed that various biological DMARDs acted at specific therapeutic time points during osteoclastic bone destruction, with different efficacies. These results enable us to grasp the real modes of action of drugs, optimising the usage of drug regimens.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by joint inflammation and progressive bone destruction. Articular bone erosion is considered to be a representative RA symptom in the early stage of disease;[1] sustained joint inflammation induces synovial proliferation and pathological bone destruction, eventually causing irreversible joint deformation and functional deterioration.[2 3] Control of joint inflammation with disease-modifying antirheumatic drugs (DMARDs) halts bone destruction in some patients.[4] To prevent RA-associated bone destruction, it is important to understand the cellular mechanism of inflammatory bone destruction *in vivo*.

Arthritic bone destruction is considered to be mediated mainly by enhanced activation of osteoclasts at inflammatory sites. Osteoclasts are multinucleated cells derived from monocyte/macrophage, haematopoietic precursor cells by stimulation with macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL).[5 6] Osteoclasts constitute a specialised subset of cells with bone-resorbing capacity and play critical roles in normal bone homoeostasis (bone remodelling) and the inflammatory bone destruction that causes bone erosion and focal bone loss.[7] During inflammatory bone destruction, a variety of cytokines control osteoclast dynamics such as precursor cell migration, differentiation and functional activation. For example, proinflammatory cytokines such as interleukin (IL) 6 and tumour necrosis factor α (TNFα) promote osteoclast differentiation by inducing RANKL in mesenchymal cells, and may directly stimulate both the osteoclastogenesis and the bone-resorptive activity of mature osteoclasts.[8–12] T helper 17 cells promote osteoclast function because they express RANKL,[13] whereas regulatory T cells control osteoclastogenesis via the expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA4), which engages directly with CD80/86 expressed on cells of the osteoclast lineage.[14 15]

Biological DMARDs, such as monoclonal antibodies (mAbs) against the anti- IL-6 receptor (IL-6R) and anti-TNFα, and CTLA4-Ig, have recently been developed to treat RA. Despite differences in the molecular targets of these drugs, they (equally) strongly inhibit bone erosion and synovitis even in patients with high disease activity.[16–18] Recent basic studies have revealed that these biologicals exert direct effects on osteoclast differentiation and function, although little is known about the differences in mode of action.[19 20]

Previously, we used intravital multiphoton microscopy to visualise osteoclasts and their precursors in living mouse bone, and found that the migration and positioning of osteoclast precursors were controlled

by the blood-enriched lipid mediator sphingosine-1-phosphate.[21] We also characterised two different populations of living mature osteoclasts in terms of their motility and function: 'static bone-resorptive' and 'moving non-resorptive' cells.[22] Furthermore, intravital imaging revealed that different antiresorptive drugs acted at specific therapeutic time points during osteoclastic bone resorption: bisphosphonates inhibited the bone-resorptive function of mature osteoclasts, whereas active vitamin D (a crucial hormone in terms of bone homoeostasis) regulated the migratory behaviour of circulating osteoclast precursors, limiting osteoclastic bone resorption.[23] This technique has the potential to clarify the modes of action of a variety of antirheumatic drugs.

In this study, using our intravital bone imaging system, we directly visualised the cellular dynamics of osteoclasts and their precursors during inflammatory bone destruction, and explored how various biological DMARDs affected osteoclast dynamics, including the migration of precursor cells, and activation of mature osteoclasts *in vivo*. We found that different biologicals acted at specific therapeutic time points during osteoclastic bone destruction, with varying efficacies; blockade of IL-6R and TNFα intensely inhibited the bone-resorptive function of mature osteoclasts, whereas CTLA4-Ig had no effect on mature osteoclasts, but mobilised circulating osteoclast precursors.

METHODS

Mice

C57BL/6 wild type (WT) mice were purchased from CLEA Japan. CX_3CR1-enhanced green fluorescent protein (EGFP) knock-in mice[24] were obtained from Jackson Laboratory. V-type H^+-ATPase a3 subunit-GFP fusion knock-in mice (a3-GFP) and tartrate-resistant acid phosphatase (TRAP)-tdTomato transgenic mice have been described previously.[22 25] All mice were maintained under a 12 hours/12 hours light/dark cycle in the specific pathogen-free animal facilities of Osaka University. All animal experiments were approved by the Institutional Animal Experimental Committee of Osaka University.

Treatment with drugs

Lipopolysaccharide (LPS) (20 mg/kg; Sigma-Aldrich) dissolved in phosphate-buffered saline (PBS) was injected into the mouse calvarial periosteum with the animals under isoflurane anaesthesia. Five days later, intravital imaging was performed. Anti-IL-6R mAb (10 mg/kg; Chugai Pharmaceutical), anti-TNFα mAb (5 mg/kg; Janssen R&D) or CTLA4-Ig (10 mg/kg; Bristol-Myers Squibb) dissolved in PBS was intraperitoneally administered at the time of LPS injection.

Intravital multiphoton bone imaging

Mouse calvarial bone tissues were examined via intravital microscopy using a protocol modified from that of a previous report.[21] The imaging system featured a Nikon upright two-photon microscope (A1R-MP) equipped with a 25 × water immersion objective (APO: numerical aperture (NA), 1.1; Nikon) and a Carl Zeiss upright two-photon microscope (LSM 780 NLO) equipped with a 20 × water immersion objective (W Plan-Apochromat: NA 1.0; Carl Zeiss). Both systems were driven by a femtosecond-pulsed infrared laser (Chameleon Vision II Ti: Sapphire; Coherent). Details of the method are also described in online supplementary information.

Immunohistochemistry

To prepare sections, mice were perfused with 4% (v/v) paraformaldehyde with 20% (w/v) sucrose for fixation, and dissected bone tissues further fixed in the same solution for 4 hours at 4°C and embedded in super cryo-embedding medium (SCEM) compound (Leica). Sections (10 μm-thick) were prepared using the Kawamoto film method, blocked with 5% (w/v) bovine serum albumin for 1 hour, stained with anti-CD80 or CD86 antibody (BioLegend) at 4°C overnight, stained with Alexa 488-conjugated secondary antibody (1:500 dilution) for 2 hours, stained with Hoechst 33342, and images acquired using a confocal microscope (A1; Nikon).

In vitro osteoclast differentiation

Bone marrow-derived macrophages (BMMs) from WT mice were obtained via culture of bone marrow collected from 8–10-week-old male tibiae and femora, as described previously, with slight modifications.[26] Details of the method are also described in online supplementary information.

Flow cytometry

BMMs of WT mice were cultured with M-CSF (30 ng/mL) and RANKL (50 ng/mL) for 48 hours, washed with PBS, and detached using enzyme-free cell dissociation buffer (Millipore) at 37°C for 5 min. After single-cell suspension, cells were incubated with anti-CD16/32 antibodies (eBioscience) for 10 min on ice, and then stained for 30 min with allophycocyanin (APC)-conjugated antimouse CD80 or CD86 antibody, or isotype control antibody (BioLegend). Cells were analysed via flow cytometry (FACS Canto II; BD) and the data analysed with the aid of FlowJo software (TreeStar).

Quantitative real-time PCR

Quantitative real-time PCR was performed with the aid of a thermal cycler dice real-time system TP800 (Takara) using the following specific primer pairs (forward and reverse, respectively): CD80 (5′-ACCCCCAACATAACTGAGTCT-3′ and 5′-TTCCAACCAAGAGAAGCGAGG-3′); CD86 (5′-TCCAGAACTTACGGAAGCACCCACG-3′ and 5′-CAGGTTCACTGAAGTTGGCGATCAC-3′); and β-actin (5′-TCCTCCCTGGAGAAGAGCTA-3′ and 5′-ATCTCCTTCTGCATCCTGTC-3′).

Statistics

The data were analysed using one-way analysis of variance or the Mann–Whitney rank-sum test. Data represent mean±SD unless otherwise specified. A p value <0.05 was considered to reflect statistical significance.

RESULTS

Biological DMARDs ameliorated inflammatory bone destruction

The calvarial bones are suitable for visualising the cellular dynamics of osteoclasts and their precursors in living mice via intravital multiphoton imaging of the endosteum.[22] In this study, we used an LPS-induced inflammatory bone destruction model to investigate the effects of various biological DMARDs on bone erosion *in vivo*. LPS was injected into the calvarial periosteum and micro–computed tomography (μCT) analysis performed 5 days later. We found bone erosive lesions on the calvaria of LPS-injected but not vehicle-injected mice (online supplementary figure 1A,B), suggesting that activated osteoclasts resorbed bone tissues at sites of LPS-induced inflammation. To explore the efficacy of biological DMARDs in terms of inhibiting LPS-induced inflammatory bone destruction, mAbs against TNFα or IL-6R, or CTLA4-Ig, were intraperitoneally administered. All of

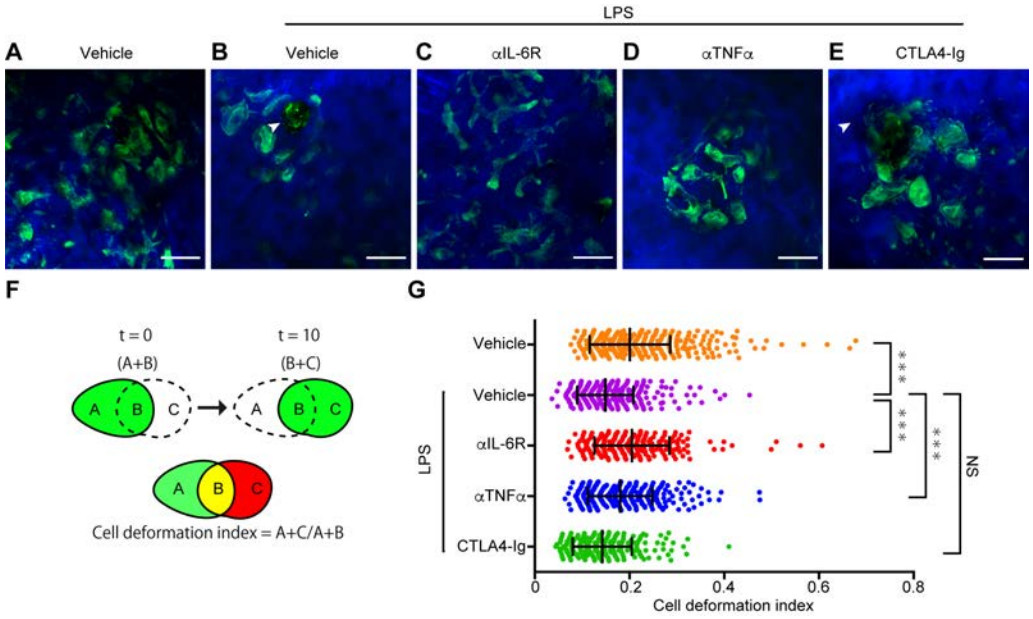


Figure 1 Anti-IL-6 receptor (IL-6R) and anti-TNFα mAbs affect the motility and function of mature osteoclasts. (A,B) Representative intravital multiphoton imaging of mouse bone tissues in a3-GFP knock-in mice injected with vehicle (A), (online supplementary video 1) or LPS (B), (online supplementary video 2) into the calvarial periosteum. Green, mature osteoclasts expressing the GFP-fused V-type H^+-ATPase a3 subunit. Blue, bone surface. Scale bars, 50 µm. The arrowhead indicates the area in which the second harmonic signal of bone was deficient. (C–E) Representative bone images of mice injected with LPS (into the calvarial periosteum) and also given intraperitoneal anti-IL-6R antibody (C), (online supplementary video 3), anti-TNFα antibody (D), (online supplementary video 4), or CTLA4-Ig (E), (online supplementary video 5). (**F**) Cell shapes were automatically recognised by the image analysis software, and three distinct areas were defined initially: (*t*=0) (A), (green); at the final time frame (*t*=10) (C), (red); and overlapping between these two time frames (B), (yellow). The cell deformation index was calculated as (A+C)/(A+B), representing the ratio of the area changed during 10 min divided by that of the previous time frame. (G) Motility of mature osteoclasts. A higher cell deformation index indicates increased motility. The data points (n=496 for vehicle injection, n=387 for LPS injection, n=235 for anti-IL-6R antibody, n=334 for anti-TNFα antibody, n=228 for CTLA4-Ig) represent the values for individual cells compiled from three independent experiments. Error bars represent mean±SD. CTLA4, cytotoxic T-lymphocyte-associated protein 4; LPS, lipopolysaccharide; mAbs, monoclonal antibodies; NS, not significant; TNFα, tumour necrosis factor α.

the biological DMARDs significantly decreased the LPS-induced bone erosion (online supplementary figure 1C–F).

Anti-IL-6R and anti-TNFα mAbs affected the motility and function of mature osteoclasts

We next examined the effects of biological DMARDs on the dynamics of living mature osteoclasts, using intravital multiphoton microscopy. To visualise mature osteoclasts, we first used fluorescent reporter mice in which GFP is expressed as a fusion protein with the vacuolar type H^+-ATPase a3 subunit (a3-GFP mice).[25] Because the a3 subunit is preferentially and abundantly expressed in mature osteoclasts,[27 28] a3-GFP mice are suitable for visualising mature osteoclasts *in vivo*.[22] In addition, as GFP was expressed as a fusion protein with a proton pump, GFP fluorescence served as a marker of mature osteoclasts, and provided information on the subcellular distribution of the proton pump in such cells. In this study, LPS was directly injected into the periostea of a3-GFP mice. Five days after LPS injection, the mouse bone tissues were visualised to assess the dynamics of GFP^+ mature osteoclasts (figure 1). Compared with vehicle-injected mice, the motility of mature osteoclasts was significantly decreased in LPS-injected ones, reflecting an increase in the population of static-resorptive (R-type) osteoclasts under inflammatory conditions (figure 1A,B,F,G and online supplementary videos 1,2). We also found some osteoclasts in the area where the second harmonic signals of bone tissues were deficient, suggesting high-level activation of osteoclasts forming bone erosions (figure 1B).

To investigate the effects of biological DMARDs on the motility and function of mature osteoclasts, each biological was administered intraperitoneally at the clinical dose into mice in which LPS-induced bone destruction was in play. In mice treated with anti-IL-6R mAb, the morphology of mature osteoclasts changed markedly (lobulation was evident) and cell motility increased (figure 1C,G and online supplementary video 3). Similarly, in mice treated with anti-TNFα mAb, the motility of mature osteoclasts also increased significantly (figure 1D,G and online supplementary video 4), indicating an increase in the population of non-resorptive (N-type) osteoclasts. In contrast, mature osteoclasts of mice given CTLA4-Ig exhibited no remarkable change in morphology or motility (figure 1E,G and online supplementary video 5). Even when the dose of CTLA4-Ig was increased twofold, osteoclast motility did not change (online supplementary figure 2).

Next, we examined the changes in acid production of mature osteoclasts using a pH-sensing chemical probe, pHocas-3, which we recently developed.[29] This probe, which emits green fluorescence only in acidic environments, can detect local low pH in bone resorption areas *in vivo*. We injected pH probes into fluorescent reporter mice in which tdTomato is expressed under the TRAP promoter[22] and quantified the bone resorptive activity of the mature osteoclasts as described previously.[30] The bone resorptive activity of mature osteoclasts was significantly activated in the LPS-treated mice compared with the vehicle-treated ones (online supplementary figure 3A,B,G). Furthermore, the bone resorptive activity was significantly inhibited by the

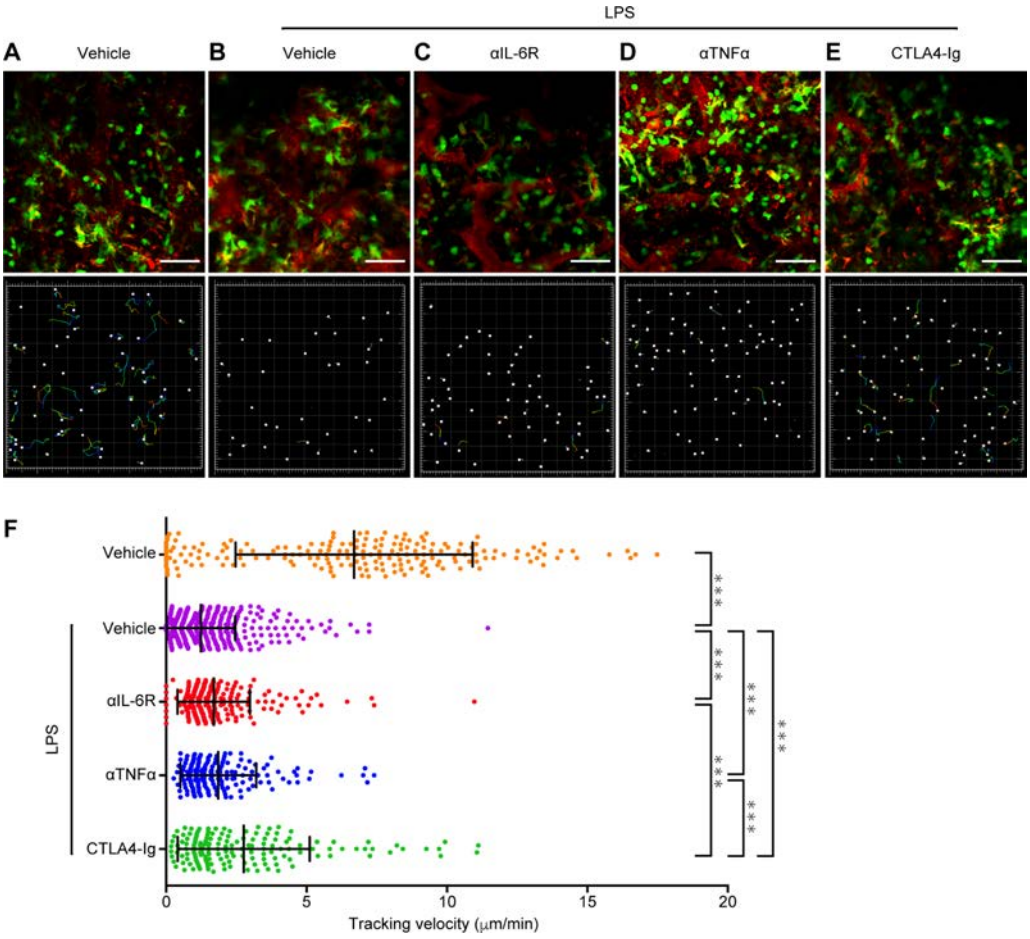


Figure 2 CTLA4-Ig mobilised osteoclast precursors. (A,B) Representative images of calvarial periosteal bone of CX_3CR1 knock-in mice injected with vehicle (A), (online supplementary video 6) or LPS (B), (online supplementary video 7). Green, CX_3CR1-EGFP-positive cells. Red, blood vessels visualised by intravenous injection of Texas Red-conjugated 70 kDa dextran. Scale bars, 50 µm (upper panels). The movements of CX_3CR1-EGFP-positive cells were tracked for 20 min. Coloured lines show the cell trajectories (lower panels). (C–E) Representative bone images of mice injected with LPS into the calvarial periosteum, and also given intraperitoneal anti-IL-6 receptor (IL-6R) antibody (C), (online supplementary video 8), anti-TNFα antibody (D), (online supplementary video 9), or CTLA4-Ig (E), (online supplementary video 10). (F) Tracking velocities of CX_3CR1-positive cells. Data points (n=194 for vehicle injection, n=601 for LPS injection, n=176 for anti-TNFα antibody, n=255 for anti-IL-6R antibody, n=157 for CTLA4-Ig) represent the values for individual cells compiled from three independent experiments. Error bars represent mean±SD. CTLA4, cytotoxic T-lymphocyte-associated protein 4; EGFP, enhanced green fluorescent protein; LPS, lipopolysaccharide; TNFα, tumour necrosis factor α.

treatment with anti-IL-6R mAb and anti-TNFα mAb, consistent with the results of the cell deformation index analysis. In comparison, the CTLA4-Ig treatment produced no remarkable change in the bone resorptive activity (online supplementary figure 3C–G), although the number of mature osteoclasts in the CTLA4-Ig-treated mice was reduced compared with the vehicle-treated ones (online supplementary figure 3H).

CTLA4-Ig mobilised osteoclast precursors

To investigate the impact of biological DMARDs on inflammatory bone destruction, we next examined the mobility of osteoclast precursors in living bone tissues using intravital multiphoton microscopy. LPS was injected into the periosteum of fluorescent reporter mice in which EGFP was expressed under the control of the CX_3CR1 promoter, labelling osteoclast precursor monocytes.[21 31 32] Five days after LPS injection, the bone tissues of the mice were visualised to assess the mobility of $EGFP^+$ monocytoid cells (figure 2). The average tracking velocity of CX_3CR1-$EGFP^+$ osteoclast precursors in the LPS-injected mice was significantly lower than that in the vehicle-injected mice (figure 2A,B,F and online supplementary videos 6,7). The osteoclast precursors adhered tightly to the endosteum in the inflammatory environment, which is thought to be a critical step during differentiation into mature osteoclasts.[33 34]

To examine the effect of biological DMARDs on the mobility of osteoclast precursors, bone destruction was induced in CX_3CR1-EGFP knock-in mice in which each biological agent was given intraperitoneally. Compared with vehicle-treated mice, the mean tracking velocity of CX_3CR1-$EGFP^+$ osteoclast precursors was slightly but significantly increased in mice treated with anti-IL6R and anti-TNFα mAbs (figure 2C,D,F and online supplementary videos 8,9). On the other hand, treatment with CTLA4-Ig markedly increased the velocity of osteoclast precursors, suggesting that *in vivo* treatment with CTLA4-Ig inhibits the firm attachment of such cells to bone surfaces, thereby limiting osteoclastic bone destruction, which is the principal therapeutic effect of CTLA4-Ig (figure 2E,F and online supplementary video 10).

CD80/86 expression on osteoclasts was suppressed during osteoclast maturation

Intravital imaging revealed that CTLA4-Ig had no remarkable effect on mature osteoclast function, but notably changed the mobility of osteoclast precursors. To explore the molecular basis of this phenomenon, we next examined the surface

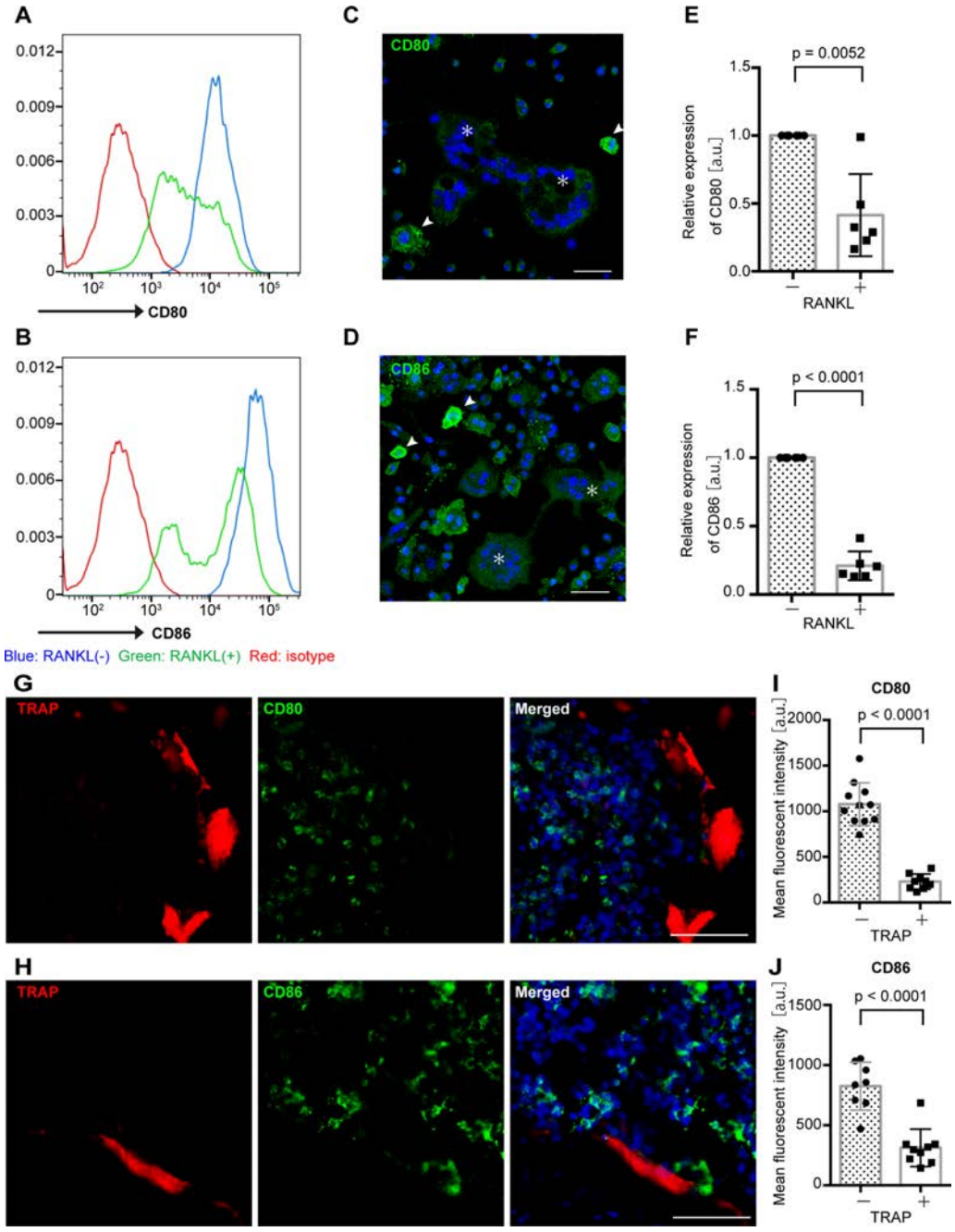


Figure 3 Expression of osteoclast CD80/86 is suppressed during osteoclast maturation. (A,B) Bone marrow-derived macrophages (BMMs) from WT mice were cultured with M-CSF in the presence or absence of RANKL for 48 hours. Harvested cells were stained with APC-conjugated anti-CD80 antibody (A) or anti-CD86 antibody (B), and analysed via flow cytometry. (C,D) Immunohistochemical staining for CD80 (C) and CD86 (D) in cultured osteoclasts. Asterisks indicate multinucleated osteoclasts. Arrowheads indicate mononuclear cells. Blue, nucleus. Green, CD80 or CD86. Scale bars, 50 µm. (E,F) The expression levels of mRNAs encoding CD80 (E) or CD86 (F) in WT BMMs cultured with M-CSF in the presence or absence of RANKL for 72 hours. Data are presented as mean±SD. P values were calculated with the aid of the Mann-Whitney rank-sum test. (G,H) Immunohistochemical staining for CD80 (G) or CD86 (H) in the femur of normal TRAP-tdTomato mice. Red, TRAP-tdTomato-positive mature osteoclasts. Blue, nucleus. Green, CD80 (G) or CD86 (H). Scale bars, 50 µm. (I,J) Mean fluorescent intensity of CD80 (I) and CD86 (J) in TRAP-negative and TRAP-positive cells shown in (G,H), respectively. P values were calculated using the Mann-Whitney rank-sum test. M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of NF-κB ligand; TRAP, tartrate-resistant acid phosphatase; WT, wild type.

expression of the molecules targeted by CTLA4-Ig, thus CD80 (B7-1) and CD86 (B7-2). The BMMs were collected from mice, cultured for 48 hours with M-CSF in the presence or absence of RANKL, and then analysed by flow cytometry. The expression levels of CD80 and CD86 decreased 48 hours after RANKL stimulation (figure 3A,B). Immunohistochemistry also showed that the CD80/86 expression levels in multinucleated osteoclasts decreased, compared with those in mononuclear cells (figure 3C,D). In agreement with these data, the levels of mRNAs encoding CD80/86 also decreased after RANKL stimulation (figure 3E,F). Finally, we determined the *in vivo* expression levels of CD80/86 on mature osteoclasts of the femur of TRAP-tdTomato mice. Consistent with the *in vitro* results, the expression levels of CD80/86 in TRAP$^+$

multinucleated osteoclasts were lower than those in mononuclear bone marrow cells (figure 3G–J). These results indicate that CD80/86, the target molecules of CTLA4-Ig, were prominently expressed only in precursor, thus not maturing, osteoclasts.

