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Rheumatoid Arthritis Therapy Reappraisal

Strategies, Opportunities and Challenges

Edited by

Rüdiger Müller

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Rheumatoid Arthritis Therapy Reappraisal

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Strategies, Opportunities and Challenges

Editor

Rüdiger Müller

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Editor

Rüdiger Müller

Division of Rheumatology,

Kantonsspital Aarau

Switzerland

Division of Rheumatology and

Clinical Immunology,

Department of Internal Medicine IV,

Ludwig-Maximilians-University

Germany

Editorial Office

MDPI

St. Alban-Anlage 66

4052 Basel, Switzerland

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How to Get the Most from Methotrexate (MTX) Treatment for Your Rheumatoid Arthritis Patient?—MTX in the Treat-to-Target Strategy

Reprinted from: *J. Clin. Med.* **2019**, *8*, 515, doi:10.3390/jcm8040515 **219**

About the Editor

Rüdiger Müller

1. Personal Data:

Date and Place of Birth: 21 February 1971, Munich, Germany

2. Clinical Career:

- 1993–2000 Medical education at the Universities of Ghent/Belgium, Amsterdam/Netherlands, and Erlangen Nuremberg/Germany
- 8/2000 Resident at the Dept. of Int. Med. III (Prof. Dr. Dr. Kalden and Prof. Dr. Grog Schett) and postdoctoral fellow in the group of Prof. Dr. Schulze-Koops
- 2/2002 Doctoral Degree, Mentor: Prof. Fleckenstein (Institute for clinical and molecular Virology/University of Erlangen). Title: Sequencing and comparison of the nef-gene of long-term-surviving HIVpos children
- 17/9/2006 Degree Internal Medicine
- 15/11/2007 Degree Rheumatologie
- 03/08–10/18 Assistant medical director at the Kantonsspital in St. Gallen/Switzerland
- 03/2009 Degree Infectiology
- 07/2012 Degree interventional pain therapy
- 08/2015 Habilitation at the University of Munich (Prof. Dr. Schulze-Koops)
- 09/18 Executive MBA University St. Gallen
- 1/19 Deputy chief physician in Aarau

3. Scientific Training:

- 1996 Introductory course on molecular methods at the Institute for Molecular and Clinical Virology of the University of Erlangen-Nuremberg (Prof. Dr. Fleckenstein)
- 1996–1998 Scientific education in the laboratory of PD Dr. Baur at the Institute of Clinical and Molecular Virology of the University Erlangen-Nuremberg
- since 8/2000 Resident at the Dept. of Int. Med. III (Prof. Dr. Dr. Kalden) and postdoctoral fellow in the group of PD Dr. Schulze-Koops
- 2/2002 Doctoral Degree, Mentor: Prof. Fleckenstein (Institute for clinical and molecular Virology/ University of Erlangen) and PD Dr. Baur. Title: Sequencing and comparison of the nef-gene of long-term-surviving HIVpos children
- 2005–2008 Scientific work in the laboratory of Prof Dr. Herrmann at the Dept. of Int. Med. III of the University Erlangen-Nuremberg (Prof. Dr. Schett)
- 8/7/2015 Venia legendi at LMU in Munich, Rheumatology, Prof. Dr. Schulze-Koops

21/8/2015 Habilitation at LMU in Munich, Rheumatology, Prof. Dr. Schulze-Koops

4. Clinical Focus:

Rheumatoid arthritis

Clinical trials and epidemiological research

Apps: Rheum-Class, Acronym Finder Rheumatology, eDrugfinder

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Rüdiger Müller MD



Editorial

Rheumatoid Arthritis from Pathogenesis to Therapeutic Strategies

Ruediger B. Mueller * and Paul Hasler *

Division of Rheumatology, Medical University Department, Kantonsspital Aarau, 5001 Aarau, Switzerland

* Correspondence: ruediger.mueller@ksa.ch (R.B.M.); Paul.Hasler@ksa.ch (P.H.)

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Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to joint destruction. Various therapeutic agents have been showed to halt disease progression in clinical studies. In this special issue, we cover subjects from the periodontal condition of RA patients [1,2] to therapeutic strategies [3], and patient related outcomes [4,5], accompanied by the most extensive review ever on methotrexate (MTX) use in RA [6].