DISCUSSION

We analysed the dynamic behaviour of osteoclasts and their precursors in a model of inflammatory bone destruction using intravital multiphoton microscopy, and revealed that anti-IL-6R and anti-TNFα mAbs affected mature osteoclasts and could switch bone-resorbing osteoclasts to the non-resorbing state. On the other hand, CTLA4-Ig had no effect on the bone resorptive activity of mature osteoclasts, but did act on osteoclast precursors to mobilise them, inhibiting their firm attachment to bone surfaces, which results in the decrease in the number of mature osteoclasts and resultant bone erosion. We also found that CD80/86 surface expression on osteoclasts was suppressed during osteoclast maturation both *in vitro* and *in vivo*, although further studies are necessary to reveal the function of CD80/86 in osteoclasts. These results indicate a novel mode of action for CTLA4-Ig-mediated suppression of inflammatory bone destruction.

Migration of osteoclast precursors is regulated by various chemoattractants and adhesion molecules. For example, TNFα is involved in the migration of CD11b-positive monocytes, including osteoclast precursors, increasing their efflux from the bone marrow into inflammatory sites.[10 35] In addition, TNFα has a profound effect on monocytes, inhibiting the migratory response towards chemotactic stimuli, which probably serves to retain monocytes at the actual sites of inflammation.[36 37] IL-6 is involved in trafficking of osteoclast precursors via upregulation of S1PR2 (which attracts the precursors to the bone surface).[38] CTLA4-Ig has been reported to regulate osteoclastogenesis, and transendothelial migration of monocytes via downregulation of adhesion molecules and altered actin organisation.[39] Such evidence suggests that all biologicals tested in this study potentially modify the migratory behaviour of osteoclast precursors, and intravital imaging showed that CTLA4-Ig had the most profound effect on osteoclast precursor migration.

Biological DMARDs improve both synovitis and bone erosion with tolerable adverse effects; although differences among the drugs and their optimal usage patterns have been vigorously investigated in various clinical trials. In this study, we propose a novel mode for selection of biologicals based on their modes of action *in vivo*. Uniquely, CTLA4-Ig does not affect mature osteoclasts, the functions of which are important in terms of physiological bone homoeostasis, bone turnover and protection against bacterial infections.[40 41] Thus, elderly patients, who are susceptible to infection and who have low levels of bone turnover, may be good candidates for CTLA4-Ig treatment. Conversely, IL-6/TNFα blockade, which strongly suppresses the function of existing mature osteoclasts, should be chosen for patients with high-level disease activity, or who are at risk of structural damage.

Finally, we should also consider the limitations of this study, as well as future perspectives. First, we used an LPS-induced inflammatory bone destruction model to evaluate the effects of various biological DMARDs. Intravital bone imaging techniques can be applied for the analysis of other inflammatory bone destruction models, such as collagen-induced arthritis, and we plan to further investigate whether our data actually reflect osteoclast dynamics in inflammatory joints in arthritis mouse models, and in patients with RA. Second, we found a direct effect of CTLA4-Ig on the mobility of osteoclast precursors, but have not clarified the molecular mechanism. To explore comprehensive gene expression changes, we performed an RNA sequence analysis of CX_3CR1-$EGFP^+$ cells from calvaria in mice treated with CTLA4-Ig or control IgG. We found that genes associated with cellular movement were increased in the CTLA4-Ig-treated group (online supplementary figure 4), but further studies are needed to determine the precise mechanism. Third, we focused mainly on the motility of osteoclast precursors, but could not evaluate osteoclast differentiation with our imaging system because the observable time is technically limited to up to 12 hours, which may be insufficient for these evaluations. If we can improve our imaging system to enable longer observation periods, we should be able to reveal whether CTLA4-Ig could directly inhibit osteoclast differentiation *in vivo*.

In conclusion, we visualised the behaviour of osteoclasts and their precursors in a model of inflammatory bone destruction using intravital multiphoton microscopy, and found that different biological DMARDs acted at specific therapeutic time points during osteoclastic bone destruction, with different efficacies. The results reveal the real modes of action of the drugs, which is informative in terms of optimal usage.

Acknowledgements This work was supported by CREST, the Japan Science and Technology Agency; a Grant-in-Aid for Scientific Research (A) from the Japan Society for the Promotion of Science (JSPS) to MI and a Grant-in-Aid for Young Scientists (A) from JSPS to JK.

Contributors MI conceived and designed the study. YM performed the imaging experiments and data analysis with the assistance of JK, YK and THa, DO performed the RNA sequence analysis. MM and KK provided the pH-sensing chemical fluorescent probe. THi and AK discussed the experiments and results. YM and JK co-wrote the initial draft. MI wrote the final draft.

Funding This research was supported by research grants from Ono Pharmaceutical, Bristol-Myers Squibb and Chugai Pharmaceutical.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Schett G, Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nat Rev Rheumatol* 2012;8:656–64.
2 Bombardier C, Barbieri M, Parthan A, *et al*. The relationship between joint damage and functional disability in rheumatoid arthritis: a systematic review. *Ann Rheum Dis* 2012;71:836–44.
3 Ødegård S, Landewé R, van der Heijde D, *et al*. Association of early radiographic damage with impaired physical function in rheumatoid arthritis: a ten-year, longitudinal observational study in 238 patients. *Arthritis Rheum* 2006;54:68–75.
4 Molenaar ET, Voskuyl AE, Dinant HJ, *et al*. Progression of radiologic damage in patients with rheumatoid arthritis in clinical remission. *Arthritis Rheum* 2004;50:36–42.
5 Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. *Nat Rev Genet* 2003;4:638–49.
6 Karsenty G, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* 2002;2:389–406.
7 Gravallese EM, Harada Y, Wang JT, *et al*. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. *Am J Pathol* 1998;152:943–51.
8 Lam J, Takeshita S, Barker JE, *et al*. TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J Clin Invest* 2000;106:1481–8.
9 Fuller K, Murphy C, Kirstein B, *et al*. TNFα potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. *Endocrinology* 2002;143:1108–18.
10 Li P, Schwarz EM, O'Keefe RJ, *et al*. RANK Signaling Is Not Required for TNFα-Mediated Increase in CD11bhi Osteoclast Precursors but Is Essential for Mature Osteoclast Formation in TNFα-Mediated Inflammatory Arthritis. *Journal of Bone and Mineral Research* 2003;19:207–13.

11 Tamura T, Udagawa N, Takahashi N, *et al*. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. *Proc Natl Acad Sci U S A* 1993;90:11924–8.
12 Palmqvist P, Persson E, Conaway HH, *et al*. IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-kappa B ligand, osteoprotegerin, and receptor activator of NF-kappa B in mouse calvariae. *J Immunol* 2002;169:3353–62.
13 Sato K, Suematsu A, Okamoto K, *et al*. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J Exp Med* 2006;203:2673–82.
14 Axmann R, Herman S, Zaiss M, *et al*. CTLA-4 directly inhibits osteoclast formation. *Ann Rheum Dis* 2008;67:1603–9.
15 Bozec A, Zaiss MM, Kagwiria R, *et al*. T cell costimulation molecules CD80/86 inhibit osteoclast differentiation by inducing the IDO/tryptophan pathway. *Sci Transl Med* 2014 6:235ra60.
16 Smolen JS, Aletaha D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat Rev Rheumatol* 2015;11:276–89.
17 Jones G, Darian-Smith E, Kwok M, *et al*. Effect of biologic therapy on radiological progression in rheumatoid arthritis: what does it add to methotrexate? *Biologics* 2012;6:155–61.
18 Schiff M, Weinblatt ME, Valente R, *et al*. Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: two-year efficacy and safety findings from AMPLE trial. *Ann Rheum Dis* 2014;73:86–94.
19 Nevius E, Gomes AC, Pereira JP. Inflammatory cell migration in rheumatoid arthritis: a comprehensive review. *Clin Rev Allergy Immunol* 2016;51:59–78.
20 Sims NA, Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. *Semin Cell Dev Biol* 2008;19:444–51.
21 Ishii M, Egen JG, Klauschen F, *et al*. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature* 2009;458:524–8.
22 Kikuta J, Wada Y, Kowada T, *et al*. Dynamic visualization of RANKL and Th17-mediated osteoclast function. *J Clin Invest* 2013;123:866–73.
23 Kikuta J, Kawamura S, Okiji F, *et al*. Sphingosine-1-phosphate-mediated osteoclast precursor monocyte migration is a critical point of control in antibone-resorptive action of active vitamin D. *Proc Natl Acad Sci U S A* 2013;110:7009–13.
24 Jung S, Aliberti J, Graemmel P, *et al*. Analysis of fractalkine receptor CX_3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* 2000;20:4106–14.
25 Sun-Wada GH, Tabata H, Kawamura N, *et al*. Direct recruitment of H^+-ATPase from lysosomes for phagosomal acidification. *J Cell Sci* 2009;122:2504–13.
26 Takegahara N, Kim H, Mizuno H, *et al*. Involvement of receptor activator of nuclear factor-κb ligand (RANKL)-induced incomplete cytokinesis in the polyploidization of osteoclasts. *J Biol Chem* 2016;291:3439–54.
27 Toyomura T, Oka T, Yamaguchi C, *et al*. Three subunit a isoforms of mouse vacuolar H^+-ATPase. Preferential expression of the a3 isoform during osteoclast differentiation. *J Biol Chem* 2000;275:8760–5.
28 Toyomura T, Murata Y, Yamamoto A, *et al*. From lysosomes to the plasma membrane. *Journal of Biological Chemistry* 2003;278:22023–30.
29 Maeda H, Kowada T, Kikuta J, *et al*. Real-time intravital imaging of pH variation associated with osteoclast activity. *Nat Chem Biol* 2016;12:579–85.
30 Furuya M, Kikuta J, Fujimori S, *et al*. Direct cell-cell contact between mature osteoblasts and osteoclasts dynamically controls their functions in vivo. *Nat Commun* 2018;9:300.
31 Kotani M, Kikuta J, Klauschen F, *et al*. Systemic circulation and bone recruitment of osteoclast precursors tracked by using fluorescent imaging techniques. *J Immunol* 2013;190:605–12.
32 Koizumi K, Saitoh Y, Minami T, *et al*. Role of CX3CL1/fractalkine in osteoclast differentiation and bone resorption. *J Immunol* 2009;183:7825–31.
33 Löwik CW, van der Pluijm G, van der Wee-Pals LJ, *et al*. Migration and phenotypic transformation of osteoclast precursors into mature osteoclasts: the effect of a bisphosphonate. *J Bone Miner Res* 1988;3:185–92.
34 Baron R, Neff L, Tran Van P, *et al*. Kinetic and cytochemical identification of osteoclast precursors and their differentiation into multinucleated osteoclasts. *Am J Pathol* 1986;122:363–78.
35 Yao Z, Li P, Zhang Q, *et al*. Tumor necrosis factor-alpha increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. *J Biol Chem* 2006;281:11846–55.
36 Kharazmi A, Nielsen H, Bendtzen K. Modulation of human neutrophil and monocyte chemotaxis and superoxide responses by recombinant TNF-alpha and GM-CSF. *Immunobiology* 1988;177:363–70.
37 Webb SE, Pollard JW, Jones GE. Direct observation and quantification of macrophage chemoattraction to the growth factor CSF-1. *J Cell Sci* 1996;109(Pt 4):793–803.
38 Tanaka K, Hashizume M, Mihara M, *et al*. Anti-interleukin-6 receptor antibody prevents systemic bone mass loss via reducing the number of osteoclast precursors in bone marrow in a collagen-induced arthritis model. *Clin Exp Immunol* 2014;175:172–80.
39 Bonelli M, Ferner E, Göschl L, *et al*. Abatacept (CTLA-4IG) treatment reduces the migratory capacity of monocytes in patients with rheumatoid arthritis. *Arthritis Rheum* 2013;65:599–607.
40 Odvina CV, Zerwekh JE, Rao DS, *et al*. Severely suppressed bone turnover: a potential complication of alendronate therapy. *J Clin Endocrinol Metab* 2005;90:1294–301.
41 Ikebe T. Pathophysiology of BRONJ: drug-related osteoclastic disease of the jaw. *Oral Science International* 2013;10:1–8.

EXTENDED REPORT

Metabolic pathways and immunometabolism in rare kidney diseases

Peter C Grayson,[1,2] Sean Eddy,[3,4] Jaclyn N Taroni,[2,5] Yaíma L Lightfoot,[1] Laura Mariani,[3,4] Hemang Parikh,[2,6] Maja T Lindenmeyer,[7] Wenjun Ju,[3,4] Casey S Greene,[2,5] Brad Godfrey,[3,4] Clemens D Cohen,[7] Jeffrey Krischer,[2,6] Matthias Kretzler,[3,4] Peter A Merkel,[2,8] the Vasculitis Clinical Research Consortium, the European Renal cDNA Bank cohort, and the Nephrotic Syndrome Study Network

Handling editor Josef S Smolen

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212935).

For numbered affiliations see end of article.

Correspondence to
Dr Peter C Grayson, National Institutes of Health, Bethesda, MD 20892, USA;
peter.grayson@nih.gov

Part of this manuscript was presented at the 2016 ACR/ARHP Annual Meeting.

Received 31 December 2017
Revised 4 April 2018
Accepted 16 April 2018
Published Online First 3 May 2018

ABSTRACT

Objectives To characterise renal tissue metabolic pathway gene expression in different forms of glomerulonephritis.

Methods Patients with nephrotic syndrome (NS), antineutrophil cytoplasmic antibody-associated vasculitis (AAV), systemic lupus erythematosus (SLE) and healthy living donors (LD) were studied. Clinically indicated renal biopsies were obtained at time of diagnosis and microdissected into glomerular and tubulointerstitial compartments. Microarray-derived differential gene expression of 88 genes representing critical enzymes of metabolic pathways and 25 genes related to immune cell markers was compared between disease groups. Correlation analyses measured relationships between metabolic pathways, kidney function and cytokine production.

Results Reduced steady state levels of mRNA species were enriched in pathways of oxidative phosphorylation and increased in the pentose phosphate pathway (PPP) with maximal perturbation in AAV and SLE followed by NS, and least in LD. Transcript regulation was isozymes specific with robust regulation in hexokinases, enolases and glucose transporters. Intercorrelation networks were observed between enzymes of the PPP (eg, transketolase) and macrophage markers (eg, CD68) ($r=0.49$, $p<0.01$). Increased PPP transcript levels were associated with reduced glomerular filtration rate in the glomerular ($r=-0.49$, $p<0.01$) and tubulointerstitial ($r=-0.41$, $p<0.01$) compartments. PPP expression and tumour necrosis factor activation were tightly co-expressed ($r=0.70$, $p<0.01$).

Conclusion This study demonstrated concordant alterations of the renal transcriptome consistent with metabolic reprogramming across different forms of glomerulonephritis. Activation of the PPP was tightly linked with intrarenal macrophage marker expression, reduced kidney function and increased production of cytokines. Modulation of glucose metabolism may offer novel immune-modulatory therapeutic approaches in rare kidney diseases.

INTRODUCTION

Activated immune cells require alterations in metabolic activity to survive, proliferate and sustain effector responses. How intracellular metabolites regulate immune cells is an emerging field of study known as immuometabolism.[1] In oncology, alteration of cancer cell metabolism to preferentially use glycolysis rather than the tricarboxylic acid (TCA) cycle for energy production is referred to as 'aerobic glycolysis' or the Warburg effect. Metabolic reprogramming of tumour cells towards enhanced glycolytic capacity is a defining characteristic of various malignancies and explains how tumours can be visualised by positron emission tomography studies coupled with radiolabelled fluorodeoxyglucose. In the context of immunity, similar alterations in metabolic pathways can promote effector functions in immune cell subsets to induce production of specific pro-inflammatory and anti-inflammatory cytokines.

Evidence of metabolic reprogramming in immune-mediated diseases is mostly limited to in vitro studies. Activation of hypoxia-inducible factor 1 alpha (HIF-1α) or stimulation of innate immune response receptors can upregulate pathways of glycolysis, promote differentiation of M1 macrophages and inform inflammatory responses via production of specific cytokines, including tumour necrosis factor (TNF).[2–7] Some studies have provided in vivo evidence of immunometabolism in rheumatologic diseases. Metabolomic profiling of serum and synovial fluid has identified specific metabolites associated with rheumatoid arthritis.[8–10] The pentose phosphate pathway (PPP) is a parallel pathway of glycolysis that may a play key role in specific inflammatory diseases. Defects in glycolytic flux due to upregulation of glucose-6-phosphate dehydrogenase (G6PD), an enzyme in the PPP, promote hyperproliferation and cytokine production in T cells from patients with rheumatoid arthritis.[11] Activated metabolism with hyperactivation of the PPP has been demonstrated in circulating lymphocytes from patients with systemic lupus erythematosus (SLE), and metabolic inhibitors can ameliorate pathology in animal models of lupus.[12–15]

Nephrotic and nephritic syndromes represent a spectrum of glomerulonephropathies characterised in part by shared end-organ kidney damage with a significant degree of activation of ischaemic injury.[16] To what extent immunometabolic changes contribute to different types of kidney disease is unknown. The objectives of this study were to compare metabolic pathways of gene transcription in renal tissue from patients with different forms

To cite: Grayson PC, Eddy S, Taroni JN, *et al*. *Ann Rheum Dis* 2018;**77**:1227–1234.

of glomerulonephritis and to determine the cellular source of specific metabolic transcription signatures in these diseases.

METHODS

Discovery cohort

Kidney biopsy samples from patients with glomerulonephritis and healthy donors were obtained from the European Renal cDNA Bank (ERCB) cohort. The ERCB is a multicentre study established to collect renal biopsy tissue for gene expression analysis at the time of a clinically indicated biopsy.[17] Biopsies were obtained from patients after informed consent with approval of the local ethics committees. For this study, patients with nephrotic syndrome (NS, n=62) and with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV, n=23), a prototypical nephritic syndrome, were included in a discovery cohort. Three forms of NSs were studied: minimal change disease (MCD, n=14), membranous glomerulonephritis (MGN, n=21) and focal segmental glomerulosclerosis (FSGS, n=25). Two forms of AAV were included: granulomatosis with polyangiitis and microscopic polyangiitis (MPA). All patients with AAVhad a positive ANCA-antibody and diagnostic confirmation of disease by renal histology. Healthy tissue obtained from living transplant donors (LD, n=21) was used as a comparator group. Detailed histology from the ERCB cohort was not available, and clinical information recorded at the time of renal biopsy was limited but included use of glucocorticoids (yes/no, categorical variable) and glomerular filtration rate (GFR).[18] To determine if gene expression signatures identified in the discovery cohort were unique to AAV or NS, relevant signatures were also queried in previously reported data from additional patients in the ERCB cohort, including patients with SLE (n=32) and patients who underwent tumour nephrectomy with donation of normal renal tissue adjacent to tumour (n=6).[19]

Validation cohort

An independent, validation cohort was studied consisting of microdissected renal biopsies from additional patients with AAV (n=57) from the ERCB cohort, additional LD (n=6), and patients with NS (n=107) recruited in the Nephrotic Syndrome Study Network (NEPTUNE). NEPTUNE is an ongoing multicentre prospective cohort study enrolling patients with glomerular diseases.[20] Detailed clinical and histopathological data from patients with nephrotic syndrome enrolled in NEPTUNE, including quantification of tubulointerstitial fibrosis, has been previously published.[21]

Kidney tissue processing and transcriptional profiling

Kidney tissue was processed prior to transcriptional profiling as previously described.[22] Briefly, collected renal tissue was stored in RNAlater (ThermoFisher) and manually microdissected into glomerular and tubulointerstitial compartments. Transcriptional data were used to assess reliability of microdissection, targeting 16-fold to 64-fold enrichment of glomerular-selective or tubulointerstitial-selective transcripts in each respective compartment. In the discovery cohort, RNA from each compartment was processed and analysed using Affymetrix GeneChip Human Genome U133A V.2.0 and U133 Plus V.2.0 platforms. In the validation cohort, samples were profiled on a Human Gene ST 2.1 array platform. Probe sets were annotated to Entrez Gene IDs using custom CDF V.19 generated from the University of Michigan Brain Array group, as previously described.[23] Expression data were quantile normalised and batch corrected using COMBAT.[24] Differential gene expression of selected gene transcripts was compared in the glomerular and tubulointerstitial compartments between patients with NS and AAV versus LD using the significance analysis of microarrays (SAM) method.[25 26] Genes were defined as significantly differentially expressed with q-value <0.05. CEL files are accessible in GEO under reference numbers: GSE104948, GSE104954 and GSE108113.

Selected metabolic and inflammatory genes

A list of 88 genes related to metabolic pathways and 25 genes related to immune cell subset markers were selected a priori for analysis.[27] Metabolic enzymes were selected to represent the following metabolic pathways: TCA cycle, glutaminolysis, fatty acid oxidation, glycolysis and the PPP. A composite gene expression score was created for each metabolic pathway by averaging z-scored transformed log2 expressed genes within the pathway. For the PPP, the composite score was created from the following genes: *G6PD, PGLS, PGD, RPE, RPIA, TKT* and *TALDO1*. A subset of enyzmes were categorised based on regulation by the transcription factor HIF-1α. A complete list of the selected genes is provided in online supplementary material.

Subset prediction from enrichment correlation analysis

Subset prediction from enrichment correlation (SPEC) was performed to determine the cell-specific source for PPP gene expression.[28] Immune cell type-specific gene sets and renal cell type-specific gene sets were derived from previously published studies.[29 30] Single-sample gene set enrichment analysis (ssGSEA) enrichment scores (ES) were calculated using each cell type-specific gene set and a PPP gene set curated from the KEGG metabolic pathways (V.5.2 from the Molecular Signatures Database). To determine how suitable a cell type gene set is for SPEC, cell type gene sets are randomly split in half ('SET A and SET B'). ssGSEA is performed on all cell types and the number of times the ES from SET B is most strongly correlated with SET A rather than any other cell type is counted. The split experiment is replicated 100 times for each cell type gene set to generate a confidence metric in the findings. Correlation between each cell type ES and the PPP ES was calculated. Higher correlation indicates that a specific cell type contributes to PPP expression in the total population of samples.

Development of TNF activation score

Previously identified candidate genes causally downstream of TNF in kidney disease with at least three literature sources of evidence were selected as a gene set representative of TNF activation.[31] A TNF activation score was generated in patient samples by transforming log2 expression profiles into z-scores and averaging z-scores of 138 TNF-dependent genes into a single metric for each patient sample.

Tissue immunofluorescence

The expression and localisation of immunometabolism-associated proteins was evaluated in paraffin-embedded kidney sections by indirect immunofluorescence in five patients with AAV. Kidney biopsies were classified based on histology as focal, crescentic, mixed or sclerotic, according to consensus guidelines.[32] Briefly, after xylene/ethanol deparaffinisation, tissue sections were pretreated with Epitope Retrieval Solution (IHC World, Woodstock, Maryland, USA) for 40 min at 60°C. Subsequently, non-specific binding was blocked with 10% Normal Goat Serum (NGS) for 30 min at room temperature. Tissue slides were double stained with combinations of antibodies specific for CD68, TKT and HIF-1α (Abcam, Cambridge, Massachusetts, USA;

Table 1 Patient characteristics for discovery and validation cohorts

	Discovery cohort					Validation cohort		
Disease group	Living donor	Nephrotic syndrome	ANCA-associated vasculitis	Systemic lupus erythematosus	Tumour nephrectomy	Living donor	Nephrotic syndrome	ANCA-associated vasculitis
Glomerular samples	21	58	23	32	6	6	90	15
Tubulointerstitial samples	21	47	21	32	0	5	107	57
Age, years (SD)	47.3 (11.5)	47.2 (17.7)	58.0 (13.8)	35.1 (13.3)	66.4 (6.8)	52.5 (6.9)	47.1 (15.7)	57.8 (9.8)
Sex (% female)	45	45	43	78	100	57	33	58
Glomerular filtration rate (SD)	104 (31)	83 (39)	46 (31)	64 (39)	58 (10.5)	108 (33)	77 (32)	31 (27)

ANCA, antineutrophil cytoplasmic antibody.