Eriksson et al. [1] describe that the subgingival plaque of RA patients with moderate/severe RA was enriched with abundant bacteria of different bacterial strains typical for periodontitis. Interestingly, ACPA also positivity correlated with moderate to severe periodontitis. Rinaudo-Gaujous et al. [2] showed that MMP-3 (matrix metalloproteinase 3), a marker of periodontal disease and bone and cartilage degradation, decreases subsequent to newly introduced infliximab therapy together with a reduction of disease activity. The interesting question is whether periodontitis primarily improves due to the reduction of disease activity, or whether improved arthritis leads to less pain during dental brushing and improved dental care remains, so far, unsolved.

Köhler et al. [7] reviewed the available methods for treatment of RA, while Mueller et al. [3] discussed how combination of the whole therapeutic armamentarium (new onset biologic agent, intra-articular and oral glucocorticoids, and optimization of conventional synthetic DMARDs) leads to a vastly improved outcome in a randomized clinical study. ACR 20, 50, and 70 response rates were achieved in 90.5%, 76.2%, 71.4%, an outcome that has so far not been achieved in a clinical trial of RA. The same group also reports the most extensive real-life experience of RA patients treated with tofacitinib in this issue [8].

Taylor et al. [6] wrote the largest and most comprehensive review on MTX, covering the pharmacology, the flexibility and efficacy and cost/benefit of the drug. Included among many other topics are the potential toxicities of MTX.

Hirter et al. [9] reviewed the literature on pseudo-erosions and came to three conclusions: (A) Pseudo-erosions may be related to normal anatomy or technical artefacts. (B) So-called calcified zones can be part of classical anatomical structures, such as subchondral, sub-tendinous or -ligamentous bone. (C) As a caveat, a real arthritic erosion can develop at the site of a pseudo-erosion.

In two post-hoc analyses of the RA BEAM Study Fautrel et al. [4] and Taylor et al. [5] demonstrated that in RA patients with moderately to severely active RA despite MTX treatment, the addition of baricitinib may be more effective in improving pain and physical function than placebo or addition of adalimumab.

In summary, in this special issue the disease of RA and its therapy is described from different angles to provide a broad and profound insight into the disease.

References

1. Eriksson, K.; Fei, G.; Lundmark, A.; Benchimol, D.; Lee, L.; Hu, Y.O.O.; Kats, A.; Saevarsdottir, S.; Catrina, A.L.; Klinge, B.; et al. Periodontal Health and Oral Microbiota in Patients with Rheumatoid Arthritis. *J. Clin. Med.* **2019**, *8*, 630. [[CrossRef](#)] [[PubMed](#)]
2. Rinaudo-Gaujous, M.; Blasco-Baque, V.; Miossec, P.; Gaudin, P.; Farge, P.; Roblin, X.; Thomas, T.; Paul, S.; Marotte, H. Infliximab Induced a Dissociated Response of Severe Periodontal Biomarkers in Rheumatoid Arthritis Patients. *J. Clin. Med.* **2019**, *8*, 751. [[CrossRef](#)] [[PubMed](#)]
3. Mueller, R.B.; Spaeth, M.; von Restorff, C.; Ackermann, C.; Schulze-Koops, H.; von Kempis, J. Superiority of a Treat-to-Target Strategy over Conventional Treatment with Fixed csDMARD and Corticosteroids: A Multi-Center Randomized Controlled Trial in RA Patients with an Inadequate Respo. *J. Clin. Med.* **2019**, *8*, 302. [[CrossRef](#)] [[PubMed](#)]
4. Fautrel, B.; Kirkham, B.; Pope, J.E.; Takeuchi, T.; Gaich, C.; Quebe, A.; Zhu, B.; de la Torre, I.; De Leonardis, F.; Taylor, P.C. Effect of Baricitinib and Adalimumab in Reducing Pain and Improving Function in Patients with Rheumatoid Arthritis in Low Disease Activity: Exploratory Analyses from RA-BEAM. *J. Clin. Med.* **2019**, *8*, 1394. [[CrossRef](#)] [[PubMed](#)]
5. Taylor, P.C.; Lee, Y.C.; Fleischmann, R.; Takeuchi, T.; Perkins, E.L.; Fautrel, B.; Zhu, B.; Quebe, A.K.; Gaich, C.L.; Zhang, X.; et al. Achieving Pain Control in Rheumatoid Arthritis with Baricitinib or Adalimumab Plus Methotrexate: Results from the RA-BEAM Trial. *J. Clin. Med.* **2019**, *8*, 831. [[CrossRef](#)] [[PubMed](#)]
6. Taylor, P.C.; Balsa Criado, A.; Mongey, A.B.; Avouac, J.; Marotte, H.; Mueller, R.B. How to Get the Most from Methotrexate (MTX) Treatment for Your Rheumatoid Arthritis Patient?-MTX in the Treat-to-Target Strategy. *J. Clin. Med.* **2019**, *8*, 515. [[CrossRef](#)] [[PubMed](#)]
7. Köhler, B.M.; Günther, J.; Kaudewitz, D.; Lorenz, H.M. Current Therapeutic Options in the Treatment of Rheumatoid Arthritis. *J. Clin. Med.* **2019**, *8*, 938. [[CrossRef](#)] [[PubMed](#)]
8. Mueller, R.B.; Caroline Hasler, C.; Popp, F.; Mattow, F.; Durmisi, M.; Souza, A.; Hasler, P.; Andrea Rubbert-Roth, A.; Schulze-Koops, H.; von Kempis, J. Effectiveness, Tolerability, and Safety of Tofacitinib in Rheumatoid Arthritis: A Retrospective Analysis of Real-World Data from the St. Gallen and Aarau Cohorts. *J. Clin. Med.* **2019**, *8*, 1548. [[CrossRef](#)] [[PubMed](#)]
9. Hirtler, L.; Rath, C.; Platzgummer, H.; Aletaha, D.; Kainberger, F. Pseudoerosions of Hands and Feet in Rheumatoid Arthritis: Anatomic Concepts and Redefinition. *J. Clin. Med.* **2019**, *8*, 2174. [[CrossRef](#)] [[PubMed](#)]