BioLegend, San Diego, California, USA) overnight at 4°C in 1% NGS. Secondary antibodies were purchased from ThermoFisher Scientific (Grand Island, New York, USA), and tissue sections incubated accordingly for 1 hour at room temperature in 1% NGS. Hoechst-stained slides were then mounted with ProLong Gold Antifade Mountant (ThermoFisher), and visualised after curing overnight.

Statistical analyses

Correlation analyses were performed using Pearson's correlation. Kruskal-Wallis with post hoc Dunn's test to account for multiple comparisons was used to compare metabolic pathway scores across multiple disease groups, and a p value <0.05 was considered significant for these analyses. Analyses were performed using GraphPad Prism V.7.0 (La Jolla, California, USA).

RESULTS

Alteration of metabolic pathways in the glomerular and tubulointerstitial compartments

Gene expression signatures for five major metabolic pathways were profiled in the glomerular and tubulointerstitial compartments between patients with NSs, AAV and healthy living donors. The clinical characteristics of the study population are presented in table 1.

In the glomerular compartment of the discovery cohort, there was a coordinated pattern of altered gene expression related to specific metabolic pathways. Gene expression of the TCA cycle, fatty acid oxidation and glutaminolysis was repressed in NS relative to LD and further downregulated in patients with AAV (figure 1A). In contrast, there was significantly increased expression of the PPP in patients with NS and AAV relative to LD ($p<0.001$), with significantly higher expression in patients with AAV compared with NS ($p<0.001$). There was increased gene expression related to glycolysis in patients with NS and AAV compared with LD but this was not statistically significant. Differential expression of the PPP was also observed in the tubulointerstitial compartment of the discovery cohort (figure 1B). There was differential upregulation of glycolytic enzymes regulated by the transcription factor HIF-1α in patients with AAV compared with LD ($p<0.001$) and patients with NS ($p<0.005$), and there were no differences in expression of glycloytic enzymes not regulated by HIF-1α across the groups (figure 1C).

In the glomerular compartment of the discovery cohort, all seven enzymes of the PPP were significantly upregulated in patients with AAV compared with controls (fold change range 1.14–1.57, $q<0.05$). In the tubulointerstitial compartment, six enzymes of the PPP were significantly upregulated in patients with AAV compared with controls (fold change range 1.05–1.52; $q<0.05$) (table 2).

Differential expression of specific metabolic isozymes

Differential expression of selected, key regulatory metabolic isozymes was compared across LD, NS and AAV groups in the discovery cohort (figure 2). Specific isozymes were significantly upregulated (*ENO2, HK1, HK2*) or downregulated (*PDK4, PFKB1, PFKFB2*) in NS and AAV compared with LD without differences between NS and AAV. Other isozymes were significantly upregulated (*GLUT3, PFKFB3*) or downregulated (*ALDOB, GLUT2, PDK2*) in AAV>NS>LD. *ENO1, HK3* and *PFKB4* were only significantly upregulated in AAV compared with NS and LD. There were no significant differences across the groups for *ALDOA, ALDOC, GLUT1, GLUT4, PDK1* and *PDK3*.

Pentose phosphate pathway gene expression signature in the validation cohort and in association with kidney function

To confirm if increased PPP expression was unique to AAV, gene expression of the PPP was compared in the discovery cohort across a broader spectrum of diseases including SLE (figure 3A and B). PPP expression did not differ between LD, normal kidney biopsy sections from patients who underwent tumour nephrectomy and patients with MCD. PPP expression in SLE was significantly greater than LD ($p<0.001$) but was not different from AAV. Among the two types of NSs with potential inflammatory components (MGN, FSGS), there was increased PPP expression compared with LDs ($p<0.01$), with lower median PPP expression values than SLE or AAV. In an analysis restricted to patients with AAV or SLE, there was no significant difference in PPP expression score between patients categorised by concomitant glucocorticoid use at the time of biopsy versus those not treated; however, there was increased variability of PPP expression in those patients taking glucocorticoids (figure 3C). Differential expression of the PPP was confirmed in the validation cohort using samples from an independent group of patients with glomerulonephritis (figure 3D). There was a significant negative association between PPP expression and GFR in the glomerular compartment ($r=-0.49$, $p<0.01$) (figure 3E) and the tubulointerstitial compartment ($r=-0.41$, $p<0.01$) (figure 3F). In analyses restricted to patients with NS in the NEPTUNE cohort where detailed histology was available for review, GFR was negatively associated with PPP expression in the glomerular ($r=-0.37$, $p<0.01$) and the tubulointerstitial compartments ($r=-0.29$, $p<0.01$), and PPP expression in

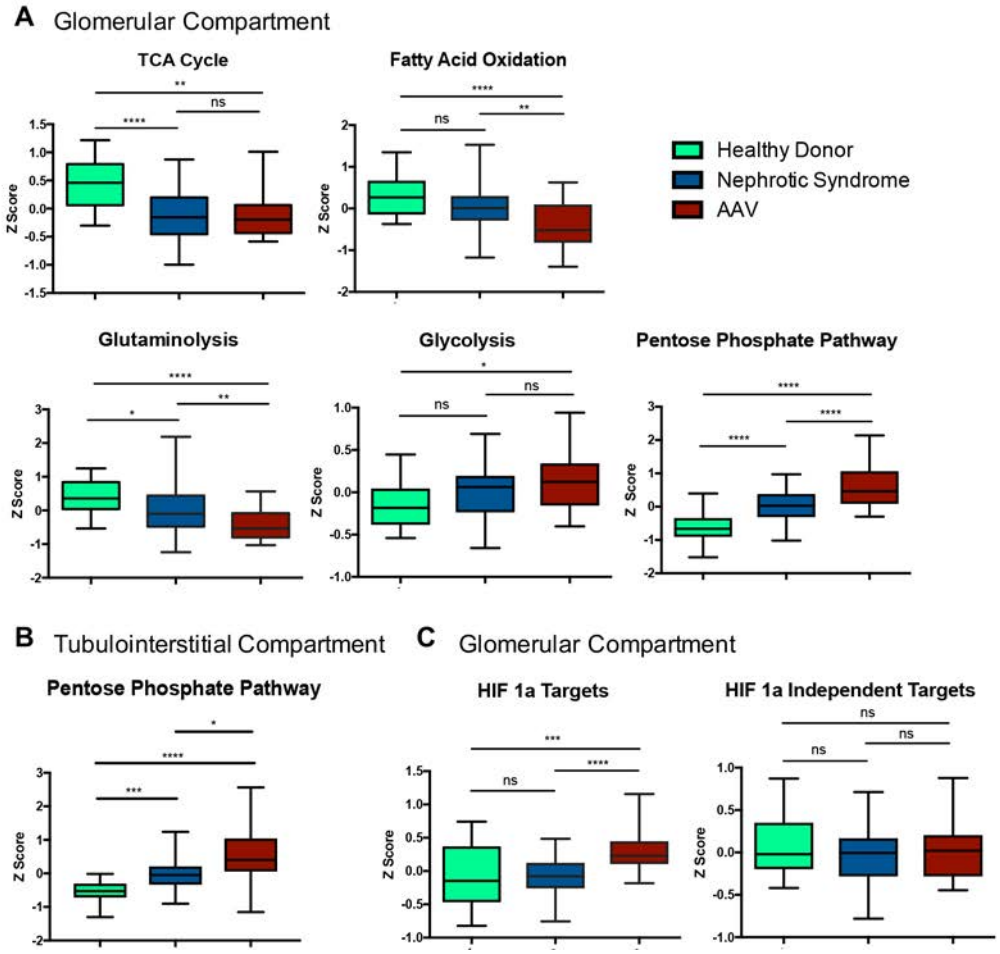


Figure 1 Bar graphs of metabolic pathways showing disease-specific differential gene expression within the glomerular compartment in the discovery cohort (A). Bar graph of pentose phosphate pathway gene expression within the tubulointerstitial compartment in the discovery cohort (B). Differences in composite gene expression were compared in the discovery cohort between hypoxia-induced factor 1-alpha (HIF-1α) glycolytic targets and HIF-1α independent glycolytic enzymes in the glomerular compartment (C). *p<0.05; **p<0.01; ***p<0.005; ****p<0.001, NS, not significant. AAV, antineutrophil cytoplasmic antibody-associated vasculitis; TCA, tricarboxylic acid.

the tubulointerstitial compartment was significantly associated with an increased degree of tubulointerstitial fibrosis on pathology (r=0.22, p=0.03).

Cellular source of the pentose phosphate pathway gene expression signature

SPEC analysis of the discovery cohort glomerular compartment dataset indicated that PPP expression was specifically increased in monocyte/macrophages and renal tubular cells (figure 4A). Correlation analyses stratified by group (NS, LD, AAV) demonstrated positive correlation between monocyte/macrophage ES and PPP ES only in patients with AAV (r=0.62, p<0.01) and positive correlation between renal tubule ES and PPP ES only in LD (r=0.49, p<0.01) and patients with NS (r=0.63, p<0.01) (figure 4B). In the glomerular compartment in patients with LD, NS and AAV, there was positive correlation between transketolase (TKT), a representative enzyme of the PPP, and CD68, a representative macrophage marker (r=0.48; p<0.01) (figure 4C). In patients with AAV with crescentic findings on kidney biopsy (n=2), protein expression of TKT and

Table 2 Glomerular compartment pentose phosphate pathway gene expression in ANCA-associated vasculitis compared with healthy donors in the discovery cohort

		Glomerular compartment		Tubulointerstitial compartment	
Enzyme	Gene symbol	Fold change	Q values	Fold change	Q values
Glucose-6-phosphate dehydrogenase	*G6PD*	1.30	<0.0001	1.10	0.0323
6-Phosphogluconolactonase	*PGLS*	1.59	<0.0001	1.06	0.0855
Phosphogluconate dehydrogenase	*PGD*	1.15	0.0210	1.16	0.0076
Ribulose-5-phosphate-3-epimerase	*RPE*	1.19	0.0002	1.18	<0.0001
Ribose-5-phosphate isomerase A	*RPIA*	1.24	<0.0001	1.19	<0.0001
Transketolase	*TKT*	1.71	<0.0001	1.61	<0.0001
Transaldolase 1	*TALDO1*	1.28	<0.0001	1.37	<0.0001

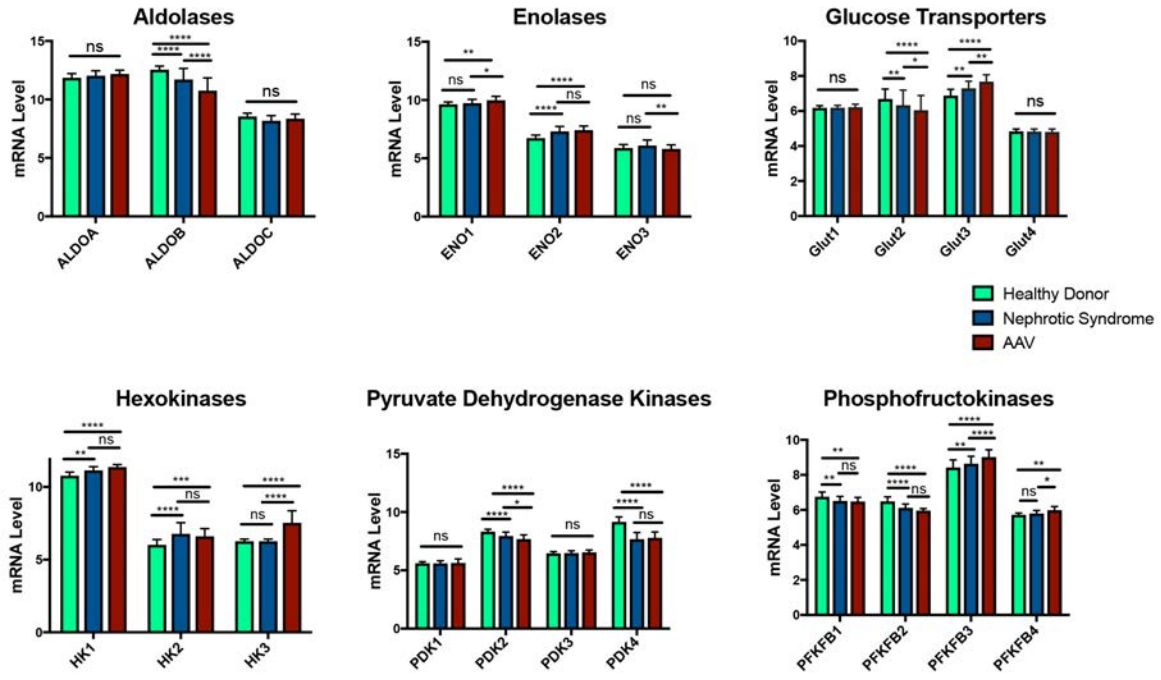


Figure 2 Comparison of differences in glomerular gene expression (log2 mRNA levels) among selected metabolic isozymes in patients with nephrotic syndrome, antineutrophil cytoplasmic antibody-associated vasculitis and healthy donors in the discovery cohort. *p<0.05; **p<0.01; ***p<0.005; ****p<0.001, NS, not significant.

HIF-1α colocalised with CD68+ macrophages, with no expression observed in renal parenchymal cells (figure 4D). In patients with AAV without crescentic histology (n=3), no protein expression of TKT or HIF-1α was observed (data not shown). To determine if PPP expression correlated with macrophage-related cytokine production, the PPP score from the glomerular

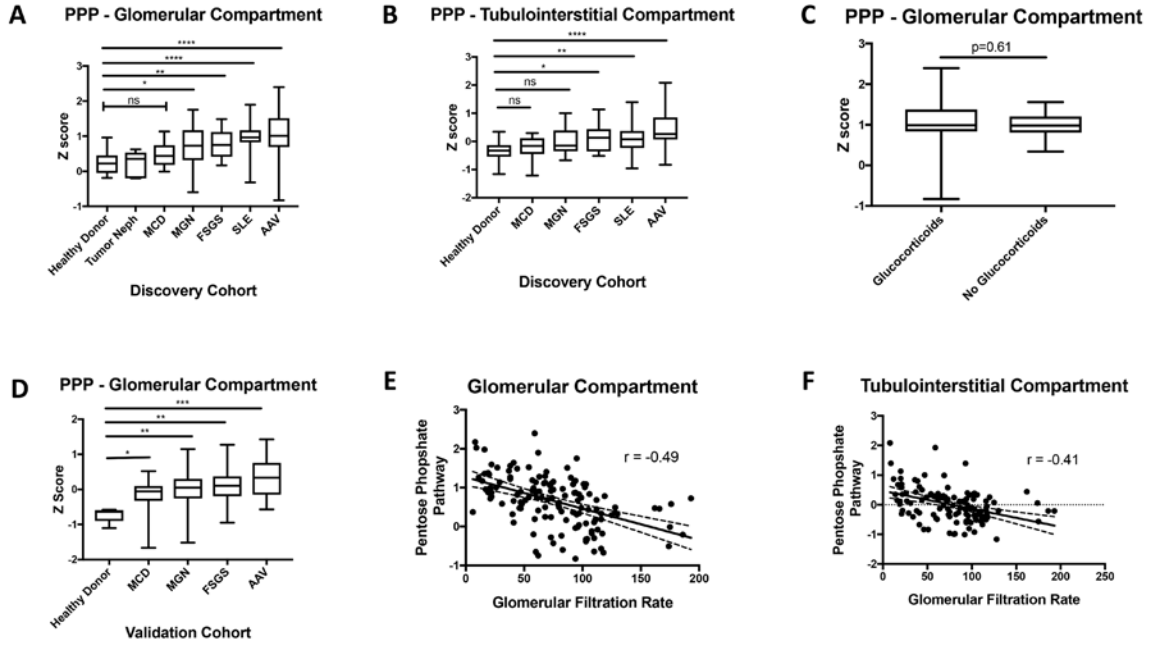


Figure 3 Pentose phosphate pathway (PPP) gene expression is differentially regulated in a variety of glomerulonephritis and is associated with impaired kidney function. PPP gene expression differences among groups collected within the discovery cohort in the glomerular (A) and tubulointerstitial (B) compartments. Comparison of PPP gene expression within the glomerular compartment in the discovery cohort between patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV) or systemic lupus erythematosus (SLE) categorised by glucocorticoid use at the time of biopsy (C). Validation of PPP gene expression differences within the glomerular compartment in the validation cohort (D). Correlation between PPP expression and glomerular filtration rate in the glomerular (E) and tubulointerstitial (F) compartments in the discovery cohort. *p<0.05; **p<0.01; ***p<0.005; ****p<0.001, NS, not significant. FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; MGN, membranous glomerulonephritis.

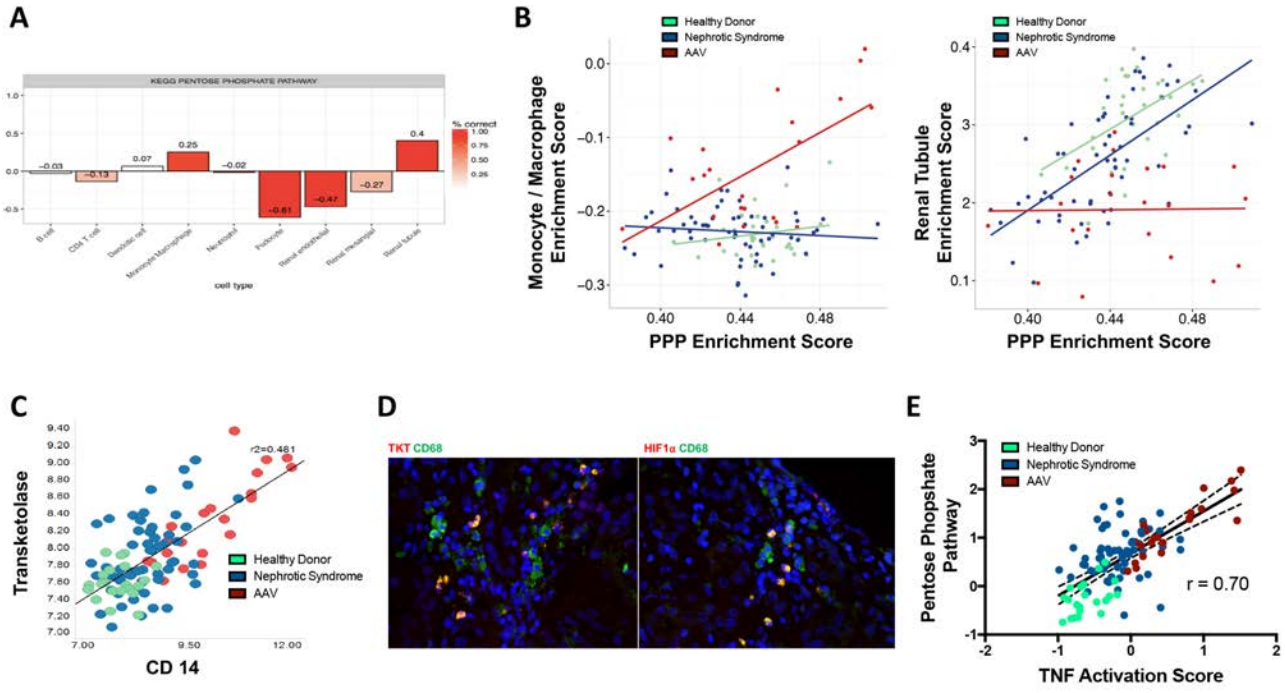


Figure 4 Myeloid cells are likely a major source of activated pentose phosphate pathway (PPP) gene expression. Subset prediction from enrichment correlation (SPEC) predicts that renal tubule and monocyte/macrophages are the likely source of PPP in the discovery cohort with the intensity of the red bar indicates degree of confidence in the bar plot correlation of cell types and PPP expression (A). The monocyte/macrophage enrichment score correlates with the PPP enrichment score in antineutrophil cytoplasmic antibody-associated vasculitis (AAV), while the tubule enrichment score correlates with the PPP enrichment score in healthy living donor and nephrotic syndrome samples (B). Transketolase (TKT), a key regulatory enzyme within the PPP, correlates with CD14, a marker for monocyte/macrophages in the glomerular compartment in the discovery cohort (C). Tissue immunofluorescence demonstrates localisation of TKT and HIF-1α within monocytes/macrophages (CD14) in the glomerular compartment (D). The PPP gene expression score is strongly associated with increased expression of a tumour necrosis factor (TNF) activation score within the glomerular compartment (E).

compartment was correlated with a TNF activation score. There was strong, positive correlation ($r=0.70$, $p<0.01$) between the PPP and TNF activation scores, with the highest values observed in patients with AAV compared with NS and LD (figure 4E).

DISCUSSION

Under conditions of cellular homeostasis, the TCA cycle serves as the most efficient source of energy production in humans. However, under conditions of cellular stress, including inflammatory microenvironments, glucose can become a preferred metabolic substrate. This study demonstrated concordant alterations of the renal transcriptome consistent with metabolic reprogramming across different forms of glomerulonpehritis. Gene expression profiling of renal tissue from the glomerular compartment revealed downregulation of pathways of cellular homeostasis, including the TCA cycle, glutaminolysis and fatty acid oxidation, and upregulation of pathways of glucose metabolism, including the PPP. Significant upregulation of HIF-1α-related gene transcripts and colocalisation of HIF-1α and CD68 by tissue immunofluorescence in the glomeruli of patients with AAV suggests that this transcription factor plays a critical role in the regulation of glycolytic pathways in glomerulonephritis.[16 33]

Activation of the PPP in both the glomerular and tubulointerstitial compartments in a discovery and validation cohort was the most striking finding in this study. Increased expression of enzymes of the PPP was demonstrated in NSs compared with healthy living donors with the highest levels of PPP expression seen in inflammatory kidney diseases. In patients with NS, increased expression of the PPP in the tubulointerstitial compartment was significantly associated with reduced kidney function and increased intensity of tubulointerstitial fibrosis. Although renal disease in AAV is typically defined by glomerular involvement, similar alterations of PPP enzyme transcription were observed in renal biopsies from patients with AAV in both the glomerular and tubulointerstitial compartments. Global alterations of the renal transcriptome across different anatomic compartments are therefore associated with renal disease in AAV. Similar levels of PPP expression in kidney biopsies from patients with AAV or SLE indicate that alterations of metabolic pathways might be shared across different forms of glomerulonephritis.

Several lines of evidence suggest that monocyte/macrophages are likely a major contributor to PPP expression in these diseases. Increased PPP expression was observed in the NS subtypes where inflammatory features on histology are most pronounced, including MGN and FSGS compared with MCD. Computational analyses showed that PPP expression strongly correlated with monocyte/macrophage surface markers, especially in patients with AAV, and protein expression of PPP enzymes colocalised to macrophages within the glomerular compartment by tissue immunofluorescence. One function of the PPP is to generate NADPH and maintain redox balance, which may be particularly important to cellular survival in activated macrophages undergoing oxidative burst. Another function of the PPP is to generate nucleic acid precursors. Production of biomass through the PPP

could facilitate generation of the necessary messenger RNA and protein to enable effector functions. Activation of the PPP is known to induce pro-inflammatory cytokine production in macrophages,[5] and in this study, strong correlation was observed between PPP expression and TNF activation within the glomerular compartment, particularly in AAV.

In addition to regulation of important metabolic pathways, differential expression of key, regulatory metabolic isozymes was observed across the conditions studied. These findings may inform future functional studies of metabolic pathways in renal disease. *PFKFB3*, which was upregulated in both NSs and AAV, has been specifically associated with the Warburg effect in tumour cells because its activity increases the rate of glycolysis.[34] Among glucose transporters, which facilitate glucose passage across plasma membranes, there was upregulation of *GLUT3* and downregulation of *GLUT2* in both NS and AAV. *GLUT3* is the highest affinity glucose receptor and therefore may play a key role in facilitating glucose metabolism in these conditions.[35] Hexokinases regulate the first step in glycolysis, and significant increased expression of *HK3* was observed in patients with AAV. *HK3* is the predominant hexokinase in myeloid cells and is upregulated in peripheral blood samples from patients with AAV in a prior transcriptomic study.[36 37] The functions of *HK3* are poorly characterised, making it an attractive candidate for future functional studies.

This study has some important potential limitations to consider. Concomitant use of glucocorticoids can affect gene expression and information about glucocorticoid dose at the time of biopsy was not available; however, no significant difference between PPP scores were observed when adjusting for glucocorticoid use as a categorical variable. Detailed pathological descriptions from renal biopsies in the ECRB cohort was not available across the cohort, precluding comparison of the renal transcriptome with histological characteristics of disease. Urinary metabolites were not studied; however, alterations of glycolysis-related transcripts in animal models of diabetes have predicted changes in glycolytic metabolites in renal cortex and urine.[38] Finally, subgroup comparisons were limited by small sample sizes.

Distinct alterations in cellular metabolism were observed in the renal transcriptome from patients with different forms of glomerulonephritis, including NSs and systemic inflammatory diseases such as AAV and SLE. Global patterns of gene expression are indicative of increased utilisation of glucose and decreased oxidative phosphorylation, especially in patients with inflammatory kidney diseases. Metabolic reprogramming of cells within affected renal tissue may constitute a form of shared molecular pathology across different types of glomerulonephritis. The strong correlations between markers of glycolysis, macrophage-related markers and inflammatory cytokines observed in this study further suggest that altered immunometabolism may also play a role in the pathophysiology across a spectrum of kidney diseases. Validation of these findings in prospective, observational cohorts with assessment of potential associations between metabolic gene expression signature, detained renal histology and long-term clinical outcomes is warranted. Modulation of glucose metabolism could offer novel approaches to the treatment of these rare syndromes.