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Article

Pseudoerosions of Hands and Feet in Rheumatoid Arthritis: Anatomic Concepts and Redefinition

Lena Hirtler ^{1,*}, Claus Rath ¹, Hannes Platzgummer ², Daniel Aletaha ³ and Franz Kainberger ²

¹ Division of Anatomy, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna 1090, Austria; claus.rath@meduniwien.ac.at

² Department of Biomedical Imaging and Image-Guided Therapy, Medical University of Vienna, Vienna 1090, Austria; hannes.platzgummer@meduniwien.ac.at (H.P.); franz.kainberger@meduniwien.ac.at (F.K.)

³ Division of Rheumatology, Department of Internal Medicine, Medical University of Vienna, Vienna 1090, Austria; daniel.aletaha@meduniwien.ac.at

* Correspondence: lena.hirtler@meduniwien.ac.at; Tel.: +43-1-40160-37570

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Abstract: Rheumatoid arthritis is a chronic inflammatory disease characterized by the development of osseous and cartilaginous damage. The correct differentiation between a true erosion and other entities—then often called “pseudoerosions”—is essential to avoid misdiagnosing rheumatoid arthritis and to correctly interpret the progress of the disease. The aims of this systematic review were as follows: to create a definition and delineation of the term “pseudoerosion”, to point out morphological pitfalls in the interpretation of images, and to report on difficulties arising from choosing different imaging modalities. A systematic review on bone erosions in rheumatoid arthritis was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The following search terms were applied in PubMed and Scopus: “rheumatoid arthritis”, “bone erosion”, “ultrasonography”, “radiography”, “computed tomography” and “magnetic resonance imaging”. Appropriate exclusion criteria were defined. The systematic review registration number is 138826. The search resulted ultimately in a final number of 25 papers. All indications for morphological pitfalls and difficulties utilizing imaging modalities were recorded and summarized. A pseudoerosion is more than just a negative definition of an erosion; it can be anatomic (e.g., a normal osseous concavity) or artefact-related (i.e., an artificial interruption of the calcified zones). It can be classified according to their configuration, shape, content, and can be described specifically with an anatomical term. “Calcified zone” is a term to describe the deep components of the subchondral, subligamentous and subtendinous bone, and may be applied for all non-cancellous borders of a bone, thus representing a third type of the bone matrix beside the cortical and the trabecular bone.