Author affiliations
[1]Vasculitis Translational Research Program, Systemic Autoimmunity Branch, National Institutes of Health/NIAMS, Bethesda, Maryland, USA
[2]Vasculitis Clinical Research Consortium, Philadelphia, Pennsylvania, USA
[3]Division of Nephrology, Michigan Medicine, University of Michigan, Ann Arbor, Michigan, USA
[4]Nephrotic Syndrome Study Network Consortia, Ann Arbor, Michigan, USA
[5]Department of Systems Pharmacology and Translational Therapeutics, Institute for Translational Medicine and Therapeutics, Institute for Biomedical Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA
[6]Health Informatics Institute, Morsani College of Medicine, University of South Florida, Tampa, Florida, USA
[7]Nephrological Center Medical Clinic and Polyclinic IV, University of Munich, Munich, Germany
[8]Division of Rheumatology and Department of Biostatistics, Epidemiology, and Informatics, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Acknowledgements The authors acknowledge Mariana Kaplan, Chief of the Systemic Autoimmunity Branch within Intramural NIAMS, for assistance in the conduct of this study. The authors also acknowledge all participating centers of the European Renal cDNA Bank, the Vasculitis Clinical Research Consortium and the Nephrotic Syndrome Study Network Consortium.

Contributors PCG, SE, JK, MK and PAM designed the study. YLL, MTL, W-JJ, BG, CDC collected the data and performed the experiments. PCG, SE, JNT, HP, CSG, JK, MK and PAM performed the statistical analysis. PCG, SE, JNT, YLL, LM, HP, CSG, JK, MK and PAM analysed the data. PCG, SE, JNT, YLL, JK, MK and PAM wrote the manuscript. All authors approved the final version of the manuscript.

Funding The Vasculitis Clinical Research Consortium (VCRC), U54 AR057319, and the Nephrotic Syndrome Study Network Consortium (NEPTUNE), U54 DK083912, are part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN), supported through collaboration between the Office of Rare Diseases Research (ORDR), NCATS and the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). This research was also supported through the Intramural Research Program at the NIAMS. Additional funding and/or programmatic support for this project has also been provided by the Else Kröner-Fresenius Foundation (ERCB), VCRC, University of Michigan, the NephCure Kidney International and the Halpin Foundation and the Applied Systems Biology Core at the University of Michigan George M O'Brien Kidney Translational Core Center. Dr Taroni is supported by the University of Pennsylvania Training Program in Rheumatic Diseases (NIAMS T32AR007442).

Competing interests None declared.

Patient consent Not required.

Ethics approval The study was approved by the ethics committees for the European Renal cDNA Bank (ERCB) and the Nephrotic Syndrome Study Network Consortium (NEPTUNE). Ethics approval for the gene expression studies was provided by the University of Michigan (HUM0002468).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement CEL files are accessible in GEO under reference numbers: GSE104948, GSE104954 and GSE108113.

REFERENCES

1 O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* 2016;16:553–65.
2 Tannahill GM, Curtis AM, Adamik J, *et al*. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 2013;496:238–42.
3 Galván-Peña S, O'Neill LA. Metabolic reprograming in macrophage polarization. *Front Immunol* 2014;5:420.
4 Moon JS, Hisata S, Park MA, *et al*. mTORC1-Induced HK1-Dependent Glycolysis Regulates NLRP3 Inflammasome Activation. *Cell Rep* 2015;12:102–15.
5 Haschemi A, Kosma P, Gille L, *et al*. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab* 2012;15:813–26.
6 Jha AK, Huang SC, Sergushichev A, *et al*. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 2015;42:419–30.
7 O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* 2016;213:15–23.
8 Kim S, Hwang J, Xuan J, *et al*. Global metabolite profiling of synovial fluid for the specific diagnosis of rheumatoid arthritis from other inflammatory arthritis. *PLoS One* 2014;9:e97501.
9 Jiang M, Chen T, Feng H, *et al*. Serum metabolic signatures of four types of human arthritis. *J Proteome Res* 2013;12:3769–79.

10 Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis. *Nat Rev Rheumatol* 2017;13:291–301.
11 Yang Z, Fujii H, Mohan SV, *et al*. Phosphofructokinase deficiency impairs ATP generation, autophagy, and redox balance in rheumatoid arthritis T cells. *J Exp Med* 2013;210:2119–34.
12 Morel L. Immunometabolism in systemic lupus erythematosus. *Nat Rev Rheumatol* 2017;13:280–90.
13 Fernandez DR, Telarico T, Bonilla E, *et al*. Activation of mammalian target of rapamycin controls the loss of TCRzeta in lupus T cells through HRES-1/Rab4-regulated lysosomal degradation. *J Immunol* 2009;182:2063–73.
14 Perl A, Hanczko R, Lai ZW, *et al*. Comprehensive metabolome analyses reveal *N*-acetylcysteine-responsive accumulation of kynurenine in systemic lupus erythematosus: implications for activation of the mechanistic target of rapamycin. *Metabolomics* 2015;11:1157–74.
15 Yin Y, Choi SC, Xu Z, *et al*. Normalization of CD4+ T cell metabolism reverses lupus. *Sci Transl Med* 2015;7:274ra18.
16 Shved N, Warsow G, Eichinger F, *et al*. Transcriptome-based network analysis reveals renal cell type-specific dysregulation of hypoxia-associated transcripts. *Sci Rep* 2017;7:8576.
17 Lindenmeyer MT, Kretzler M, Boucherot A, *et al*. Interstitial vascular rarefaction and reduced VEGF-A expression in human diabetic nephropathy. *J Am Soc Nephrol* 2007;18:1765–76.
18 Levey AS, Coresh J, Greene T, *et al*. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006;145:247–54.
19 Ju W, Greene CS, Eichinger F, *et al*. Defining cell-type specificity at the transcriptional level in human disease. *Genome Res* 2013;23:1862–73.
20 Gadegbeku CA, Gipson DS, Holzman LB, *et al*. Design of the Nephrotic Syndrome Study Network (NEPTUNE) to evaluate primary glomerular nephropathy by a multidisciplinary approach. *Kidney Int* 2013;83:749–56.
21 Mariani LH, Martini S, Barisoni L, *et al*. Interstitial fibrosis scored on whole-slide digital imaging of kidney biopsies is a predictor of outcome in proteinuric glomerulopathies. *Nephrol Dial Transplant* 2018;33:310–8.
22 Cohen CD, Frach K, Schlöndorff D, *et al*. Quantitative gene expression analysis in renal biopsies: a novel protocol for a high-throughput multicenter application. *Kidney Int* 2002;61:133–40.
23 Dai M, Wang P, Boyd AD, *et al*. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. *Nucleic Acids Res* 2005;33:e175.
24 Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007;8:118–27.
25 Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A* 2001;98:5116–21.
26 Schmid H, Boucherot A, Yasuda Y, *et al*. Modular activation of nuclear factor-kappaB transcriptional programs in human diabetic nephropathy. *Diabetes* 2006;55:2993–3003.
27 Caspi R, Billington R, Ferrer L, *et al*. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Res* 2016;44:D471–D480.
28 Bolen CR, Uduman M, Kleinstein SH. Cell subset prediction for blood genomic studies. *BMC Bioinformatics* 2011;12:258.
29 Newman AM, Liu CL, Green MR, *et al*. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods* 2015;12:453–7.
30. Renal tubule cells. http://nano.princeton.edu/standard/view/renal-tubule/
31 Eddy S, Mariani L, Martini S, *et al*. An Integrative genomics approach to predict patients with TNF activation in progressive nephrotic syndrome. Kidney Precision meeting abstract. http://www.niddk.nih.gov/news/events-calendar/Documents/abstract.pdf.
32 Berden AE, Ferrario F, Hagen EC, *et al*. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 2010;21:1628–36.
33 Marín-Hernández A, Gallardo-Pérez JC, Ralph SJ, *et al*. HIF-1 alpha modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms. *Mini Rev Med Chem* 2009;9:1084–101.
34 Atsumi T, Chesney J, Metz C, *et al*. High expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (iPFK-2; PFKFB3) in human cancers. *Cancer Res* 2002;62:5881–7.
35 Simpson IA, Dwyer D, Malide D, *et al*. The facilitative glucose transporter GLUT3: 20 years of distinction. *Am J Physiol Endocrinol Metab* 2008;295:E242–E253.
36 Federzoni EA, Humbert M, Torbett BE, *et al*. CEBPA-dependent HK3 and KLF5 expression in primary AML and during AML differentiation. *Sci Rep* 2014;4:4261.
37 Cheadle C, Berger AE, Andrade F, *et al*. Transcription of proteinase 3 and related myelopoiesis genes in peripheral blood mononuclear cells of patients with active Wegener's granulomatosis. *Arthritis Rheum* 2010;62:1744–54.
38 Sas KM, Kayampilly P, Byun J, *et al*. Tissue-specific metabolic reprogramming drives nutrient flux in diabetic complications. *JCI Insight* 2016;1:e86976.

Shared epitope positivity is related to efficacy of abatacept in rheumatoid arthritis

Abatacept, a soluble fusion protein consisting of the extracellular domain of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the Fc portion of human IgG1, has been approved for the treatment of rheumatoid arthritis (RA). It acts by binding to cluster of differenciation (CD)80/86 (B7-1/B7-2) on antigen-presenting cells (APCs) and blocking the B7:CD28 interaction. Meanwhile, HLA-DRB1 shared epitope (SE) has been proposed to be associated with the production of anticyclic citrullinated peptide antibody (ACPA) via major histocompatibility complex-based antigen presentation.[1 2] Moreover, the efficacy of abatacept is associated with positivity and titer for ACPA.[3 4] Therefore, we hypothesised that the efficacy of abatacept may be associated with patients' HLA-DRB1 SE positivity. To test this idea, we have retrospectively underwent exploratory analysis of the association between the clinical efficacy of abatacept and HLA-DRB1 genotype. HLA-DRB1 genotype could be identified in 72 patients. The study was approved by the ethics review board of Matsuyama Red Cross Hospital, Japan and was conducted as a retrospective observation study. All patients' consents were obtained. HLA-DRB1 *0101, *0102, *0401, *0404, *0405, *0408 and *1001 were defined as SE.[5]

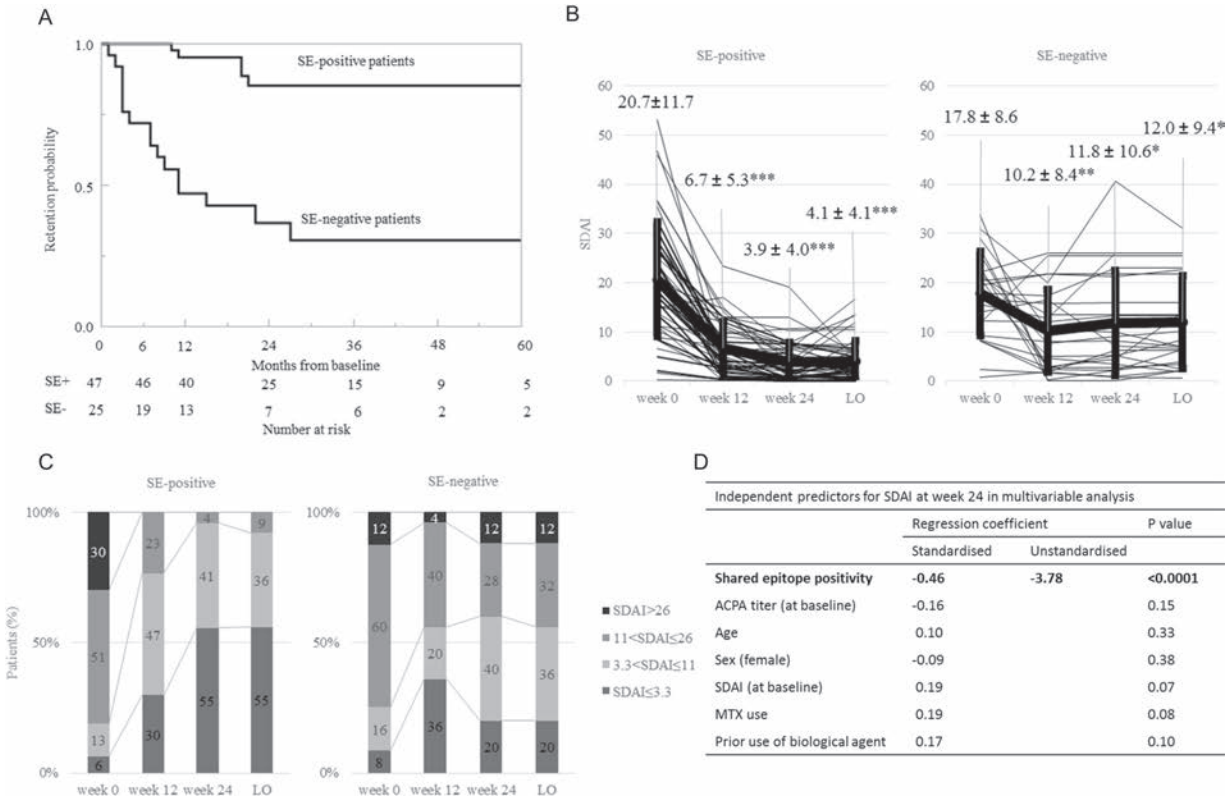
Table 1 shows characteristics of the study patients. Of 72 patients, SE-positive and SE-negative subjects were 47 and 25, respectively. Compared with SE-negative patients, SE-positive patients had a higher retention rate of abatacept treatment ($p<0.0001$, log-rank test, figure 1A). The numbers of SE-positive and SE-negative patients discontinued abatacept due to lack of efficacy were 5 and 16, respectively. In multivariable Cox hazard regression models, SE-negative patients had a significantly higher abatacept discontinuation due to lack of efficacy than SE-positive patients (crude HR 9.26, 95% CI 3.37 to 25.4; age and sex adjusted HR 10.83, 95% CI 3.53 to 33.2 and fully adjusted (age, sex, ACPA titer, prior use of biological agents, methotrexate (MTX) use) HR 9.64, 95% CI 3.13 to 29.7). Due to a high number of covariates compared with the number of events (6 compared with 21), the fully adjusted estimate should be interpreted with caution. The changes in Simple Disease Activity Index (SDAI) recorded over 24 weeks are shown in figure 1B. Despite the fact that SE-positive patients could reduce their corticotherapy significantly more (prednisolone dose equivalent at week 24, 3.5 ± 3.6 to 2.8 ± 2.8 and 4.9 ± 3.5 to 4.6 ± 3.0 mg/day, $p=0.04$ and $p=0.13$, in SE-positives and SE-negatives, respectively), the mean SDAI values were 3.87 ± 4.03 and 11.79 ± 10.57 at week 24 ($p<0.0001$) in SE-positive and SE-negative patients, respectively. The achievement ratio of SDAI remission at week 24 was significantly higher in SE-positive patients than in SE-negative patients (55.3% vs 20.0%, respectively, $p=0.01$, figure 1C). An independent inverse association was observed between SE-positivity and SDAI at week 24 after adjustment for ACPA titer, age, sex, SDAI at baseline, MTX use and prior use of biological agent in multiple regression analysis (figure 1D). Including SE positivity in this multiple regression analysis, the highest ACPA quartile (730–4627 IU/L[4]) also did not become significant predictor for SDAI at week 24 ($p=0.10$).

Previous studies have demonstrated increased efficacy of abatacept with ACPA positivity and high titers of autoantibodies. Our

Table 1 Baseline characteristics of patients

Variables (n=72)	SE-positive patients (n=47)	SE-negative patients (n=25)	p Value
Age (years)	60.5±14.5	64.0±10.3	0.29
Female, n (%)	36 (76.6)	23 (92.0)	0.20
Disease duration (years)	10.4±9.3	10.6±10.0	0.93
Stage I/II, n (%)	12 (25.5)	8 (32.0)	0.56
TJC, 0–28	4.9±4.8	3.8±4.3	0.30
SJC, 0–28	5.0±4.2	3.3±2.1	0.03
GH, VAS 0–100 mm	48±27	52±29	0.54
EGA, VAS 0–100 mm	40±23	43±24	0.59
CRP (mg/dl)	1.99±2.67	0.94±1.32	0.03
SDAI	20.7±11.7	17.6±8.6	0.24
DAS28-CRP	4.08±1.22	3.66±1.14	0.16
MHAQ	0.61±0.62	0.55±0.56	0.70
ACPA positive, n (%)	43 (91.5)	22 (88.0)	0.69
ACPA (IU/L)	645±880	323±401	0.04
RF positive, n (%)	40 (85.1)	21 (84.0)	1.00
RF (IU/L)	142±251	133±200	0.87
MTX use, n (%)	30 (63.8)	18 (72.0)	0.48
MTX dose (mg/week)	8.5±3.1	8.0±3.6	0.63
Oral steroid use, n (%)	27 (57.4)	21 (84.0)	0.03
Oral steroid use (mg/day*)	3.5±3.6	4.9±3.5	0.11
Bio naive (%)	26 (55.3)	13 (52.0)	0.79

*Prednisolone equivalents.
Results are shown as mean ±SD unless stated otherwise.
Significance was determined by means of the t-test for continuous variables, and the Chi-square or Fisher's exact test for categorical variables.
ACPA, anticyclic citrullinated peptide antibody; CRP, C-reactive protein; DAS28, 28-joint count disease activity score; EGA, evaluator global assessment of disease activity; GH, patient's global assessment of general health; MHAQ, Modified Health Assessment Questionnaire; MTX, methotrexate; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale.



Independent predictors for SDAI at week 24 in multivariable analysis

	Regression coefficient		P value
	Standardised	Unstandardised	
Shared epitope positivity	**-0.46**	**-3.78**	**<0.0001**
ACPA titer (at baseline)	-0.16		0.15
Age	0.10		0.33
Sex (female)	-0.09		0.38
SDAI (at baseline)	0.19		0.07
MTX use	0.19		0.08
Prior use of biological agent	0.17		0.10

Figure 1 (A) Retention rate of abatacept treatment with the subject population stratified according to SE positivity (Kaplan-Meier plots, p<0.0001, log-rank test). (B) Time course of Simplified Disease Activity Index (SDAI). Error bars indicate 95% CI. *p<0.05, **p<0.01, ***p<0.0001. (C) Disease activity stratified according to SDAI. (D) Independent predictors for SDAI at week 24 by multiple regression analysis after adjustment for anticyclic citrullinated peptide antibody (ACPA) titer, age, sex, SDAI at baseline, methotrexate (MTX) use and prior use of biological agent. Only SE positivity was significantly associated with SDAI at 24 week of abatacept treatment.

results suggests that the observed effect of seropositivity could be mediated by the SE, as high titers of ACPA are associated with the presence of SE.[6] Because ACPA titer is also affected by non-SE alleles such as HLA-DRB1*15 and 0901,[7–9] SE positivity may be more accurate predicting factor for abatacept efficacy. Indeed, 26% and 24% of patients had HLA-DRB1*15 and 0901 in our study, respectively. We believe that the prior identification of HLA-DRB1 could increase the precision of expectation for the efficacy of abatacept in patients with RA and ultimately contribute to the development of 'personalised medicine' for this disease.

Kensuke Oryoji,[1] Kenji Yoshida,[1] Yusuke Kashiwado,[2] Keiko Tanaka,[3,4] Shin-ichi Mizuki,[1] Hiroshi Tsukamoto,[2] Kazuo Kamada,[1] Koichi Akashi[2]

[1]Center for Rheumatic Diseases, Matsuyama Red Cross Hospital, Matsuyama, Ehime, Japan
[2]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Fukuoka, Japan
[3]Department of Epidemiology and Preventive Medicine, Ehime University Graduate School of Medicine, Toon, Ehime, Japan
[4]Epidemiology and Medical Statistics Unit, Translational Research Center, Ehime University Hospital, Toon, Ehime, Japan

Correspondence to Dr Kensuke Oryoji, Center for Rheumatic diseases, Matsuyama Red Cross Hospital, Matsuyama, Ehime 7908524, Japan; oryoji@matsuyama.jrc.or.jp

Handling editor Tore K Kvien.

Contributors KO contributed to the study design, overall review, writing of the manuscript, and the other authors were involved in performance of the study coordination. KY, YK, KK and SM enrolled and managed patients in the clinic. All authors read and approved the final manuscript.

Competing interests KO and SM have received speaking fees from Abbvie, Chugai, Tanabe-Mitsubishi, Astellas, Eisai, Janssen, Pfizer, UCB and Ono.

Patient consent Obtained.

Ethics approval Matsuyama Red Cross Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Oryoji K, Yoshida K, Kashiwado Y, *et al*. *Ann Rheum Dis* 2018;**77**:1234–1236.

Received 7 March 2017
Accepted 15 August 2017
Published Online First 22 August 2017

Ann Rheum Dis 2018;**77**:1234–1236. doi:10.1136/annrheumdis-2017-211430

REFERENCES

1 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011;365:2205–19.
2 Sakkas LI, Bogdanos DP, Katsiari C, *et al*. Anti-citrullinated peptides as autoantigens in rheumatoid arthritis-relevance to treatment. *Autoimmun Rev* 2014;13:1114–20.
3 Gottenberg JE, Ravaud P, Cantagrel A, *et al*. Positivity for anti-cyclic citrullinated peptide is associated with a better response to abatacept: data from the 'Orencia and Rheumatoid Arthritis' registry. *Ann Rheum Dis* 2012;71:1815–9.
4 Sokolove J, Schiff M, Fleischmann R, *et al*. Impact of baseline anti-cyclic citrullinated peptide-2 antibody concentration on efficacy outcomes following treatment with subcutaneous abatacept or adalimumab: 2-year results from the AMPLE trial. *Ann Rheum Dis* 2016;75:709–14.

5 Raychaudhuri S, Sandor C, Stahl EA, *et al*. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet* 2012;44:291–6.
6 van der Helm-van Mil AH, Verpoort KN, Breedveld FC, *et al*. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. *Arthritis Rheum* 2006;54:1117–21.
7 Laki J, Lundström E, Snir O, *et al*. Very high levels of anti-citrullinated protein antibodies are associated with HLA-DRB1*15 non-shared epitope allele in patients with rheumatoid arthritis. *Arthritis Rheum* 2012;64:2078–84.
8 Okada Y, Suzuki A, Yamada R, *et al*. HLA-DRB1*0901 lowers anti-cyclic citrullinated peptide antibody levels in Japanese patients with rheumatoid arthritis. *Ann Rheum Dis* 2010;69:1569–70.
9 Terao C, Ikari K, Ohmura K, *et al*. Quantitative effect of HLA-DRB1 alleles to ACPA levels in Japanese rheumatoid arthritis: no strong genetic impact of shared epitope to ACPA levels after stratification of HLA-DRB1*09:01. *Ann Rheum Dis* 2012;71:1095–7.

Seropositivity combined with smoking is associated with increased prevalence of periodontitis in patients with rheumatoid arthritis

An association between periodontitis and rheumatoid arthritis (RA) has been proposed based on observations of increased risk of periodontitis in patients with RA as well as the presence of antibodies to citrullinated protein antigens (ACPAs) and rheumatoid factor (RF) in serum and gingiva of patients with periodontitis.[1–3] Additionally, smoking is one of the most important risk factors for both periodontitis and RA, and predispose for the development of seropositive RA.[4–6] We have previously reported that smokers with RA have increased prevalence of periodontitis as compared with never smokers in the Swedish population-based case–control study EIRA (Epidemiological Investigation of Rheumatoid Arthritis).[7] The objective of the current study was to further investigate the effects of smoking on the risk of periodontitis in seropositive and seronegative (ACPA/RF) subsets of RA.

Data on periodontal status (years 2008–2012) were retrieved from the Swedish Dental Health Registry (DHR) for 2327 patients with established RA (1469/852 ACPA-positive/ACPA-negative and 1505/822 RF-positive/RF-negative, respectively) included in the EIRA study (years 1996–2009) as previously described.[7] Periodontal diagnosis was based on diagnostic codes for periodontitis, peri-implantitis and increased risk of periodontitis/peri-implantitis, registered by the patients' dentists in the DHR.[7] The diagnosis of RA was confirmed by the rheumatologist at the time of the recruitment into EIRA; blood samples were collected to determine ACPA/RF status.[8] Detailed information on smoking status was collected by a self-administered questionnaire at the time of enrolment to EIRA.[8] For the association between smoking status, seropositive/seronegative RA and periodontitis, we calculated OR with 95% CI adjusted for age, gender, education and residential area.

In ACPA-positive RA, smoking was associated with a significantly ($p<0.05$) higher prevalence of periodontitis, mainly in current smokers (OR=1.9, 95% CI 1.5 to 2.5) (table 1). The

Table 1 Association between periodontal diagnostic codes and smoking habits compared with never smokers in EIRA RA cases, in relation to ACPA status and gender*

		ACPA-positive RA (n=1469)		ACPA-negative RA (n=852)	
Smoking habits		No with periodontitis (%)†	OR (95% CI)‡	No with periodontitis (%)†	OR (95% CI)‡
Total					
	All	773 (100)		458 (100)	
	Women	557 (100)		331 (100)	
	Men	216 (100)		127 (100)	
Never smokers					
	All	196 (25.4)	1.0 (ref)	155 (33.8)	1.0 (ref)
	Women	156 (28.0)	1.0 (ref)	115 (34.7)	1.0 (ref)
	Men	40 (18.5)	1.0 (ref)	40 (31.5)	1.0 (ref)
Ex-smokers					
	All	285 (36.9)	1.7 (1.3 to 2.2)§	140 (30.6)	0.9 (0.7 to 1.3)
	Women	200 (35.9)	1.8 (1.4 to 2.4)§	88 (26.6)	1.0 (0.7 to 1.5)
	Men	85 (39.4)	1.8 (1.0 to 3.1)§	52 (40.9)	0.7 (0.4 to 1.3)
Ever smokers					
	All	577 (74.6)	1.6 (1.3 to 2.0)§	303 (66.2)	1.1 (0.9 to 1.4)
	Women	401 (72.0)	1.6 (1.3 to 2.1)§	216 (65.3)	1.3 (0.9 to 1.7)
	Men	176 (81.5)	1.9 (1.2 to 3.0)§	87 (68.5)	0.8 (0.5 to 1.3)
Current smokers					
	All	232 (30.0)	1.9 (1.5 to 2.5)§	111 (24.2)	1.2 (0.9 to 1.6)
	Women	157 (28.2)	1.8 (1.3 to 2.4)§	85 (25.7)	1.4 (0.9 to 2.0)
	Men	75 (34.7)	2.9 (1.6 to 5.3)§	26 (20.5)	0.7 (0.4 to 1.4)

*The periodontal diagnostic codes include periodontitis, peri-implantitis and increased risk for periodontitis/peri-implantitis.
†Number (%) of ACPA-positive or ACPA-negative RA cases with periodontal diagnostic codes.
‡ORs, with a 95% CI, were adjusted for age, gender, education and residential area.
§p Value <0.05 for association between periodontal diagnostic codes and smoking habits as compared with never smokers among ACPA-positive and ACPA-negative RA cases.
ACPA, anticitrullinated protein antibody; EIRA, Epidemiological Investigation of Rheumatoid Arthritis; RA, rheumatoid arthritis; ref, reference group.

Table 2 Association between periodontal diagnostic codes and smoking habits compared with never smokers in EIRA RA cases, in relation to double seropositive or negative antibody status and gender*

		ACPA-positive and RF-positive RA (n=1261)		ACPA-negative and RF-negative RA (n=616)	
Smoking habits		No with periodontitis (%)†	OR (95% CI)‡	No with periodontitis (%)†	OR (95% CI)‡
Total					
	All	667 (100)		328 (100)	
	Women	479 (100)		234 (100)	
	Men	188 (100)		94 (100)	
Never smokers					
	All	162 (24.3)	1.0 (ref)	122 (37.2)	1.0 (ref)
	Women	130 (27.1)	1.0 (ref)	90 (38.5)	1.0 (ref)
	Men	32 (17.0)	1.0 (ref)	32 (34.0)	1.0 (ref)
Ex-smokers					
	All	254 (38.1)	1.8 (1.4 to 2.3)§	94 (28.7)	0.8 (0.6 to 1.1)
	Women	178 (37.2)	1.9 (1.4 to 2.5)§	53 (22.6)	0.8 (0.5 to 1.2)
	Men	76 (40.4)	1.9 (1.1 to 3.4)§	41 (43.6)	0.7 (0.4 to 1.3)
Ever smokers					
	All	505 (75.7)	1.7 (1.4 to 2.1)§	206 (62.8)	1.0 (0.7 to 1.2)
	Women	349 (72.9)	1.7 (1.3 to 2.2)§	144 (61.5)	1.1 (0.8 to 1.5)
	Men	156 (83.0)	2.0 (1.2 to 3.3)§	62 (66.0)	0.7 (0.4 to 1.1)
Current smokers					
	All	200 (30.0)	2.0 (1.5 to 2.7)§	76 (23.2)	1.0 (0.7 to 1.5)
	Women	133 (27.8)	1.8 (1.3 to 2.5)§	61 (26.1)	1.3 (0.8 to 1.9)
	Men	67 (35.6)	3.3 (1.8 to 6.2)§	15 (16.0)	0.5 (0.2 to 1.1)

*The periodontal diagnostic codes include periodontitis, peri-implantitis and increased risk for periodontitis/peri-implantitis.
†Number (%) of ACPA-positive and RF-positive or ACPA-negative and RF-negative RA cases with periodontal diagnostic codes.
‡ORs, with a 95% CI, were adjusted for age, gender, education and residential area.
§p <0.05 for association between periodontal diagnostic codes and smoking habits as compared to never smokers among ACPA-positive and RF-positive or ACPA-negative and RF-negative RA cases.
ACPA, anticitrullinated protein antibody; EIRA, Epidemiological Investigation of Rheumatoid Arthritis; RA, rheumatoid arthritis; ref, reference group; RF, rheumatoid factor.

highest prevalence of periodontitis, with almost a threefold increased risk, was observed among current smoking ACPA-positive men (OR=2.9, 95%CI 1.6 to 5.3). For ACPA-negative RA, no convincing association between smoking and periodontitis was observed (table 1). Similar associations ($p<0.05$) were observed in analysis based on RF status (RF-positive current smokers; OR=1.9, 95%CI 1.5 to 2.5) with the highest OR observed in RF-positive current smoking men (OR=2.9, 95%CI 1.6 to 5.2) (table not shown).

Interestingly, the OR for periodontitis increased even further among patients double positive for ACPA and RF antibodies, with OR of 3.3 (95% CI 1.8 to 6.2) observed in current smoking men compared with never smokers (table 2).

We herein demonstrate that the previously observed association between smoking and periodontitis in RA[7] is confined to patients with seropositive RA, especially those with both ACPA and RF antibodies. One reason for the increased risk of periodontitis in seropositive RA may be due to enhanced ACPA and/or RF titres in smokers since smoking is reported to be associated with increased risk for seropositive RA and higher titres of ACPA/RF in RA, and furthermore, periodontitis has been associated with increased levels of ACPA/RF in patients with RA.[1 4 5 9 10] Smoking did not, however, significantly increase the prevalence of periodontitis in ACPA-negative/RF-negative RA, suggesting different pathophysiological mechanisms depending on autoantibody status in patients with RA. Our results are in line with previous findings that seropositive and seronegative RA represent distinct disease subsets differing in several aspects, including the association between seropositive RA with specific genetic and environmental risk factors such as human leukocyte antigen (HLA)-shared epitope and smoking.[4 5] In summary, the highest risk of periodontitis in patients with established RA was observed among seropositive current smokers, especially those double positive for ACPA and RF antibodies, a finding that warrants awareness by clinicians and their patients as well as further investigations on the mechanisms behind this association.

Kaja Eriksson,[1] Lena Nise,[2] Lars Alfredsson,[2,3] Anca Irinel Catrina,[4] Johan Askling,[4,5] Karin Lundberg,[4] Lars Klareskog,[4] Tülay Yucel-Lindberg[1]

[1]Division of Periodontology, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden
[2]Unit of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[3]Centre of Environmental and Occupational Medicine, Stockholm County Council, Stockholm, Sweden
[4]Rheumatology Unit, Department of Medicine, Karolinska University Hospital, Stockholm, Sweden
[5]Clinical Epidemiology Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Correspondence to Dr Tülay Yucel-Lindberg and Dr Kaja Eriksson, Division of Periodontology, Department of Dental Medicine, Karolinska Institutet, Box 4064, SE-141 04 Huddinge, Sweden; tulay.lindberg@ki.se, Kaja.eriksson@ki.se

Acknowledgements The authors thank all the EIRA participants, clinicians and research nurses and the investigators and founders of the EIRA study. We also thank Lena Israelsson and Hiba Mahdi for analysing the presence of anti-CCP2 antibodies in EIRA.

Contributors TY-L, LK, LA, KL, JA and AIC conceived and designed the study. TY-L, LN and KE acquired and analysed the data. The statistical analysis was conducted by LN. Interpretation of data was made by TY-L, KE, LA, LK, KL, JA and AIC. KE and TY-L drafted the manuscript. All authors revised the article for important intellectual content and approved the final version for publication.

Funding This work was supported by the collaborative European Union's FP7 Research Projects—Gums & Joints (grant agreement no FP7-HEALTH-2010-261460) and TRIGGER (grant agreement no FP7-HEALTH-2013-306029), the Stockholm County Council (SOF and ALF), the Swedish Research Council, the Swedish Dental Society, the Swedish Rheumatic Foundation and Karolinska Institutet.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Regional Ethics Board in Stockholm approved the study design and written consent was obtained from all subjects.

Provenance and peer review Not commissioned; externally peer reviewed.

OPEN ACCESS

To cite Eriksson K, Nise L, Alfredsson L, *et al*. *Ann Rheum Dis* 2018;**77**:1236–1238.

Received 17 July 2017
Revised 5 September 2017
Accepted 8 September 2017
Published Online First 6 October 2017

Ann Rheum Dis 2018;**77**:1236–1238. doi:10.1136/annrheumdis-2017-212091

REFERENCES

1 Mikuls TR, Payne JB, Yu F, *et al*. Periodontitis and Porphyromonas gingivalis in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:1090–100.
2 Lappin DF, Apatzidou D, Quirke AM, *et al*. Influence of periodontal disease, Porphyromonas gingivalis and cigarette smoking on systemic anti-citrullinated peptide antibody titres. *J Clin Periodontol* 2013;40:907–15.
3 Gargiulo AV, Robinson J, Toto PD, *et al*. Identification of rheumatoid factor in periodontal disease. *J Periodontol* 1982;53:568–77.
4 Gerlag DM, Norris JM, Tak PP. Towards prevention of autoantibody-positive rheumatoid arthritis: from lifestyle modification to preventive treatment. *Rheumatology* 2016;55:607–14.
5 Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *Nat Rev Immunol* 2017;17:60–75.
6 Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *J Periodontol* 2000;71:743–51.
7 Eriksson K, Nise L, Kats A, *et al*. Prevalence of periodontitis in patients with established rheumatoid arthritis: a Swedish population based case–control study. *PLoS One* 2016;11:e0155956.
8 Klareskog L, Stolt P, Lundberg K, *et al*. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum* 2006;54:38–46.
9 Mikuls TR, Hughes LB, Westfall AO, *et al*. Cigarette smoking, disease severity and autoantibody expression in African Americans with recent-onset rheumatoid arthritis. *Ann Rheum Dis* 2008;67:1529–34.
10 Gonzalez SM, Payne JB, Yu F, *et al*. Alveolar bone loss is associated with circulating anti-citrullinated protein antibody (ACPA) in patients with rheumatoid arthritis. *J Periodontol* 2015;86:222–31.

Amount of smoking, duration of smoking cessation and their interaction with silica exposure in the risk of rheumatoid arthritis among males: results from the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study

Cigarette smoking is a well-established environmental risk factor for rheumatoid arthritis (RA),[1–3] particularly anticitrullinated protein antibody (ACPA)-positive RA.[2] We previously observed an association between silica exposure and increased risk of seropositive RA.[4 5] While the additive interaction between silica exposure and smoking has been demonstrated,[5] considerably less is known regarding the dose of smoking required to elicit this effect. In this study, we extend our previous findings on smoking and silica exposure by investigating the additive interaction between silica exposure and dose of smoking as well as between silica exposure and duration of smoking cessation, with regard to the risk of developing ACPA-positive RA.

Our study is based on the Swedish Epidemiological Investigation of Rheumatoid Arthritis project,[3] an ongoing population-based study comprising incident cases (American College of Rheumatology 1987 or 2010[6 7] criteria) and controls aged ≥18 years living in Sweden between 1996 and 2014. Information on occupational silica exposure and cigarette smoking were collected through questionnaire. Additive interaction was evaluated by calculating the attributable proportion due to interaction (AP).[8]

Data from 599 incident male ACPA-positive RA cases and 1530 male controls were analysed. Consistent with the previous findings, the proportion of current smokers, past smokers and individuals ever exposed to silica were higher among the cases (30%, 39% and 16%, respectively) as compared with the controls (16%, 31% and 10%, respectively). A lower median value of years of smoking cessation was observed in ACPA-positive RA cases (12 years) than in controls (19 years). The amount of pack-years of smoking (median) were relatively similar between the cases and the controls (approximately 27 pack-years) who were smokers.

A high risk of developing ACPA-positive RA was observed among silica-exposed current smokers (OR=7.5 (4.2–13.2)), with a significant additive interaction (AP=0.5 (0.2–0.8)) (table 1). The magnitude of silica–smoking interaction increased as pack-year of smoking increased, with the highest AP observed for smoking ≥28 pack-years (AP=0.7 (0.4–0.9)) (table 1). Current smokers who had smoked 1–20 pack-years and past smokers who quitted smoking <10 years ago had comparable AP (0.5 (−0.1–0.9)) (table 2).

The strengths of this study include: a population-based case–control design, enrolling incident RA cases, with a relatively short mean time from disease onset to diagnosis (10 months), and high participation proportion among both cases (91%) and controls (72%). These strengths substantially reduced the magnitude of potential selection bias.

The number of silica-exposed non-smokers is relatively low. Therefore, the combined effect should be interpreted with caution. It is likely that our results might be subjected to recall bias to some extent, since the study included retrospective self-reported exposures. However, we consider the influence of

such potential bias on the results to be of minor magnitude, since silica exposure is easy to recall as it is related to specific types of occupations and work environments. We consider subjects exposed to rock drilling, stone crushing and stone dust, which have previously been documented to be associated with high degree of silica exposure,[9 10] as silica exposed.

In summary, we found that the interaction between smoking and silica exposure regarding ACPA-positive RA depended on the cumulative dose of smoking. The additive interaction effect between these two exposures might take more than 10 years to disappear. Since silica dust is difficult to remove once it deposited in the lungs, our study strengthens the rationale to advice silica exposed persons to avoid cigarette smoking. Our study also strengthen the concept that a multitude of lung-affecting agents may trigger the development of ACPA-positive RA.

Table 1 Additive interaction between smoking and silica exposure regarding risk of ACPA-positive RA among males, by pack-years of smoking

Pack-years of smoking	Non-smokers/silica non-exposed (reference group)	Non-smokers/silica exposed		Current smokers/silica non-exposed		Current smokers/silica exposed			p Value for AP
	Case/control	Case/control	OR* (95% CI)	Case/control	OR* (95% CI)	Case/control	OR* (95% CI)	AP (95% CI)	
All current smokers	135/622	17/57	1.4 (0.8 to 2.5)	140/220	3.4 (2.5 to 4.6)	35/25	7.5 (4.2 to 13.2)	0.5 (0.2 to 0.8)	0.001
1 pack-year	135/622	17/57	1.4 (0.8 to 2.5)	140/216	3.4 (2.5 to 4.7)	35/25	7.5 (4.2 to 13.2)	0.5 (0.2 to 0.8)	0.002
2 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	138/214	3.4 (2.5 to 4.7)	35/24	7.8 (4.4 to 13.9)	0.5 (0.2 to 0.8)	0.001
3 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	136/210	3.5 (2.6 to 4.7)	35/24	7.9 (4.4 to 14.0)	0.5 (0.2 to 0.8)	0.001
4 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	136/203	3.6 (2.7 to 4.9)	35/24	7.9 (4.4 to 14.0)	0.5 (0.2 to 0.8)	0.002
5 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	136/200	3.7 (2.7 to 5.1)	35/24	8.0 (4.5 to 14.2)	0.5 (0.2 to 0.8)	0.002
6 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	136/196	3.8 (2.8 to 5.2)	35/24	8.0 (4.5 to 14.2)	0.5 (0.2 to 0.8)	0.003
7 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	133/193	3.8 (2.8 to 5.2)	34/24	7.7 (4.3 to 13.7)	0.5 (0.1 to 0.8)	0.006
8 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	133/192	3.8 (2.8 to 5.2)	34/24	7.7 (4.3 to 13.8)	0.5 (0.1 to 0.8)	0.006
9 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	130/190	3.8 (2.8 to 5.2)	33/23	8.0 (4.4 to 14.4)	0.5 (0.2 to 0.8)	0.004
10 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	127/188	3.8 (2.7 to 5.2)	33/23	8.0 (4.5 to 14.5)	0.5 (0.2 to 0.8)	0.003
11 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	122/185	3.7 (2.7 to 5.1)	33/23	8.0 (4.4 to 14.4)	0.5 (0.2 to 0.8)	0.002
12 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	119/183	3.6 (2.6 to 5.0)	33/23	8.0 (4.4 to 14.4)	0.5 (0.2 to 0.8)	0.002
13 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	117/178	3.7 (2.7 to 5.1)	33/20	9.2 (5.0 to 17.0)	0.6 (0.3 to 0.8)	<0.001
14 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	113/173	3.7 (2.7 to 5.2)	33/20	9.2 (5.0 to 17.0)	0.6 (0.3 to 0.8)	<0.001
15 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	110/167	3.7 (2.7 to 5.2)	32/19	9.6 (5.1 to 17.9)	0.6 (0.3 to 0.8)	<0.001
16 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	108/161	3.8 (2.7 to 5.4)	32/19	9.6 (5.1 to 18.0)	0.6 (0.3 to 0.8)	<0.001
17 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	105/155	3.9 (2.8 to 5.5)	30/19	9.1 (4.8 to 17.1)	0.5 (0.2 to 0.8)	0.001
18 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	103/152	4.0 (2.8 to 5.6)	30/19	9.2 (4.9 to 17.4)	0.5 (0.2 to 0.8)	0.001
19 pack-years	135/622	17/57	1.4 (0.8 to 2.6)	99/144	4.0 (2.8 to 5.7)	30/18	9.7 (5.1 to 18.5)	0.5 (0.2 to 0.8)	0.001
20 pack-years	135/622	17/57	1.4 (0.8 to 2.6)	95/137	4.0 (2.8 to 5.8)	29/18	9.5 (5.0 to 18.2)	0.5 (0.2 to 0.8)	0.001
21 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	93/131	4.1 (2.9 to 5.9)	29/17	10.1 (5.2 to 19.5)	0.6 (0.2 to 0.9)	<0.001
22 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	91/130	4.1 (2.8 to 5.8)	29/17	10.2 (5.3 to 19.6)	0.6 (0.3 to 0.9)	<0.001
23 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	84/125	3.9 (2.7 to 5.7)	29/17	10.2 (5.3 to 19.6)	0.6 (0.3 to 0.9)	<0.001
24 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	77/122	3.7 (2.5 to 5.4)	27/17	9.4 (4.8 to 18.2)	0.6 (0.3 to 0.9)	<0.001
25 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	73/121	3.6 (2.4 to 5.2)	25/14	10.5 (5.2 to 21.4)	0.6 (0.3 to 0.9)	<0.001
26 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	72/115	3.7 (2.5 to 5.5)	24/12	11.6 (5.5 to 24.5)	0.6 (0.4 to 0.9)	<0.001
27 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	67/110	3.6 (2.4 to 5.3)	22/12	10.9 (5.1 to 23.2)	0.6 (0.3 to 0.9)	<0.001
28 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	59/105	3.3 (2.2 to 4.9)	22/12	10.7 (5.0 to 22.8)	0.7 (0.4 to 0.9)	<0.001
29 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	54/102	3.1 (2.0 to 4.7)	22/11	11.5 (5.3 to 24.9)	0.7 (0.4 to 0.9)	<0.001
30 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	52/98	3.1 (2.0 to 4.7)	21/11	10.8 (5.0 to 23.6)	0.7 (0.4 to 0.9)	<0.001
31 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	50/91	3.2 (2.1 to 5.0)	18/11	8.9 (4.0 to 19.8)	0.6 (0.2 to 0.9)	0.001
32 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	48/87	3.3 (2.1 to 5.1)	17/10	9.1 (3.9 to 20.9)	0.6 (0.2 to 1.0)	0.001
33 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	46/80	3.5 (2.2 to 5.5)	17/9	10.1 (4.3 to 23.9)	0.6 (0.3 to 1.0)	0.001
34 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	45/73	3.8 (2.4 to 6.1)	17/9	10.2 (4.3 to 24.2)	0.6 (0.2 to 1.0)	0.002
35 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	41/72	3.5 (2.2 to 5.6)	14/9	8.8 (3.6 to 21.4)	0.6 (0.1 to 1.0)	0.01
36 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	38/70	3.3 (2.0 to 5.3)	13/7	10.9 (4.1 to 28.8)	0.7 (0.3 to 1.0)	<0.001
37 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	32/62	3.2 (1.9 to 5.3)	9/7	7.7 (2.7 to 21.9)	0.5 (0.0 to 1.1)	0.042
38 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	31/56	3.4 (2.0 to 5.8)	9/6	8.9 (3.0 to 26.5)	0.6 (0.1 to 1.1)	0.023
39 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	30/52	3.5 (2.1 to 6.0)	8/6	7.8 (2.6 to 23.8)	0.5 (–0.1 to 1.1)	0.108
40 pack-years	135/622	17/57	1.4 (0.8 to 2.5)	27/48	3.4 (2.0 to 5.9)	7/6	6.8 (2.2 to 21.3)	0.4 (–0.2 to 1.1)	0.209

*OR adjusted for age and residency.

ACPA, anticitrullinated protein/peptide antibodies; AP, attributable proportion due to interaction; RA, rheumatoid arthritis.

Table 2 Additive interaction between smoking cessation and silica exposure regarding risk of ACPA-positive RA among males, by duration of smoking cessation

Duration of smoking cessation	Non-smoker/ silica non-exposed (reference group)	Non-smoker/ silica exposed	OR*	Ex-smoker/ silica non-exposed		Ex-smoker/ silica exposed			p Value for AP
	Case/control	Case/control	(95% CI)	Case/control	OR* (95% CI)	Case/control	OR* (95% CI)	AP (95% CI)	
All ex-smokers	135/622	17/57	1.4 (0.8 to 2.5)	196/411	2.6 (1.9 to 3.3)	38/55	3.9 (2.4 to 6.2)	0.2(–0.2 to 0.60)	0.226
0–10 years	135/622	17/57	1.4 (0.8 to 2.5)	83/131	3.2 (2.2 to 4.5)	16/13	7.1 (3.2 to 15.6)	0.5 (0.1 to 0.9)	0.017
>10 years	135/622	17/57	1.4 (0.8 to 2.5)	111/278	2.2 (1.6 to 3.1)	22/42	3.0 (1.7 to 5.3)	0.1 (–0.4 to 0.7)	0.679
5 years	135/622	17/57	1.4 (0.8 to 2.5)	49/69	3.5 (2.3 to 5.3)	8/4	11.4 (3.2 to 40.5)	0.7 (0.2 to 1.1)	0.003
6 years	135/622	17/57	1.4 (0.8 to 2.5)	53/80	3.2 (2.1 to 4.9)	11/7	8.7 (3.2 to 24.0)	0.6 (0.1 to 1.0)	0.009
7 years	135/622	17/57	1.4 (0.8 to 2.5)	66/92	3.6 (2.4 to 5.2)	11/7	8.9 (3.2 to 24.8)	0.6 (0.1 to 1.0)	0.019
8 years	135/622	17/57	1.4 (0.8 to 2.5)	68/107	3.1 (2.2 to 4.6)	13/9	8.5 (3.4 to 21.1)	0.6 (0.2 to 1.0)	0.004
9 years	135/622	17/57	1.4 (0.8 to 2.5)	76/119	3.2 (2.2 to 4.6)	14/10	8.0 (3.4 to 19.1)	0.6 (0.2 to 1.0)	0.007
10 years	135/622	17/57	1.4 (0.8 to 2.5)	83/131	3.2 (2.2 to 4.5)	16/13	7.1 (3.2 to 15.6)	0.5 (0.1 to 0.9)	0.017
11 years	135/622	17/57	1.4 (0.8 to 2.5)	92/137	3.4 (2.4 to 4.7)	18/16	6.5 (3.1 to 13.4)	0.4 (0.0 to 0.9)	0.062
12 years	135/622	17/57	1.4 (0.8 to 2.5)	101/149	3.4 (2.5 to 4.7)	19/19	5.9 (2.9 to 11.7)	0.4 (–0.1 to 0.8)	0.142
13 years	135/622	17/57	1.4 (0.8 to 2.4)	112/157	3.6 (2.6 to 5.0)	19/21	5.3 (2.7 to 10.3)	0.2 (–0.3 to 0.8)	0.388
14 years	135/622	17/57	1.4 (0.8 to 2.5)	116/163	3.6 (2.7 to 5.0)	19/22	5.1 (2.6 to 9.9)	0.2 (–0.3 to 0.8)	0.461
15 years	135/622	17/57	1.4 (0.8 to 2.5)	118/169	3.6 (2.6 to 5.0)	21/23	5.4 (2.8 to 10.3)	0.3 (–0.2 to 0.8)	0.312
16 years	135/622	17/57	1.4 (0.8 to 2.5)	122/173	3.7 (2.7 to 5.0)	22/24	5.4 (2.9 to 10.2)	0.3 (–0.2 to 0.7)	0.318
17 years	135/622	17/57	1.4 (0.8 to 2.4)	128/186	3.6 (2.7 to 4.9)	22/24	5.4 (2.9 to 10.2)	0.3 (–0.2 to 0.8)	0.273
18 years	135/622	17/57	1.4 (0.8 to 2.5)	135/206	3.5 (2.6 to 4.7)	23/25	5.3 (2.9 to 9.9)	0.3 (–0.2 to 0.7)	0.253
19 years	135/622	17/57	1.4 (0.8 to 2.5)	139/223	3.3 (2.5 to 4.5)	24/26	5.3 (2.9 to 9.8)	0.3 (–0.1 to 0.8)	0.178
20 years	135/622	17/57	1.4 (0.8 to 2.5)	145/235	3.3 (2.5 to 4.4)	25/27	5.3 (2.9 to 9.7)	0.3 (–0.1 to 0.7)	0.159
21 years	135/622	17/57	1.4 (0.8 to 2.5)	149/241	3.3 (2.5 to 4.4)	25/31	4.6 (2.6 to 8.2)	0.2 (–0.3 to 0.7)	0.409
22 years	135/622	17/57	1.4 (0.8 to 2.5)	157/258	3.2 (2.4 to 4.3)	25/32	4.5 (2.5 to 8.0)	0.2 (–0.3 to 0.7)	0.441
23 years	135/622	17/57	1.4 (0.8 to 2.4)	162/266	3.2 (2.4 to 4.3)	25/36	3.9 (2.2 to 6.9)	0.1 (–0.5 to 0.6)	0.747
24 years	135/622	17/57	1.4 (0.8 to 2.4)	166/276	3.2 (2.4 to 4.2)	27/39	3.8 (2.2 to 6.6)	0.1 (–0.5 to 0.6)	0.781
25 years	135/622	17/57	1.4 (0.8 to 2.5)	169/286	3.1 (2.4 to 4.2)	27/40	3.8 (2.2 to 6.6)	0.1 (–0.5 to 0.6)	0.77
26 years	135/622	17/57	1.4 (0.8 to 2.5)	172/297	3.1 (2.3 to 4.1)	28/42	3.8 (2.2 to 6.5)	0.1 (–0.4 to 0.6)	0.721
27 years	135/622	17/57	1.4 (0.8 to 2.5)	175/309	3.0 (2.3 to 4.0)	29/43	3.9 (2.3 to 6.6)	0.1 (–0.4 to 0.6)	0.589
28 years	135/622	17/57	1.4 (0.8 to 2.5)	179/320	3.0 (2.3 to 3.9)	30/44	4.0 (2.3 to 6.7)	0.2 (–0.3 to 0.6)	0.53
29 years	135/622	17/57	1.4 (0.8 to 2.5)	182/329	2.9 (2.2 to 3.9)	32/46	4.0 (2.4 to 6.7)	0.2 (–0.3 to 0.6)	0.445
30 years	135/622	17/57	1.4 (0.8 to 2.5)	183/336	2.9 (2.2 to 3.8)	33/48	3.9 (2.4 to 6.5)	0.2 (–0.3 to 0.6)	0.452

*OR adjusted for age and residency.