Keywords: rheumatoid arthritis; pseudoerosions; hand; foot; ultrasonography; radiography; computed tomography; magnetic resonance imaging

1. Introduction

Rheumatoid arthritis (RA) manifests with three types of structural joint damage: joint space narrowing, erosions, and capsular abnormalities in the form of synovial proliferation and subluxations [1–4]. The diagnosis of erosions and their quantification as part of radiographic scoring systems is an accepted surrogate biomarker of structural progression of arthritis [4,5]. Erosions in RA have been defined in consensus statements and in studies with high-resolution peripheral quantitative computed tomography (HRpQCT) as cortical defects, breaks, or other discontinuities with underlying trabecular bone loss and characteristic locations that can be identified with imaging [6–12]. On radiographs, according to the 2010 ACR/EULAR (American College of Rheumatology/European

League Against Rheumatism) rheumatoid arthritis classification criteria [13,14], erosions have to be seen at least at three separate joints at the interphalangeal (PIP), metacarpophalangeal (MCP), wrist (counted as one joint), or metatarsophalangeal (MTP) joints [15,16]. For ultrasound (US) and magnetic resonance imaging (MRI), the operational OMERACT (outcome measures in rheumatology) definition requests the abnormality being visible in two planes [17,18].

At the wrist, the most frequent locations are the capitate, ulna, lunate, triquetrum, and scaphoid [19–26], at the ankle the distal fibular notch, the navicular, cuneiform and cuboid bones are often involved, the talus and calcaneus less frequently [27,28]. Why erosions occur at these sites is commonly explained by immunological and anatomical models [29–31]. The latter mainly refer to the thinning of cartilage near capsular insertions at bones (bare areas) and to microdamage [32–36]. Following immunologically-based concepts, erosion formation is explained by increased bone resorption and decreased bone formation at certain locations in the subchondral bone [37]. Werner et al. [32] showed a correlation between cortical micro-channels and the occurrence of bone erosions in bare areas.

Especially in early, preclinical or undifferentiated arthritis with small or no erosions, it is necessary to differentiate a true rheumatic erosion from the various forms of normal erosion-simulating concavities of the bony surface and therefor avoid false-positive statements [38,39]. Such so-called pseudoerosions [40] have been described to be smooth and well demarcated on radiographs, ultrasound, computed tomography (CT) and MRI [41]. The effect of misinterpreting a normal anatomic concavity as an erosion or vice versa may be estimated from the intra- and inter-reader variations of scoring systems and has been directly mentioned for the RAMRIS (rheumatoid arthritis MRI score) [42,43]. The spectrum of MRI “erosion-like” lesions is broad: Ejbjerg et al. [44] observed them in 1.9% of healthy persons, whereas Olech et al. [45] saw them in 65%. Rothschild [46] questioned if such findings should be interpreted as true erosions, old erosions from earlier diseases without clinical significance, or other. For the US, a 30% false-positive rate of erosion detection has been reported [47]. The computer-assisted assessment of erosions was considered helpful, but difficulties in discriminating those from normal bony concavities were observed [48,49].

The aim of this systematic review was (1) to evaluate the frequency of specifically stated difficulties arising in the interpretation of imaging modalities in search for bone erosions, (2) to define the characteristic anatomic appearances and patterns of pseudoerosions with respect to the potential pitfalls in the diagnosis of RA as reported in the literature and (3) to develop an anatomic concept for improving the accuracy and precision of imaging assessment.

2. Materials and Methods

A systematic review on bone erosions in RA was performed based on the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement and was registered accordingly (No. 138826) [50].

2.1. Search Strategy

The search was performed in PubMed (Medline) and Scopus with the following search terms: “rheumatoid arthritis”, “bone erosion”, “ultrasonography”, “radiography”, “computed tomography” and “magnetic resonance imaging” (example for search in PubMed: “rheumatoid arthritis” AND bone AND erosion AND (ultrasonography OR radiography OR “computed tomography” OR “magnetic resonance imaging”). No specific date was defined as starting point, the end of search was 31 May 2019. English language was defined as a required criterium.

2.2. Selection Criteria

All original studies investigating the diagnosis of RA with X-ray, sonography, CT or MRI and describing false positive diagnoses of bone erosions and erosion-like changes published before 31 May 2019 were included. Exclusion criteria were animal studies, feasibility studies, other inflammatory diseases, clinical studies comparing therapeutic measurements in RA, studies comparing the sensitivity

of imaging modalities without report of false positive diagnosed erosions or erosion-like lesions, surgical procedures or longitudinal studies without direct reference to this topic, case reports and conference papers. Additionally, all papers without full text availability were excluded from the analysis.