ACPA, anticitrullinated protein/peptide antibodies; AP, attributable proportion due to interaction; RA, rheumatoid arthritis.

Pingling Zeng,[1] Zuomei Chen,[1] Lars Klareskog,[2] Lars Alfredsson,[1,3] Camilla Bengtsson,[1] Xia Jiang[1]

[1]Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden
[2]Rheumatology Unit, Department of Medicine, Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden
[3]Center for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden

Correspondence to Pingling Zeng, Institute of Environmental Medicine, Karolinska Institute, 171 77, Stockholm, Sweden; pingling.zeng@ki.se

Acknowledgements We would like to thank all the participants of the EIRA study.

Contributors All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. PZ and XJ have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding This work was supported by grants from the Swedish Research Council for Health, Working Life and Welfare; the Swedish Foundation for Strategic Research; the COMBINE public–private research programme; Swedish Research Council; and EU-IMI BTCure.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics committee of the Karolinska Institutet.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Zeng P, Chen Z, Klareskog L, *et al*. *Ann Rheum Dis* 2018;**77**:1238–1241.
Received 28 July 2017

Revised 14 August 2017
Accepted 15 August 2017
Published Online First 15 September 2017

Ann Rheum Dis 2018;**77**:1238–1241. doi:10.1136/annrheumdis-2017-212145

REFERENCES

1 Costenbader KH, Feskanich D, Mandl LA, *et al*. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *Am J Med* 2006;119:503.e1–503.e9.
2 Källberg H, Ding B, Padyukov L, *et al*. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Ann Rheum Dis* 2011;70:508–11.
3 Stolt P, Bengtsson C, Nordmark B, *et al*. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann Rheum Dis* 2003;62:835–41.

4 Stolt P, Källberg H, Lundberg I, *et al*. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann Rheum Dis* 2005;64:582–6.
5 Stolt P, Yahya A, Bengtsson C, *et al*. Silica exposure among male current smokers is associated with a high risk of developing ACPA-positive rheumatoid arthritis. *Ann Rheum Dis* 2010;69:1072–6.
6 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
7 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010:69:1580–8.
8 Rothman KJ, Greenland S, Walker AM. Concepts of interaction. *Am J Epidemiol* 1980;112:467–70.
9 Guénel P, Breum NO, Lynge E. Exposure to silica dust in the Danish stone industry. *Scand J Work Environ Health* 1989;15:147–53.
10 Healy CB, Coggins MA, Van Tongeren M, *et al*. Determinants of respirable crystalline silica exposure among stoneworkers involved in stone restoration work. *Ann Occup Hyg* 2014;58:6–18.

High-dose ustekinumab for severe childhood deficiency of interleukin-36 receptor antagonist (DITRA)

Deficiency of the interleukin-36 receptor antagonist (DITRA) is an autosomal recessive disease caused by mutations of *IL36RN* gene.[1] Patients suffer from flares of acute generalised pustular psoriasis and systemic inflammation. We present two paediatric cases of DITRA with a severe clinical course, resistant to multiple therapies in whom the use of high doses of ustekinumab (a monoclonal antibody against the p40 subunit of both IL-12 and IL-23) lead to a persistent control of the disease.

CASE 1

A 4-year-old boy, born from unrelated parents, presented at the age of 3 years with inverse psoriasis in the genital area. After some months, he developed diffuse pustular lesions associated with fever, elevation of acute phase reactants and poor general condition, requiring parenteral antibiotics and high-dose steroids (figure 1). Compound heterozygosity for the *IL36RN* P76L/S113L mutations was detected. Different treatments (acitretin, high-dose ciclosporin, anakinra, thalidomide and dapsone)[1–7] could not control disease flares (figure 2). High-dose steroids had resulted in a clear cushingoid appearance (figure 1). After approval and parental consent, ustekinumab was therefore started, added at a dose of 0.75 mg/kg every 2 months with a good clinical response. Due to mild relapses observed few days before the scheduled administration, the dose was increased (1.5 mg/kg every 2 months) followed by complete remission, persisting for a total follow-up of 15 months despite discontinuation of steroids (figures 1 and 2A).

CASE 2

A 5-year-old girl from consanguineous Moroccan parents presented with erythroderma and pustular lesions, covering more than 85% of her body surface when she was 1 month old

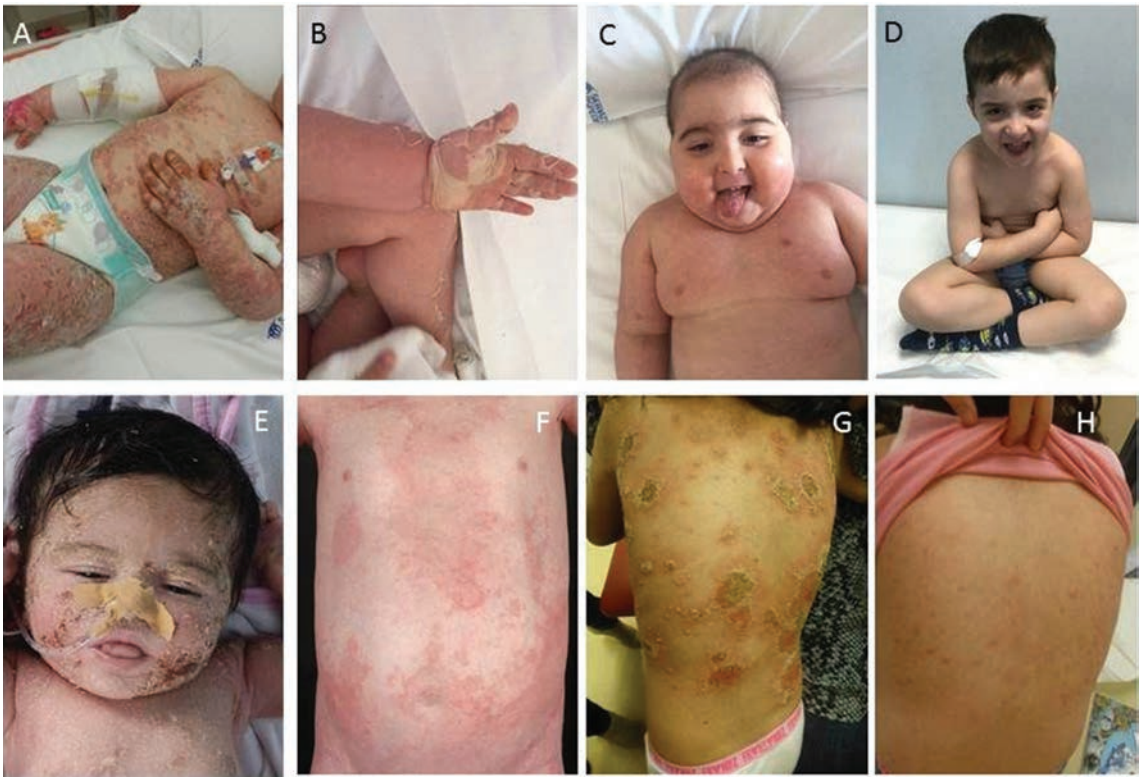


Figure 1 Clinical manifestations associated to deficiency of the interleukin-36 receptor antagonist in two children. Panels A–D refer to patient 1. (A) Flare of generalised pustular psoriasis at admission, (B) diffuse desquamation after a skin flare, (C) clinical picture after few weeks of treatment (March 2016) with ustekinumab, showing an amelioration of the skin manifestations and a severe cushingoid appearance secondary to previous prolonged steroid treatment and (D) general condition at last follow-up (February 2017). Panels E–H refer to patient 2. (E) Severe systemic involvement at the age of 4 months, (F) incomplete response during the treatment with etanercept (December 2014), (G) severe skin flare before ustekinumab treatment and (H) clinical picture after 4 months of treatment with ustekinumab.

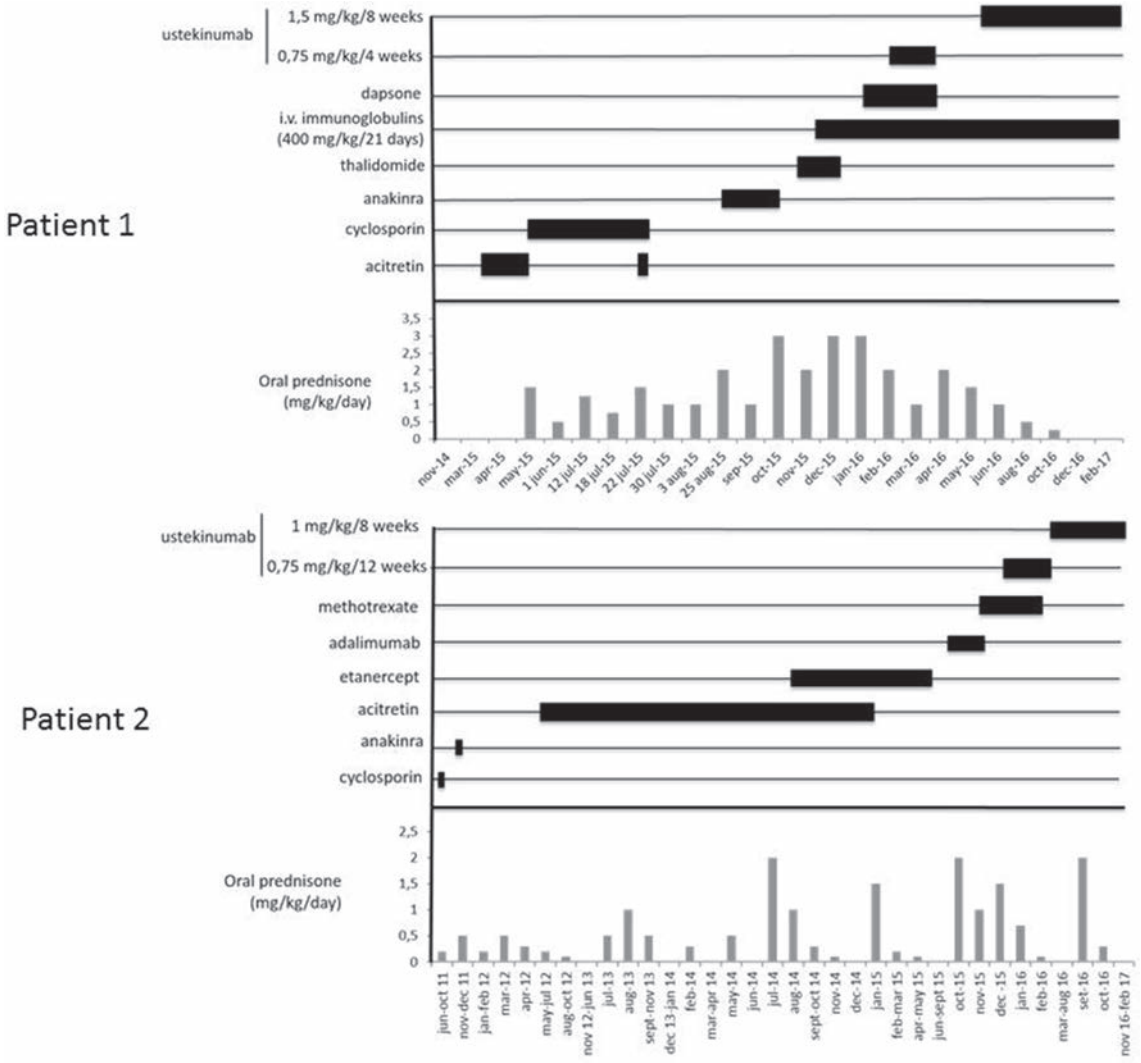


Figure 2 Drug history of the two patients before and after ustekinumab treatment.

(figure 1). Fever, elevation of acute phase reactants and failure-to-thrive were also present. Genetic analysis showed a homozygous L27P mutation in the *IL36RN* gene. High-dose systemic steroids could control disease activity, but with severe side effects. Treatment with topical steroids, acitretin, ciclosporin, methotrexate, anakinra, etanercept and adalimumab were ineffective (figure 2B). At the age of 5 years, on ethical approval and parental consent, ustekinumab (0.75 mg/kg every 12 weeks) was started in combination with methotrexate 7.5 mg/week. Within weeks, the pustular lesions disappeared and only a few erythematous patches remained, which could be controlled with topical steroids (figure 1). Methotrexate was slowly reduced and ultimately discontinued. Due to a disease flare after 5 months dosing interval of ustekinumab was shortened to every 8 weeks and the dosage was increased to 1 mg/kg, with a good disease control and discontinuation of steroids.

Our experience confirms the efficacy of the blockade of the IL-23/T helper cell (Th)17 axis in patients with DITRA,[8 9] although suggesting the need of higher doses in children. Unexpected for an autoinflammatory disease, a treatment targeting adaptive immunity (Th1 and Th17 cells) appears more effective than therapies targeting the innate immune system (IL-1β and tumour necrosis factor alpha). The crucial role of IL-17 in the development of an experimental model of psoriasiform dermatitis induced by imiquimod via toll-like receptor 7 has been demonstrated. In this model, IL-36R–deficient mice were protected from the disease in an IL-1 independent manner, showing the close crosstalk between IL-36 and the IL-23/Th17 axis.[10]

Nadia Bonekamp,[1] Roberta Caorsi,[2] Gian Maria Viglizzo,[3] Marlies de Graaf,[4] Francesca Minoia,[2] Alice Grossi,[5] Paolo Picco,[2] Isabella Ceccherini,[5] Joost Frenkel,[1] Marco Gattorno[2]

[1]Department of Pediatrics, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands
[2]Rheumatology Unit, G Gaslini Institute, Genoa, Italy
[3]Dermatology Service, G Gaslini Institute, Genoa, Italy
[4]Department of Dermatology, University Medical Center Utrecht, Utrecht, The Netherlands
[5]UOC Medical Genetics, G Gaslini Institute, Genoa, Italy

Correspondence to Dr Marco Gattorno, UO Pediatria 2, G Gaslini Institute, Largo G Gaslini 5, Genova, Italy; marcogattorno@gaslini.org

Contributors NB, RC, JF and MG analysed the data and wrote the manuscript. GMV, MdG, FM and PP followed the patient, collected the data and approved the manuscript. AG and IC performed the genetic analysis and approved the manuscript.

Competing interests None declared.

Patient consent Guardian consent.

Ethics approval G Gaslini IRCCS Ethics Board.

Provenance and peer review Not commissioned; externally peer reviewed.

NB and RC contributed equally.
JF and MG contributed equally.

To cite Bonekamp N, Caorsi R, Viglizzo GM, *et al*. *Ann Rheum Dis* 2018;**77**:1241–1243.

Received 16 May 2017
Revised 8 August 2017
Accepted 10 August 2017
Published Online First 2 September 2017

Ann Rheum Dis 2018;**77**:1241–1243. doi:10.1136/annrheumdis-2017-211805

REFERENCES

1 Marrakchi S, Guigue P, Renshaw BR, *et al*. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. *N Engl J Med* 2011;365:620–8.
2 Pan J, Qiu L, Xiao T, *et al*. Juvenile generalized pustular psoriasis with IL36RN mutation treated with short-term infliximab. *Dermatol Ther* 2016;29:164–7.
3 Sugiura K, Endo K, Akasaka T, *et al*. Successful treatment with infliximab of sibling cases with generalized pustular psoriasis caused by deficiency of interleukin-36 receptor antagonist. *J Eur Acad Dermatol Venereol* 2015;29:2054–6.
4 Podlipnik S, de la Mora L, Alsina M, *et al*. *Pneumocystis jirovecii* pneumonia in a patient with pustular psoriasis with an IL-36RN deficiency treated with infliximab: case report and review of the literature. *Australas J Dermatol* 2017;58:e44–e47.
5 Aksentijevich I, Masters SL, Ferguson PJ, *et al*. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* 2009;360:2426–37.
6 Rossi-Semerano L, Piram M, Chiaverini C, *et al*. First clinical description of an infant with interleukin-36-receptor antagonist deficiency successfully treated with anakinra. *Pediatrics* 2013;132:e1043–e1047.
7 Hüffmeier U, Wätzold M, Mohr J, *et al*. Successful therapy with anakinra in a patient with generalized pustular psoriasis carrying IL36RN mutations. *Br J Dermatol* 2014;170:202–4.
8 Arakawa A, Ruzicka T, Prinz JC. Therapeutic efficacy of interleukin 12/interleukin 23 blockade in generalized pustular psoriasis regardless of IL36RN mutation status. *JAMA Dermatol* 2016;152:825–8.
9 Cordoro KM, Ucmak D, Hitraya-Low M, *et al*. Response to interleukin (IL)-17 inhibition in an adolescent with severe manifestations of IL-36 receptor antagonist deficiency (DITRA). *JAMA Dermatol* 2017;153:106–8.
10 Tortola L, Rosenwald E, Abel B, *et al*. Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. *J Clin Invest* 2012;122:3965–76.

Use of urate-lowering therapies is not associated with an increase in the risk of incident dementia in older adults

Few recent studies[1–3] reported that hyperuricemia may be protective against dementia, a common disease in the elderly associated with significant morbidity and mortality.[4 5] We hypothesised that allopurinol or febuxostat use (the two most common urate-lowering therapies (ULTs)) in the elderly will be associated with a higher risk of dementia.

Study methods for this new user design study (a more robust design than a prevalent user design) were similar to those previously reported.[6] Patients were eligible for this retrospective cohort study if they were (1) US residents enrolled in Medicare fee-for-service (covers all Americans ≥65 years) with pharmacy coverage, ie, enrolled in Medicare parts A (inpatient care), B (outpatient doctor and laboratory service) and D (prescription drugs) and not enrolled in a Medicare Advantage Plan (a standard approach for analysis)[7] during 2006–2012 and (2) filled a new allopurinol (or febuxostat) prescription with a clean baseline period of 365 days with no exposure to either drug. Incident dementia was identified by new occurrence (no diagnosis in the 183-day baseline period) of an International Classification of Diseases, 9th Revision code, 290.xx, 294.1x or 331.2, a valid approach for dementia studies.[8 9] We followed each eligible patient until the loss of Medicare coverage, dementia, death or the end of the study period, whichever came first. We used multivariable-adjusted Cox proportional hazard models to adjust for demographics, comorbidity and medication use.

For the 2591 eligible treatment episodes that ended in incident dementia, the cohort mean age was 81 years, 42% were men and the mean follow-up was 683 days. Compared with neither drug, allopurinol or febuxostat use was not significantly associated with dementia (table 1). The following sensitivity analyses replicated the main findings: (1) additionally adjusting for coronary artery disease and risk factors (table 1); (2) limiting to gout: HRs for allopurinol and febuxostat were 0.98 (95% CI 0.90 to 1.08) and 0.85 (95% CI 0.65 to 1.12), respectively; (3) with death as a competing risk, 1.01 (95% CI 0.93 to 1.10) and 0.88 (95% CI 0.68 to 1.15); and (4) limiting of the dementia code to 290.xx only (data not shown).

Reassuringly, we found that compared with neither drug, ULT use (allopurinol or febuxostat) was not associated with any increase in the risk of dementia. Further research is needed to investigate whether significant lowering of serum urate (sUA) with ULTs can potentially increase the risk of dementia.[10]

A higher age related to Medicare sample (≥65 years) indicates that these findings can only be generalised to the elderly. There was a higher proportion of women compared to men among people with incident dementia in our sample. Due to the non-availability of laboratory data, we were unable to examine the baseline sUA (disease severity marker) or the extent of sUA-lowering. Frequent suboptimal urate-lowering with ULTs in the real world meant limiting our ability to examine the effect of optimal ULT use, as per treatment guidelines.[10] Only the long-lasting hyperuricemia may have protective effect on the development of dementia (with a long asymptomatic period), which could not be addressed by this shorter term study. Other limitations were our inability to control for education level (residual confounding), the lack of cognitive function scores and the inability to adjust for ULT dose/duration.

Key study strengths were the use of a representative sample of the elderly, who are at risk of dementia, and the use of a new user design, which avoids missing early events and confounding bias seen with a prevalent user design.

In conclusion, our study shows that in an elderly population, new use of ULTs was not associated with any increase in the risk of dementia. More studies should examine the effect of longer term ULT use and the role of baseline sUA levels and change in sUA level on the risk of dementia in people with gout.

Jasvinder A Singh,[1,2,3] John D Cleveland[2]

[1]Medicine Service, Birmingham Veterans Affairs Medical Center, Birmingham, Alabama, USA
[2]Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA
[3]Division of Epidemiology, University of Alabama at Birmingham, Birmingham, Alabama, USA

Correspondence to Dr Jasvinder A Singh, University of Alabama, Birmingham, Alabama, USA; jasvinder.md@gmail.com

Acknowledgements We thank Jeffrey Curtis of the UAB Division of Rheumatology, who permitted us to reuse the 5% Medicare data.

Table 1 Association of new allopurinol use* or febuxostat use* versus neither medication with incident dementia in new users of allopurinol or febuxostat

	Univariate		Multivariable-adjusted (model 1)		Multivariable-adjusted (model 2)	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (in years)						
65 to <75	Ref		Ref		Ref	
75 to <85	**2.79 (2.52 to 3.09)**	**<0.0001**	**2.69 (2.43 to 2.98)**	**<0.0001**	**2.70 (2.44 to 2.99)**	**<0.0001**
≥85	**6.04 (5.42 to 6.71)**	**<0.0001**	**5.78 (5.18 to 6.43)**	**<0.0001**	**5.66 (5.07 to 6.32)**	**<0.0001**
Gender						
Male	Ref		Ref		Ref	
Female	**1.46 (1.35 to 1.58)**	**<0.0001**	**1.14 (1.06 to 1.24)**	**0.001**	**1.16 (1.07 to 1.25)**	**0.0004**
Race						
White	Ref		Ref		Ref	
Black	**1.38 (1.24 to 1.53)**	**<0.0001**	**1.46 (1.31 to 1.63)**	**<0.0001**	**1.44 (1.29 to 1.61)**	**<0.0001**
Other	1.01 (0.88 to 1.16)	0.92	1.02 (0.89 to 1.17)	0.77	1.03 (0.90 to 1.19)	0.65
Charlson-Romano score, per unit change	**1.15 (1.13 to 1.17)**	**<0.0001**	**1.14 (1.12 to 1.15)**	**<0.0001**	**1.13 (1.11 to 1.15)**	**<0.0001**
Statins	0.82 (0.67 to 1.00)	0.054	0.93 (0.76 to 1.14)	0.48	0.97 (0.79 to 1.19)	0.74
Beta blockers	0.89 (0.74 to 1.09)	0.26	0.92 (0.76 to 1.14)	0.42	0.91 (0.76 to 1.12)	0.42
Diuretics	0.85 (0.70 to 1.04)	0.11	0.82 (0.67 to 1.01)	0.06	0.82 (0.67 to 1.01)	0.06
ACE inhibitor	0.88 (0.70 to1.11)	0.30	1.06 (0.84 to 1.34)	0.63	1.06 (0.84 to 1.34)	0.64
Hypertension	**1.18 (1.08 to 1.29)**	**0.0004**			1.04 (0.94 to 1.14)	0.47
Hyperlipidaemia	**0.77 (0.71 to 0.83)**	**<0.0001**			**0.77 (0.71 to 0.84)**	**<0.0001**
Tobacco use disorder	**1.40 (1.08 to 1.83)**	**0.013**			**1.79 (1.37 to 2.34)**	**<0.0001**
Coronary Artery Disease (CAD)	**1.33 (1.22 to 1.44)**	**<0.0001**			**1.11 (1.01 to 1.21)**	**0.024**
Neither allopurinol nor febuxostat	Ref		Ref		Ref	
Allopurinol	1.01 (0.93 to 1.10)	0.87	1.01 (0.93 to 1.10)	0.81	1.02 (0.93 to 1.11)	0.73
Febuxostat	0.83 (0.64 to 1.08)	0.17	0.83 (0.64 to 1.08)	0.16	0.84 (0.65 to 1.09)	0.19

Model 1=ULT use+age+gender+race+Charlson-Romano index+statins+beta blockers+diuretics+ACE inhibitor.
Model 2=model 1+CAD+hypertension+hyperlipidaemia+tobacco use disorder.
*Patients were considered exposed for 30 days after the last filled allopurinol (or febuxostat) prescription, to account for the residual biological effect and allow for use of extra supplies on hand that patients frequently have. Gaps of >30 days between prescription fills led to the start of a new allopurinol (or febuxostat) episode. If a patient had prescriptions for both drugs, then they were considered exposed to the medication that was prescribed second; for example, if a patient was taking allopurinol and got a new prescription of febuxostat, then he/she was considered to be on febuxostat only as of the febuxostat fill date.
ACE, angiotension converting enzyme; Ref, referent category.
Bold indicates statistically significant at p<0.05.

Contributors JAS designed the study, developed study protocol, reviewed analyses and wrote the first draft of the paper. JC performed the data abstraction and data analyses. Both authors made revisions to the manuscript, read and approved the final manuscript.

Funding This material is the result of work supported by research funds from the Division of Rheumatology at the University of Alabama at Birmingham and the resources and use of facilities at the Birmingham VA Medical Center, Birmingham, Alabama, USA. The funding body did not play any role in design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Disclaimer The views expressed inthis article are those of the authors and do not necessarily reflect theposition or policy of the Department of Veterans Affairs or the United Statesgovernment.

Competing interests JAS has received research grants from Takeda and Savient, and consultant fees from Savient, Takeda, Regeneron, Merz, Iroko, Bioiberica, Crealta/Horizon and Allergan pharmaceuticals, WebMD, UBM LLC and the American College of Rheumatology. JAS serves as the principal investigator for an investigator-initiated study funded by Horizon Pharmaceuticals through a grant to DINORA, a 501 (c)(3) entity. JAS is a member of the executive of OMERACT, an organisation that develops outcome measures in rheumatology and receives arm's length funding from 36 companies; a member of the American College of Rheumatology's (ACR) Annual Meeting Planning Committee (AMPC); Chair of the ACR Meet-the-Professor, Workshop and Study Group Subcommittee; and a member of the Veterans Affairs Rheumatology Field Advisory Committee. JAS is the editor and Director of the UAB Cochrane Musculoskeletal Group Satellite Center on Network Meta-analysis. JC has no conflicts to declare. There are no non-financial competing interests for any of the authors.

Patient consent The IRB waived the need for written informed consent of patients for this database study.

Ethics approval The University of Alabamaat Birmingham's Institutional Review Board approved this study and allinvestigations were conducted in conformity with ethical principles ofresearch. The IRB waived the need for written informed consent of patients forthis database study.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement These data can be obtained from the Centers for Medicare and Medicaid Services (CMS) Chronic Condition Data Warehouse. We are ready to share the data with colleagues, after obtaining appropriate permissions from the Centers for Medicare and Medicaid Services (CMS) Chronic Condition Data Warehouse and the University of Alabama at Birmingham (UAB) Ethics Committee, related to HIPAA and privacy policies.

To cite Singh JA, Cleveland JD. *Ann Rheum Dis* 2018;**77**:1243–1245.

Received 18 July 2017
Revised 16 August 2017
Accepted 19 September 2017
Published Online First 26 September 2017

Ann Rheum Dis 2018;**77**:1243–1245. doi:10.1136/annrheumdis-2017-212094

REFERENCES

1 Hong JY, Lan TY, Tang GJ, *et al*. Gout and the risk of dementia: a nationwide population-based cohort study. *Arthritis Res Ther* 2015;17:139.
2 Lu N, Dubreuil M, Zhang Y, *et al*. Gout and the risk of Alzheimer's disease: a population-based, BMI-matched cohort study. *Ann Rheum Dis* 2016;75:547–51.

3 Euser SM, Hofman A, Westendorp RG, *et al*. Serum uric acid and cognitive function and dementia. *Brain* 2009;132:377–82.
4 Johnson NB, Hayes LD, Brown K, *et al*. CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors-United States, 2005-2013. *MMWR Suppl* 2014;63:3–27.
5 Ballard C, Holmes C, McKeith I, *et al*. Psychiatric morbidity in dementia with Lewy bodies: a prospective clinical and neuropathological comparative study with Alzheimer's disease. *Am J Psychiatry* 1999;156:1039–45.
6 Singh JA, Yu S. Are allopurinol dose and duration of use nephroprotective in the elderly? A Medicare claims study of allopurinol use and incident renal failure. *Ann Rheum Dis* 2017;76:133–9.
7 National Center for Health Statistics, Office of Analysis and Epidemiology. *Analytic Issues in Using the Medicare Enrollment and Claims Data Linked to NCHS Surveys*. Hyattsville, Maryland, 2012. http://www.cdc.gov/nchs/data/datalinkage/cms_medicare_analytic_issues_final.pdf. (cited 14 Aug 2017).
8 Quan H, Li B, Saunders LD, *et al*. Assessing validity of ICD-9-CM and ICD-10 administrative data in recording clinical conditions in a unique dually coded database. *Health Serv Res* 2008;43:1424–41.
9 Taylor DH, Østbye T, Langa KM, *et al*. The accuracy of Medicare claims as an epidemiological tool: the case of dementia revisited. *J Alzheimers Dis* 2009;17:807–15.
10 Richette P, Doherty M, Pascual E, *et al*. 2016 updated EULAR evidence-based recommendations for the management of gout. *Ann Rheum Dis* 2017;76:29–42.

'Twitterland': a brave new world?

In an era of rapidly expanding digital technologies and social media (SM), considerable transformations in the delivery of healthcare are taking place. SM channels such as Facebook and Twitter represent a generation of online platforms that foster user-generated content, social interaction and real-time collaboration.[1] In this letter, we review the advantages and disadvantages of engaging with Twitter within the rheumatology field.

The ease of access to a wide range of SM platforms provides a dynamic medium for professional interaction. Twitter is gaining increasing attention by healthcare professionals as a platform for information sharing and professional networking, learning and communication. Twitter journal clubs (@RheumJC[2] and @EULAR_JC[3]) are some examples of novel educational uses of Twitter.

Despite the many positive applications of Twitter, potential hazards cannot be underestimated. The restricted character count of a tweet (140 characters) represents an important limitation, posing a risk to the content and validity of the information shared.[4] This limitation received high attention culminating in the recent doubling of the character count of tweets. Furthermore, the possibility to include links for further reading has overcome the limitations of not being able to present necessary assumptions under which the research findings hold.

The lack of knowledge on how to use SM professionally represents an unmet need.[5] Scientific Journals have dedicated teams supporting and advising on the use of SM and facilitating a target-orientated and appropriate way of using these platforms. For example, Twitter is now used by publishers to disseminate their latest or 'online first' articles (see table 1). One potential bias with journals may be the selection of more provocative/attention-grabbing articles to be publicised, which would appeal to a much wider base over perhaps more methodological/scientific articles, which have a niche appeal but are still worthy of SM dissemination. Furthermore, there are concerns regarding potential information misuse.[4] It is paramount that we take full responsibility to inform the society responsibly, minimising the risk of wrong and unreliable information being disseminated.

While the lack of control of what/how information is shared on Twitter is a limitation, the professional advantages of instant access to a wealth of information at a person's fingertips are considerable. As an example, survey data suggest a significant role of SM in knowledge acquisition by young urologists[6] highlighting the need to strive for educational content dissemination.

Although the majority of scientific accounts recognise as their target population physicians/experts in the field, one needs to remember that this information can also be easily accessed by patients and the wider community. This can be problematic due to ambiguous and misleading tweets. As with all SM, one needs to be mindful of anonymising identifiable patient data and restrict online discussions about patients.[7] The distribution of articles' lay summaries on SM is a way of informing patients of scientific work and allowing them to access a source they can understand and make use of (http://promotions.bmj.com/ardsummaries/).

We have previously demonstrated the 'power' of Twitter during key rheumatology conferences.[5] Anecdotally, this kind of interaction enables a 'virtual feel' of the scientific content of a conference remotely. However, other needs remain; for example, to understand the influence of SM on citations.[4] In this

Table 1 Advantages and disadvantages of the use of social media from the perspective of publishers

Advantages	Disadvantages
Enhances visibility for the scientific content of the journal	Imbalance of visibility across scientific content driven by individual choice and not necessarily scientific credit
Potential to showcase scientific material of excellent quality	Potential bias to select more provocative but less scientifically robust papers
Fast dissemination of information and ability to reach out the wider audience (eg, online first articles)	Information overload
Provides a channel for reaching out to the journal and its content	Benefit restricted to Twitter users
Increases the number of article downloads	Potential source of dissatisfaction if the article is not open access
Allows publishers to identify material that is relevant to the audience	Potential negative influence on publication choice by journal boards
Promotion of other journal activities (podcasts, webinars, blogs and others)	Information overload; only appealing to a select group of individuals familiar with these activities
Increases the readership and followers of the journal	Potential for scientific manuscript submission overload
Provides alternative ways to measure journal impact	Non-representative measure of scientific kudos
It provides a good and discrete means of observing 'competitor' activity	Potential for misinterpretation of external activity affecting marketing strategies
It provides an interactive platform for the engagement of the journal during scientific activities	'Manpower' is necessary to initiate and maintain the journal presence at these events
Provides novel opportunities for scientific interaction (eg, Twitter journal club)	Only relevant to a selected group 'those who use and those who understand this'
It helps to identify the target audience	Potential for misinterpretation by target audience
It can be a port of dissemination for lay summaries	Difficult to reach out to the target audience (eg, patients)

respect, where the impact of research is much broader than citation numbers, we advocate the use of additional and alternative metrics that capture impact on policy and wider stakeholders.[8]

To conclude, Twitter and other SM are clearly becoming increasingly used as a source of medical and scientific information. Despite potential caveats, these platforms will continue to provide novel ways for opinion sharing, learning and development. They are inevitably claiming a role in modern rheumatology practice and in healthcare in general.

Elena Nikiphorou,[1,2] Paul Studenic,[3] Alessia Alunno,[4] Mary Canavan,[5] Meghna Jani,[6] Francis Berenbaum[7]

[1]Department of Academic Rheumatology, King's College London, London, UK
[2]Department of Rheumatology, Whittington Hospital, London, UK
[3]Division of Rheumatology, Department of Internal Medicine, Medical University Vienna, Vienna, Austria
[4]Rheumatology Unit, Department of Medicine, University of Perugia, Perugia, Italy
[5]Department of Molecular Rheumatology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland
[6]School of Biological Sciences, Faculty of Biology, Medicine and Health, Arthritis Research UK Centre for Epidemiology, Centre for Musculoskeletal Research, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK
[7]Department of Rheumatology, Inflammation–Immunopathology–Biotherapy Department, Pierre and Marie Curie University, Saint-Antoine hospital, Sorbonne University, Paris, France

Correspondence to Dr Elena Nikiphorou, Academic Rheumatology Department, King's College London, London, UK; enikiphorou@gmail.com

Handling editor Tore K Kvien

Twitter @ElenaNikiUK

Contributors EN conceived the idea for this article and produced a first draft. All coauthors critically reviewed and revised the first draft producing subsequent drafts. All authors approved the final version of the article before submission.

Competing interests All authors have supported the Annals of Rheumatic Diseases (ARD) in social media activities relating to dissemination of educational and scientific content. EN, PS, AA, MC and MJ are part of ARD's social media advisory team.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Nikiphorou E, Studenic P, Alunno A, *et al*. *Ann Rheum Dis* 2018;**77**:1245–1246.

Received 23 August 2017
Revised 15 November 2017
Accepted 19 November 2017
Published Online First 4 December 2017

Ann Rheum Dis 2018;**77**:1245–1246. doi:10.1136/annrheumdis-2017-212273

REFERENCES

1 George DR, Rovniak LS, Kraschnewski JL. Dangers and opportunities for social media in medicine. *Clin Obstet Gynecol* 2013;56:453–62.
2 #RheumaJC. Impact of Sustained Remission on the Risk of Serious Infection in Patients With Rheumatoid Arthritis. http://rheumjc.com/
3 Emeunet. EULAR – EMEUNET Journal Club. http://emeunet.eular.org/emeunet_journal_club.cfm
4 Bukhari M, Galloway J. Twitter, #alternativefacts, careless whispers and Rheumatology. *Rheumatology* 2017. doi: 10.1093/rheumatology/kex091.[Epub ahead of print 29 Mar 2017].
5 Nikiphorou E, Studenic P, Ammitzbøll CG, *et al*. Social media use among young rheumatologists and basic scientists: results of an international survey by the Emerging EULAR Network (EMEUNET). *Ann Rheum Dis* 2017;76:712–5.
6 Rivas JG, Socarras MR, Patruno G, *et al*. Perceived role of social media in urologic knowledge acquisition among young urologists: a european survey. *Eur Urol Focus* 2017. doi: 10.1016/j.euf.2016.11.010. [Epub ahead of print 27 Jul 2017].
7 Rimmer A. Hidden risks your smartphone poses to your career. *BMJ* 2017;359:j4896.
8 Almetric. Who's talking about your research? https://www.altmetric.com/

Discussion of Methotrexate Dosage

We thank Safy *et al*[1] for their recent article discussing clinical outcomes in early treatment of rheumatoid arthritis with methotrexate and 10 mg daily of prednisolone versus methotrexate alone. This was a post-trial follow-up of the CAMERA II trial, which monitored for radiographic evidence of disease progression, use of biologic disease-modifying antirheumatic drugs (DMARDs) and incidence of glucocorticoid comorbidities. We appreciate the work that went into the review of up to 11 years worth of data; however, we feel there are outstanding issues worth discussion.

It was noted with interest that despite the careful collection of data for the follow-up analysis, there was no description of methotrexate dosage in either study group. Are we to assume dosages were comparable between the two groups? If so, what were the median doses of methotrexate? Previous research has shown improved clinical outcomes from using intensive methotrexate treatment strategies with rapid dose increase[2] as compared with lower induction doses and slower titration regimes. For this reason, information about the methotrexate dosed must be available prior to conclusions about the additional benefit of steroid being drawn from the data presented.

As this study collected data over an 11-year period, it should be acknowledged that trends in methotrexate prescribing has significantly changed over this time period. Current European League Against Rheumatism (EULAR) guidelines advise the use of methotrexate in doses up to 25–30 mg per week.[3]

This study had a number of merits, which we read with interest. This is the first study to examine potential rebound of disease activity following weaning of prednisolone and commencement of bDMARDs. Although Safy *et al* demonstrated lower use of bDMARDs in the patient population studied, we would question if these findings were affected by the close monitoring of disease activity using a treat to target approach as opposed to the use of prednisolone.

Sinead A Maguire,[1] Claire Marie Sheehy[2]

[1]Department of Rheumatology, University Hospital Waterford, Waterford, Ireland
[2]Department of Rheumatology, Waterford Regional Hospital, Waterford, Ireland

Correspondence to Dr Sinead A Maguire, Department of Rheumatology SPR, University Hospital Waterford, Waterford, Ireland; sineadmaguire@rcsi.ie

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Maguire SA, Sheehy CM. *Ann Rheum Dis* 2018;**77**:e47.

Received 8 September 2017
Accepted 11 September 2017
Published Online First 22 September 2017

► http://dx.doi.org/10.1136/annrheumdis-2017-212380

Ann Rheum Dis 2018;**77**:e47. doi:10.1136/annrheumdis-2017-212358

REFERENCES

1 Safy M, Jacobs J, IJff ND, *et al*. Long-term outcome is better when a methotrexate-based treatment strategy is combined with 10 mg prednisone daily: follow-up after the second Computer-Assisted Management in Early Rheumatoid Arthritis trial. *Ann Rheum Dis* 2017;76:1432–5.
2 Verstappen SM, Jacobs JW, van der Veen MJ, *et al*. Intensive treatment with methotrexate in early rheumatoid arthritis: aiming for remission. Computer Assisted Management in Early Rheumatoid Arthritis (CAMERA, an open-label strategy trial). *Ann Rheum Dis* 2007;66:1443–9.
3 Smolen JS, Landewé R, Breedveld FC, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;73:492–509.

Response to eLetter: 'Discussion of methotrexate dosage' by Maguire *et al*

In their letter to the editor 'Discussion of Methotrexate Dosage', Maguire *et al*[1] raised three issues regarding our recent paper.[2] We appreciate their interest in our study and will address these issues here.

First, regarding the methotrexate dosage, we reiterate what we have discussed in the discussion section of our paper: 'As in the post-trial follow-up period, the controlled situation was lost and treatment was open to the rheumatologists, long-term outcomes in our study may have been influenced by the use of different antirheumatic drugs'. We did not systematically record the methotrexate dosages during the post-trial follow-up period after the Computer Assisted Management in Early Rheumatoid Arthritis (CAMERA)-II trial. However, since all patients were treated to target during the post-trial follow-up period, we see no convincing argument to assume that this lack would disqualify our findings.

Next, Maguire *et al* raised the issue that it should be acknowledged that trends in methotrexate prescribing have significantly changed over the 11-year study period and that current European League Against Rheumatism (EULAR) guidelines advise the use of methotrexate in doses up to 25–30 mg per week. Importantly, already in our first CAMERA trial, which was published in 2007 and conceived several years before, the maximum dose of 30 mg methotrexate per week was applied.[3] Also in CAMERA-II and its post-trial follow-up, we applied the maximum dose of 30 mg,[4] which is still recommended in the newest EULAR guidelines.[5] Finally, Maguire *et al* questioned if the lower use of biological disease-modifying antirheumatic drugs (bDMARDs) observed in the former methotrexate and prednisone compared with the methotrexate and placebo treatment strategy group was affected by the close monitoring of disease activity utilising a treat to target approach as opposed to the use of prednisone.

Of course, a tight control regime applying the full range of dosing of methotrexate and of other conventional synthetic DMARDs could be bDMARD sparing, compared with less strict regimes.

However, in the CAMERA-II trial, both treatment strategy groups were tightly controlled. In the post-trial follow-up period, all patients were treated to target; so the difference in outcome between the two groups can only be ascribed to the only difference between the two groups, which is the use of prednisone or placebo during the study period, tapered off and stopped in most patients during post-trial follow-up.

We thus hope to have answered the issues raised by Maguire *et al* satisfyingly.

Mary Safy, J W G Jacobs, Nicole D Ijff, J W J Bijlsma, J M van Laar, Maria J H de Hair, Society for Rheumatology Research Utrecht (SRU)

Department of Rheumatology and Clinical Immunology, University Medical Center, Utrecht, the Netherlands

Correspondence to Mary Safy, Department of Rheumatology and Clinical Immunology, F.02.127, University Medical Center Utrecht, Box 85500, 3508 GA, Utrecht, the Netherlands; m.safy@umcutrecht.nl

Competing interests JMvL received honoraria from MSD, Roche, Pfizer, BMS, Eli Lilly. JWJB received honoraria from AbbVie, BMS, MSD, Pfizer, Roche, SUN, UCB. MS was supported by a research grant from Astra Zeneca. Astra Zeneca was not involved in this study.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Safy M, Jacobs JWG, Ijff ND, *et al*. *Ann Rheum Dis* 2018;**77**:e48.

Received 1 October 2017
Accepted 2 October 2017
Published Online First 9 October 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212358

Ann Rheum Dis 2018;**77**:e48. doi:10.1136/annrheumdis-2017-212380

REFERENCES

1 Maguire SA, Sheehy CM. Discussion of methotrexate dosage. *Ann Rheum Dis* 2018;77:e47.
2 Safy M, Jacobs J, IJff ND, *et al*. Long-term outcome is better when a methotrexate-based treatment strategy is combined with 10 mg prednisone daily: follow-up after the second computer-assisted management in early rheumatoid arthritis trial. *Ann Rheum Dis* 2017;76:1432–5.
3 Verstappen SM, Jacobs JW, van der Veen MJ, *et al*. Intensive treatment with methotrexate in early rheumatoid arthritis: aiming for remission. Computer assisted management in early rheumatoid arthritis (CAMERA, an open-label strategy trial). *Ann Rheum Dis* 2007;66:1443–9.
4 Bakker MF, Jacobs JW, Welsing PM, *et al*. Low-dose prednisone inclusion in a methotrexate-based, tight control strategy for early rheumatoid arthritis: a randomized trial. *Ann Intern Med* 2012;156:329–39.
5 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.

Risk of invasive melanoma in patients with rheumatoid arthritis treated with biologics: an updated meta-analysis

We read with interest the report by Mercer *et al* in the February 2017 issue of *ARD*[1] which examined the risk of invasive melanoma in patients with rheumatoid arthritis (RA) treated with biologics. In a collaborative analysis of data from nine European countries (11 biologics registers), the authors report a pooled standardised incidence ratio (SIR) for tumour necrosis factor inhibitor (TNFi)-exposed patients of 1.2 (95% CI 0.99 to 1.6) compared with the general population (country specific) and an incidence rate ratio (IRR) of 1.1 (0.8–1.6) for TNFi-exposed patients compared with biologic naive patients. While they allowed that an increased melanoma risk in patients with RA treated with TNFi could not be ruled out completely, the authors concluded that the previous signal of an increased risk of melanoma reported by the Swedish[2] and Danish[3] registries was not confirmed.

Just prior to the publication of this report, we had conducted a systematic review of observational studies reporting the risk of melanoma in patients with RA treated with TNFi and found a 90% increased risk compared with the general population (pooled SIR 1.9 (95% CI 1.5 to 2.3); n=6 studies) and a 60% increased risk compared with patients treated with non-biologic disease-modifying anti-rheumatic drugs (nbDMARDS) (pooled IRR 1.6 (95% CI 1.2 to 2.2); n=5 studies).[4]

We have now repeated our meta-analysis including the estimates from the European collaborative project. Data from two European registries included in the analysis by Mercer *et al*[1] had been included in our meta-analysis,[2 3 5] and so we removed the previously published estimates from our analyses to avoid double counting.

Our updated meta-analysis showed an attenuated association, but the pooled SIR for TNFi-exposed patients compared with the general population remained significant (1.7 (95% CI 1.2 to 2.3)), with evidence of heterogeneity (Phet 0.04). The updated pooled IRR for TNFi-exposed patients compared with biologic naive patients was 1.4 (95% CI 0.8 to 2.3) with no significant heterogeneity (Phet 0.18).

Thus, the aggregated evidence from observational studies supports a 70% increased risk of melanoma in patients with RA treated with TNFi in comparison with the general population, and it remains possible that patients with RA treated with TNFi are at a raised risk of melanoma in comparison with patients treated with nbDMARDS given the wide 95% CI around the raised IRR.

We note that a second report from the same collaborative study observed similarly raised risks for patients with TNFi-exposed spondyloarthritis compared with both the general population and TNFi-naive patients (SIR 1.3 (95% CI 0.7 to 2.3) and 1.4 (95% CI 0.7 to 2.6), respectively), while patients with TNFi-naive spondyloarthritis did not have an elevated risk in comparison with the general population.[6]

Melanomas are highly immunogenic tumours, with a higher mutation load and consequent production of more neoantigens[7] than other cancers. In light of the evolving immune-modulatory treatments for melanoma itself, further data from prospective studies of patients with RA treated with TNFi are required. The same premise applies to other patient populations treated with TNFi. We therefore recommend a cautious interpretation of the currently available data which cannot be viewed as reassuring. We further recommend that meta-analyses on this topic be updated frequently to include new evidence as it becomes available.

Catherine M Olsen,[1] Adele C Green[2,3]

[1]Cancer Control Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4029, Australia
[2]Cancer and Population Studies Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4029, Australia
[3]Cancer Research UK Manchester Institute and Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom

Correspondence to Catherine M Olsen, Cancer Control Group, QIMR Berghofer Medical Research Institute, Brisbane, Queensland 4029, Australia; Catherine.Olsen@qimrberghofer.edu.au

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Olsen CM, Green AC. *Ann Rheum Dis* 2018;**77**:e49.

Received 9 August 2017
Accepted 13 August 2017
Published Online First 22 August 2017

Ann Rheum Dis 2018;**77**:e49. doi:10.1136/annrheumdis-2017-212205

REFERENCES

1 Mercer LK, Askling J, Raaschou P, *et al*. Risk of invasive melanoma in patients with rheumatoid arthritis treated with biologics: results from a collaborative project of 11 European biologic registers. *Ann Rheum Dis* 2017;76:386–91.
2 Raaschou P, Simard JF, Holmqvist M, *et al*. Rheumatoid arthritis, anti-tumour necrosis factor therapy, and risk of malignant melanoma: nationwide population based prospective cohort study from Sweden. *BMJ* 2013;346:f1939.
3 Dreyer L, Mellemkjær L, Andersen AR, *et al*. Incidences of overall and site specific cancers in TNFα inhibitor treated patients with rheumatoid arthritis and other arthritides – a follow-up study from the DANBIO Registry. *Ann Rheum Dis* 2013;72:79–82.
4 Olsen CM, Hyrich KL, Knight LL, *et al*. Melanoma risk in patients with rheumatoid arthritis treated with tumour necrosis factor alpha inhibitors: a systematic review and meta-analysis. *Melanoma Res* 2016;26:517–23.
5 Askling J, Fored CM, Brandt L, *et al*. Risks of solid cancers in patients with rheumatoid arthritis and after treatment with tumour necrosis factor antagonists. *Ann Rheum Dis* 2005;64:1421–6.
6 Hellgren K, Dreyer L, Arkema EV, *et al*. Cancer risk in patients with spondyloarthritis treated with TNF inhibitors: a collaborative study from the ARTIS and DANBIO registers. *Ann Rheum Dis* 2017;76:105–11.
7 McArthur GA, Ribas A. Targeting oncogenic drivers and the immune system in melanoma. *J Clin Oncol* 2013;31:499–506.

Antisynthetase syndrome or what else? Different perspectives indicate the need for new classification criteria

We read with great interest the extended report by Lilleker *et al*[1] on the EuroMyositis registry. Our attention was particularly addressed to antisynthetase syndrome (ASSD) because in the last years we and all members of the AENEAS (American and European Network of Antisynthetase Syndrome) collaborative group strongly contributed[2–7] to increase the knowledge on this peculiar disease.

We think that a comparison between our cohorts could be of interest and useful for clinicians, even if the lack of some data in the EuroMyositis paper does not allow us to perform statistical analysis. Both groups collected a very large number of patients (AENEAS collaborative group 813 cases, EuroMyositis 512 cases). The comparison of available data seems to indicate that in the AENEAS and in the EuroMyositis cohort, patients' age at disease onset (mean±SD: 51±14 vs 48±15 years), female sex (74% vs 69%), Raynaud's phenomenon (RP) (44% vs 51%) and mechanic's hands (37% vs 38%) prevalence are similar, whereas interstitial lung diseases (82% vs 71%) and, in particular, arthritis (68% vs 51%) seem to be more common in our cohort. However, these differences are intrinsic to our different politics: muscle involvement is the more common reason for patients' inclusion in the EuroMyositis registry, whereas muscle involvement is not mandatory for the inclusion in our registry. Thanks to this choice, we showed that muscle involvement is not the most frequent onset finding in ASSD.[2–4 6] In fact, in our cohort, 381 patients (47%) had no muscle involvement at disease onset and 186 (23%) were without muscle involvement and also without accompanying findings (RP, cutaneous manifestations and fever). On the other hand, we also showed that the occurrence of ex novo clinical findings during the follow-up is a typical hallmark of ASSD.[2–6] However, the main problem that involves all groups working on ASSD is the lack of well-established clinicoserological classification criteria. This is not a secondary issue because patients with ASSD could be classified also as interstitial pneumonia with autoimmune features[3] or, by considering clinical characteristics,[4 6 8] as rheumatoid arthritis (RA),[9] thus potentially entering in clinical trials addressed to other conditions. Also, the heterogeneity of commercially available testing tools, and the limited use of the gold-standard methodology, immunoprecipitation (IP), for antisynthetase antibodies (ARS) determination, add further variability in disease definition. In particular, IP is able to identify ARS positivity also when commercially available kits are negative.[10] These considerations, together with the possible occurrence of other up to now not recognised ARS,[11] suggest that the practice of defining ASSD based on simple positivity or negativity of these antibodies may lead to patients' misclassification. In fact, we need classification criteria based on differential weights for various clinical, pathological and serological variables, such as that developed by the American College of Rheumatology and European League Against Rheumatism for RA.[9] We think that this result will be achieved by the assessment of the steadily increasing number of patients with ASSD included in most recent reports. Thanks to the closer collaboration among the centres and groups interested in ASSD, the scientific community is now ready to work together to develop these much needed classification criteria.

Lorenzo Cavagna,[1] Santos Castañeda,[2,3] Carlo Sciré,[4] Miguel A Gonzalez-Gay,[5] On Behalf of the AENEAS Collaborative Group Members

[1]Department of Rheumatology, University and IRCCS Foundation Policlinico S. Matteo, Pavia, Italy
[2]Department of Rheumatology, Hospital Universitario La Princesa, Madrid, Spain
[3]Department of Rheumatology, FJD, Madrid, Spain
[4]Dipartimento di Scienze Mediche, Universita degli Studi di Ferrara, Ferrara, Emilia-Romagna, Italy
[5]Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, Santander, Cantabria, Spain

Correspondence to Dr Lorenzo Cavagna, Departement of Rheumatology, University and IRCCS Foundation Policlinico S. Matteo, Pavia 27100, Italy; lorenzo.cavagna@unipv.it

Acknowledgements The authors thank all AENEAS collaborative group members for their crucial support, work and collaboration in data collection and research aspects.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Obtained.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Cavagna L, Castañeda S, Sciré C, *et al*. *Ann Rheum Dis* 2018;**77**:e50.

Received 11 September 2017
Accepted 11 September 2017
Published Online First 25 September 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212382

Ann Rheum Dis 2018;**77**:e50. doi:10.1136/annrheumdis-2017-212368

REFERENCES

1 Lilleker JB, Vencovsky J, Wang G, *et al*. The EuroMyositis registry: an international collaborative tool to facilitate myositis research. *Ann Rheum Dis* 2018;77:30–9.
2 Cavagna L, Nuño L, Scirè CA, *et al*. Clinical spectrum time course in anti Jo-1 positive antisynthetase syndrome: results from an international retrospective multicenter study. *Medicine* 2015;94:e1144.
3 Scirè CA, Gonzalez-Gay MA, Selva-O'Callaghan A, *et al*. Clinical spectrum time course of interstitial pneumonia with autoimmune features in patients positive for antisynthetase antibodies. *Respir Med* 2017 (accessed 31 Mar 2017)
4 González-Gay MA, Montecucco C, Selva-O'Callaghan A, *et al*. Timing of onset affects arthritis presentation pattern in antisyntethase syndrome. *Clin Exp Rheumatol* 2017 (accessed 26 Jul 2017)
5 Bartoloni E, Gonzalez-Gay MA, Scirè C, *et al*. Clinical follow-up predictors of disease pattern change in anti-Jo1 positive anti-synthetase syndrome: results from a multicenter, international and retrospective study. *Autoimmun Rev* 2017;16:253–7.
6 Cavagna L, Nuño L, Scirè CA, *et al*. Serum Jo-1 autoantibody and isolated arthritis in the antisynthetase syndrome: review of the literature and report of the experience of AENEAS collaborative group. *Clin Rev Allergy Immunol* 2017;52:71–80.
7 Monti S, Montecucco C, Cavagna L. Clinical spectrum of anti-Jo-1-associated disease. *Curr Opin Rheumatol* 2017:1 (accessed 8 Aug 2017)
8 Cavagna L, Monti S, Grosso V, *et al*. The multifaceted aspects of interstitial lung disease in rheumatoid arthritis. *Biomed Res Int* 2013;2013:1–13.
9 Aletaha D, Neogi T, Silman AJ, *et al*. rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;2010:1580–8.
10 Noguchi E, Uruha A, Suzuki S, *et al*. Skeletal muscle involvement in antisynthetase syndrome. *JAMA Neurol* 2017;74:992–9.
11 Ibba M, Soll D. Aminoacyl-tRNA synthesis. *Annu Rev Biochem* 2000;69:617–50.

Response to: 'Antisynthetase syndrome or what else? Different perspectives indicate the need for new classification criteria' by Cavagna *et al*

We thank Cavagna *et al*[1] for their thoughtful analysis relating to our recent publication.[2] Several important points are raised, many of which we also referred to. It is of great interest and reassurance that the demographics and clinical features of antisynthetase syndrome (ASS) are broadly similar between the AENEAS (American and European NEtwork of Anti-Synthetase syndrome) and EuroMyositis cohorts. Clearly the frequency of interstitial lung disease and arthritis differs due to the sources of case ascertainment. This highlights the importance when evaluating the natural history and demographics of a disease presenting with heterogeneity that one must use more than one source of case ascertainment.

The paradigm of classification of the idiopathic inflammatory myopathies (IIM), particularly with regard to ASS, is a subject of much debate. This reflects our developing understanding of these disorders and the growing agreement regarding the importance of autoantibody status in determining a phenotype. One unanswered question relates to the heterogeneity within the ASS spectrum and the underlying mechanisms that explain why certain antisynthetase antibodies (ASAs) are associated with certain clinical phenotypes. We also agree that if the shift towards disease definition according to clinicoserological syndrome is to continue, harmonisation of antibody testing methodologies is required. However, exceptions are required for patients displaying typical clinical features of ASS, but without a detectable ASAs, in some cases almost certainly the consequence of the presence of a hitherto unrecognised antibody or the limitations of current antibody testing methodologies.

To develop a well-rounded knowledge of ASS, future collaboration across multiple disease specialities is vital. Together with other groups such as the International Myositis Assessment and Clinical Studies Group (https://www.niehs.nih.gov/research/resources/imacs/index.cfm) and the European Reference Network for Rare Diseases, future standardised classification criteria will be developed. We agree that the presence of skeletal muscle inflammation should not be a prerequisite for the diagnosis of ASS and that labelling such patients as having an IIM is becoming less intuitive. More accurate clinicoserological criteria will allow more homogeneous participant groups in research studies, and in the future allow for more targeted therapies.

We look forward to broadening the existing collaboration between our groups, facilitated through the recently awarded Foundation for Research in Rheumatology grant (http://www.foreum.org/prg_13_myositis_transition.cfm). It is only with such collaborative efforts that we can advance our search for a more accurate diagnosis and better treatment options in the future.

James B Lilleker,[1,2] Jiri Vencovsky,[3] Guochun Wang,[4] Lucy R Wedderburn,[5] Louise P Diederichsen,[6] Jens Schmidt,[7] Paula Jordan,[8] Olivier Benveniste,[9] Maria Giovanna Danieli,[10] Katalin Dankó,[11] Nguyen Thi Phuong Thuy,[12] Monica Vázquez-Del Mercado,[13] Helena Andersson,[14] Boel De Paepe,[15] Jan L De Bleecker,[15] Britta Maurer,[16] Liza J McCann,[17] Nicolo Pipitone,[18] Neil McHugh,[19,20] Zoe Betteridge,[19,20] Paul New,[21] Robert G Cooper,[21,22] William E Ollier,[22] Janine A Lamb,[22] Niels Steen Krogh,[23] Ingrid E Lundberg,[24] Hector Chinoy,[25,26] On behalf of all EuroMyositis contributors

[1]Division of Musculoskeletal and Dermatological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, Centre for Musculoskeletal Research, School of Biological Sciences, The University of Manchester, Manchester, UK
[2]Greater Manchester Neurosciences Centre, Salford Royal NHS Foundation Trust, Stott Lane, UK
[3]Institute of Rheumatology, Prague, Czech Republic
[4]Department of Rheumatology, China-Japan Friendship Hospital, Beijing, China
[5]University College London GOS Institute of Child Health and NIHR GOSH Biomedical Research Centre, Great Ormond Street Hospital for Children NHS Trust, London, UK
[6]Department of Rheumatology, Odense University Hospital, Odense, Denmark
[7]Department of Neurology, University Medical Center Göttingen, Göttingen, Germany
[8]Myositis UK, Southampton, UK
[9]Département de Médecine Interne et Immunologie Clinique, Hôpital Pitié-Salpêtrière, AP-HP, UPMC, Paris, France
[10]Dipartimento di Scienze Cliniche e Molecolari, Clinica Medica, Università Politecnica delle Marche and Ospedali Riuniti, Ancona, Italy
[11]Division of Immunology, University of Debrecen, Debrecen, Hungary
[12]Department of Rheumatology, Bach Mai Hospital, Hanoi Medical University, Hanoi, Vietnam
[13]División de Medicina Interna, Servicio de Reumatología, PNPC 004086, CONACyT, Hospital Civil Dr Juan I Menchaca, Guadalajara, Jalisco, Salvador Quevedo y Zubieta S/N, Guadalajara, Mexico
[14]Department of Rheumatology, Oslo University Hospital, Oslo, Norway
[15]Department of Neurology, Ghent University Hospital, Ghent, Belgium
[16]Department of Rheumatology, University Hospital Zurich, Zurich, Switzerland
[17]Department of Rheumatology, Alder Hey Children's NHS Foundation Trust, Liverpool, UK
[18]Department of Rheumatology, Arcispedale S Maria Nuova-IRCCS of Reggio Emilia, Reggio Emilia, Italy
[19]Royal National Hospital for Rheumatic Diseases, Royal United Hospitals Bath, Bath, UK
[20]Department of Pharmacy and Pharmacology, University of Bath, Bath, UK
[21]MRC-ARUK Institute for Ageing and Chronic Disease, University of Liverpool, Liverpool, UK
[22]Division of Population Health, Health Services Research and Primary Care, Faculty of Biology, Medicine and Health, School of Health Sciences, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK
[23]ZiteLab ApS, Frederiksberg, Denmark
[24]Unit of Rheumatology, Department of Medicine, Karolinska University Hospital, Solna, Karolinska Institutet, Stockholm, Sweden
[25]Rheumatology Department, Salford Royal NHS Foundation Trust, Manchester Academic Health Science Centre, Salford, UK
[26]The National Institute for Health Research Manchester Musculoskeletal Biomedical Research Unit, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK

Correspondence to Dr Hector Chinoy, Centre for Musculoskeletal Research, The University of Manchester, Manchester M13 9PT, UK; hector.chinoy@manchester.ac.uk

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Lilleker JB, Vencovsky J, Wang G, *et al*. *Ann Rheum Dis* 2018;**77**:e51.

Received 22 September 2017
Revised 29 November 2017
Accepted 3 December 2017
Published Online First 19 December 2017

► http://dx.doi.org/10.1136/annrheumdis-2017-212368

Ann Rheum Dis 2018;**77**:e51. doi:10.1136/annrheumdis-2017-212382

REFERENCES

1 Cavagna L, Castañeda S, Sciré C, *et al*. Antisynthetase syndrome or what else? Different perspectives indicate the need for new classification criteria. *Ann Rheum Dis* 2018;77:e50.
2 Lilleker JB, Vencovsky J, Wang G, *et al*. The EuroMyositis registry: an international collaborative tool to facilitate myositis research. *Ann Rheum Dis* 2018;77:30–9.

Obesity and CRP

I commend the authors for this very important study.[1] There has been an association between obesity and C-reactive protein (CRP) levels. USA has a higher obesity rate than Europe and not infrequently we see obese women for fibromyalgia like symptoms with elevated CRP, even greater than the 30 mg/dL cut-off in the study. It would be interesting to know what were the weights of the inflammation of unknown origin subjects in this study and if weight had any relation to the CRP levels? Also, if there were obese patients in the study with elevated CRP levels, did their scans reveal any findings, even if non-specific?

Fawad Aslam

Division of Rheumatology, Mayo Clinic, Scottsdale, Arizona, USA

Correspondence to Dr Fawad Aslam, Division of Rheumatology, Mayo Clinic, Scottsdale, Arizona, USA; aslam.fawad@mayo.edu

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Aslam F. *Ann Rheum Dis* 2018;**77**:e52.

Received 22 September 2017
Accepted 27 September 2017
Published Online First 4 October 2017

► http://dx.doi.org/10.1136/annrheumdis-2017-212520

Ann Rheum Dis 2018;**77**:e52. doi:10.1136/annrheumdis-2017-212425

REFERENCE

1 Schönau V, Vogel K, Englbrecht M, *et al*. The value of (18)F-FDG-PET/CT in identifying the cause of fever of unknown origin (FUO) and inflammation of unknown origin (IUO): data from a prospective study. *Ann Rheum Dis* 2018;77:70–7.

Response to: the value of 18(F)-FDG-PET/CT in identifying the cause of fever of unknown origin (FUO) and inflammation of unknown origin (IUO): data from a prospective study

We thank Dr Aslam for his thoughtful comments[1] regarding our article entitled 'The value of 18F-FDG-PET/CT in identifying the cause of fever of unknown origin (FUO) and inflammation of unknown origin (IUO): data from a prospective study'.[2] Dr Aslam mentions that due to increasing prevalence of obesity in the industrialised world, average C reactive protein levels rise merely because of the increased volume of adipose tissue in the body, even in the absence of concomitant inflammatory disease. This association has been supported by epidemiological studies[3] and is likely based on the proinflammatory role of adipose tissue, which represents a source for proinflammatory mediators such as cytokines and adipokines.[4] Hence, obese individuals could have an elevated C reactive protein without underlying inflammatory disease complicating diagnostics.

In our cohort, body weight ranged from 49 kg to 131 kg, with a body mass index (BMI) range of 17.0–45.9. This range was very similar in the group of patients with inflammation of unknown origin group (IUO; 51–131 kg; BMI 17.0–44.6). The average body weight in the IUO group was 74.1 kg and the average BMI was 25.6, which is rather low and per definition exactly at transition between normal body weight and overweight. Only 19 (13.3%) patients were obese, defined as BMI >30 kg/m^2. Out of these 19 patients, only 2 (1.4%) had a BMI between 35 and 40 kg/m^2 (obesity class II), and only 3 (2.1%) had a BMI of more than 40 kg/m^2 (obesity class III). The characteristics of the five patients with obesity class II and III are shown in table 1.

In obese patients with a BMI >30 kg/m^2, we had 7 patients (of a total of 19) where positron emission tomography (PET)-CT was not helpful in establishing a diagnosis. Two are already listed in table 1. The other five patients are shown in table 2.

Taken together, only two obese patients (P5 and P8) with IUO remained with no specific diagnosis and had a non-diagnostic PET-CT. In these two patients it cannot be excluded that inflammation was caused by obesity. Overall the characteristics of a rather lean IUO population with an average BMI of 25.6 suggest that C reactive protein elevation due to obesity is not a major issue in this cohort. Nonetheless, the situation may be different in a population with high prevalence of obesity, where inflammation/'IUO' may occur more frequently due to cytokine and adipokines production from adipose tissue. This situation is also diagnostically challenging as one can hardly rely that obesity is the only reason for elevated systemic inflammation markers unless the presence of underlying disease has been thoroughly ruled out.

Verena Schönau, Georg Schett

Department of Internal Medicine 3 and Institute for Clinical Immunology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Correspondence to Professor Georg Schett, Department of Internal Medicine 3, Rheumatology and Immunology, University Clinic of Erlangen-Nuremberg, Erlangen; Ulmenweg 18, 91054 Erlangen, Germany; georg.schett@uk-erlangen.de

Contributors VS and GS analysed the data and wrote the letter.

Competing interests None declared.

Patient consent Obtained.

Ethics approval University Clinic Erlangen.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Schönau V, Schett G. *Ann Rheum Dis* 2018;**77**:e53.

Received 12 October 2017
Revised 29 October 2017
Accepted 30 October 2017
Published Online First 16 November 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212425

Ann Rheum Dis 2018;**77**:e53. doi:10.1136/annrheumdis-2017-212520

REFERENCES

1 Aslam F. Obesity and CRP. *Ann Rheum Dis* 2017.
2 Schönau V, Vogel K, Englbrecht M, *et al*. The value of (18)F-FDG-PET/CT in identifying the cause of fever of unknown origin (FUO) and inflammation of unknown origin (IUO): data from a prospective study. *Ann Rheum Dis* 2017.
3 Visser M, Bouter LM, McQuillan GM, *et al*. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999;282:2131–5.
4 Reilly SM, Saltiel AR. Adapting to obesity with adipose tissue inflammation. *Nat Rev Endocrinol* 2017;13:633–43.

Table 1 Characteristics of IOU patients with class II or III obesity

Number	Diagnosis	CRP (mg/L)	Weight (kg)	BMI (kg/m^2)	PET helpful	PET finding
P1 (♂)	Endocarditis	50.0	112	35.3	No	No pathological FDG uptake
P2 (♀)	Femoral head necrosis and coxitis	98.8	96	35.6	Yes	Coxitis and bursitis
P3 (♀)	Polymyalgia rheumatica	76.0	97	41.0	Yes	Bursitis trochanterica
P4 (♀)	Metastatic breast cancer	39.0	131	44.3	Yes	Multiple pleural and bone lesions
P5 (♀)	IUO (no diagnosis)	32.0	126	44.6	No	Diffuse bone marrow activation

BMI, body mass index; CRP, C reactive protein; FDG, fluorodeoxyglucose; IUO, inflammation of unknown origin; PET, positron emission tomography.

Table 2 Characteristics of IOU patients with obesity and not helpful PET-CT

	Diagnosis	CRP (mg/L)	Weight (kg)	BMI (kg/m^2)	PET helpful	PET finding
P6 (♂)	Erythema nodosum	92.7	94	30.3	No	Axillary lymph nodes and bone marrow
P7 (♂)	Undifferentiated connective tissue disease	76.2	85	31.2	No	No pathological FDG uptake
P8 (♀)	IUO (no diagnosis)	34.2	89	32.7	No	Patchy FDG uptake in the liver
P9 (♂)	Polymyalgia rheumatica	50.1	105	34.3	No	No pathological FDG uptake
P10 (♂)	Autoimmune hepatitis	38.1	99	34.3	No	Minimal mesenterial FDG uptake

BMI, body mass index; CRP, C reactive protein; FDG, fluorodeoxyglucose; IUO, inflammation of unknown origin; PET, positron emission tomography.

Chondroitin sulfate is superior to placebo in symptomatic knee osteoarthritis

I read with great interest the article by Reginster and colleagues[1] regarding the effectiveness of chondroitin sulfate (CS) in the treatment of symptomatic knee osteoarthritis (OA). This randomised, double-blind trial demonstrated that pharmaceutical-grade CS is superior to placebo and equivalent to celecoxib in reducing pain and improving function over 6 months in patients with symptomatic knee OA, indicating that this formulation of CS should be considered a first-line treatment in the management of knee OA1. The results of Reginster *et al* are in agreement with a recent systematic review conducted by the Cochrane Group, which showed that CS, alone or in combination with GS, is better than placebo in improving pain in patients with OA, as reported in short-term studies.[2] However, there are some noteworthy issues in this regard. First, the duration of the study for the symptomatic treatment of knee OA is relatively short. In addition, published studies regarding the efficacy of CS in knee OA beyond the 6-month duration are sparse. Long-term prospective studies, ideally those performed over 2 or 3 years or more, are warranted.[3] Second, CS is most commonly used in combination with glucosamine sulfate (GS) to treat OA. However, a recent randomised, double-blind, placebo-controlled study has failed to demonstrate the superiority of CS/GS combination therapy over placebo in terms of reducing joint pain and functional impairment in patients with symptomatic knee OA over 6 months.[4] Third, CS is available as pharmaceutical-grade and nutraceutical-grade products. The results of this study were obtained using prescription drugs containing highly purified CS produced by pharmaceutical companies. Since nutraceutical-grade products are known to show marked variations in their preparation, composition, content and purity, this result should not be extrapolated to nutraceutical-grade products. Although I respect the work done by the authors, I am unsure whether the use of CS in routine clinical practice should be encouraged. Considering that medication for OA is usually taken for a long time, the findings of this study must be confirmed by a long-term trial. I am looking forward to further evaluation to clarify the efficacy of this agent in long-term trials.

Young Ho Lee

Correspondence to Professor Young Ho Lee, Division of Rheumatology, Korea University Medical Center, Seoul, Republic of Korea; lyhcgh@korea.ac.kr

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Lee YH. *Ann Rheum Dis* 2018;**77**:e54.

Received 27 September 2017
Accepted 28 September 2017
Published Online First 9 October 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212460

Ann Rheum Dis 2018;**77**:e54. doi:10.1136/annrheumdis-2017-212452

REFERENCES

1 Reginster JY, Dudler J, Blicharski T, *et al*. Pharmaceutical-grade chondroitin sulfate is as effective as celecoxib and superior to placebo in symptomatic knee osteoarthritis: the ChONdroitin versus CElecoxib versus Placebo Trial (CONCEPT). *Ann Rheum Dis* 2017;76:1537–43.
2 Singh JA, Wilt T, MacDonald R. Chondroitin for osteoarthritis. *Cochrane Library* 2006.
3 Lee YH, Woo JH, Choi SJ, *et al*. Effect of glucosamine or chondroitin sulfate on the osteoarthritis progression: a meta-analysis. *Rheumatol Int* 2010;30:357–63.
4 Roman-Blas JA, Castañeda S, Sánchez-Pernaute O, *et al*. Combined treatment with chondroitin sulfate and glucosamine sulfate shows no superiority over placebo for reduction of joint pain and functional impairment in patients with knee osteoarthritis: a six-month multicenter, randomized, double-blind, placebo-controlled clinical trial. *Arthritis Rheumatol* 2017;69:77–85.

Differentiation between various Chondroitin sulfate formulations in symptomatic knee osteoarthritis

We are very grateful to Professor Lee[1] for his senseful comments regarding our recently published paper showing that pharmaceutical-grade chondroitin sulfate is superior to placebo and similar to celecoxib in reducing pain and improving function in patients with knee osteoarthritis.[2] We fully agree with Professor Lee that results obtained with pharmaceutical-grade chondroitin sulfate cannot be extrapolated to low-grade nutriceuticals, generics or over-the-counter products. This was extensively discussed in the recent algorithm published by the European Society for Clinical and Economic Aspect of Osteoporosis and Osteoarthritis, for the management of knee osteoarthritis.[3] This algorithm also re-emphasises the need for extreme caution when using combination products including chondroitin and glucosamine. The two studies, recently conducted and combining chondroitin sulfate and glucosamine sulfate or hydrochloride, did not use pharmaceutical-grade products, and subsequently their negative[4] or doubtful[5] results do not support a claim for superiority or even equivalence of the combination treatment compared with pharmaceutical-grade chondroitin sulfate or pharmaceutical-grade glucosamine sulfate, used as a stand-alone treatment. We fully agree with Professor Lee that our study encourages the use of pharmaceutical-grade chondroitin sulfate in the management of knee osteoarthritis but that it cannot, at any rate, be considered as supportive of the use of low-grade formulations.

Jean Yves Reginster[1,2,3]

[1]Department of Public Health, Epidemiology and Health Economics, University of Liege, Liege, Belgium
[2]Bioethics and Societal Medicine, University of Liege, Liege, Belgium
[3]WHO Collaborating Center for Public Health aspects of muskulo-skeletal health and aging, University of Liege, Liege, Belgium

Correspondence to Professor Jean Yves Reginster, Public Health, Epidemiology and Health Economics, WHO Collaborating Center for Public Health aspects of muskulo-skeletal health and aging, University of Liege-4020, Liege, Belgium; jyreginster@ulg.ac.be

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Reginster JY. *Ann Rheum Dis* 2018;**77**:e55.

Received 20 October 2017
Accepted 22 October 2017
Published Online First 1 November 2017

► http://dx.doi.org/10.1136/annrheumdis-2017-212452

Ann Rheum Dis 2018;**77**:e55. doi:10.1136/annrheumdis-2017-212460

REFERENCES

1 Lee YH. Chondroitin sulfate is superior to placebo in symptomatic knee osteoarthritis. *Ann Rheum Dis* 2018;77:e54.
2 Reginster JY, Dudler J, Blicharski T, *et al*. Pharmaceutical-grade Chondroitin sulfate is as effective as celecoxib and superior to placebo in symptomatic knee osteoarthritis: the ChONdroitin versus CElecoxib versus Placebo Trial (CONCEPT). *Ann Rheum Dis* 2017;76:1537–43.
3 Bruyère O, Cooper C, Pelletier JP, *et al*. A consensus statement on the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) algorithm for the management of knee osteoarthritis-from evidence-based medicine to the real-life setting. *Semin Arthritis Rheum* 2016;45(4 Suppl):S3–S11.
4 Roman-Blas JA, Castañeda S, Sánchez-Pernaute O, *et al*. Combined treatment with chondroitin sulfate and glucosamine sulfate shows no superiority over placebo for reduction of joint pain and functional impairment in patients with knee osteoarthritis: A six-month multicenter, randomized, double-blind, placebo-controlled clinical trial. *Arthritis Rheumatol* 2017;69:77–85.
5 Hochberg MC, Martel-Pelletier J, Monfort J, *et al*. Combined chondroitin sulfate and glucosamine for painful knee osteoarthritis: a multicentre, randomised, double-blind, non-inferiority trial versus celecoxib. *Ann Rheum Dis* 2016;75:37–44.