Data extraction was performed by using a standardized Excel (Microsoft Corporation, Redmont, WA, USA) data extraction form: first author, year of publication, country, study population, number of patients, imaging modality, joints evaluated, reported sensitivity of imaging modalities, reported false positive or false negative diagnosis of bone erosions, reported limitations in image interpretation with respect to anatomy, the differential diagnosis to other erosive diseases, artifacts and signal-to-noise reduction.

3. Results

The search with the defined terms resulted in a total of 1487 results. An additional number of 59 papers were added after reference-screening. The flow diagram of the literature review may be seen in Figure 1. Ultimately, only 25 papers reported specifically on false-positive results or erosion-like changes.



Figure 1. Flow diagram of the literature review.

Based on the information gathered in the remaining papers, the false-positive results were subdivided into anatomic pseudoerosions, if the explanation for the false-positive diagnosis was

described as a morphological phenomenon, and into artifact-related pseudoerosions, if the explanation for the false-positive diagnosis was related to the respective imaging technique.

3.1. Anatomic Pseudoerosions

Anatomic pseudoerosions, i.e., normal concavities of a bone with a potential for misinterpreting them as arthritis-related erosions, were described in twelve original papers and reviews and may be classified into four types according to their anatomic form and configuration (Table 1): (1) a groove or notch or its incomplete form, i.e., a jutting, (2) a sulcus as part of an osteofibrous channel, (3) a subcapital neck on long bones, or (4) a nutritional channel or a zonal roughness [3,11,41,51–57]. According to their shape, they may be grouped into (1) shallow or broad concavities and (2) subchondral cysts, if en-face displayed on an image and occasionally with a small opening to the joint space, or (3) channel-like structures (Figure 2a,c) [3,54,55]. The anatomic location of pseudoerosions is predominantly at the carpal bones, the MCP- and the MTP-joints. Almost always they are linked to a ligament insertion (Figure 2b), a mucosal fold fixation or the hood of a tendon sheath, and occur at the noncortical bone, also known calcified zones (i.e., borders of the subchondral and enthesial calcified bone with the adjacent underlying trabecular structures). The content of pseudoerosions is visible with US and MRI and may be normal or degenerated ligament tissue, or blood vessels [44,56] and the development of edematous changes [58]. With contrast media, a slight enhancement can be observed, however, in one publication rare cases of strong enhancement was documented [56].

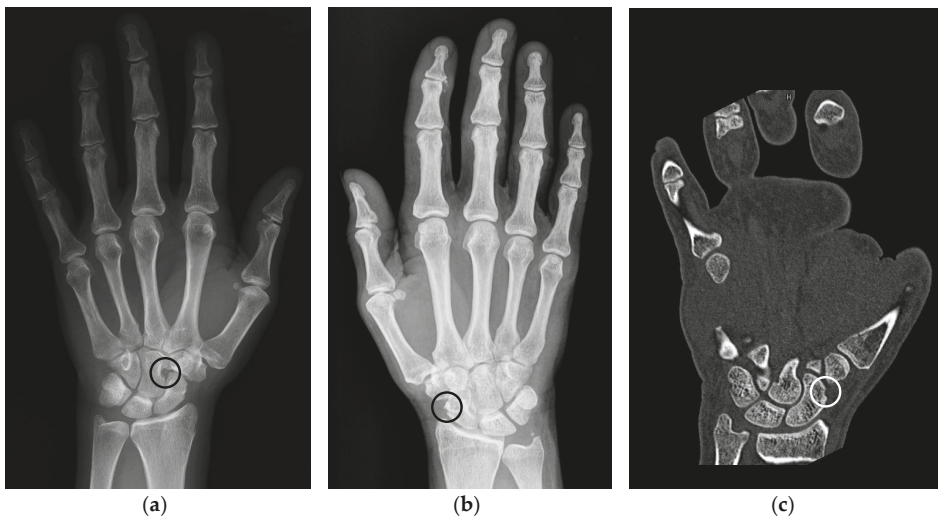


Figure 2. Examples of anatomical pseudoerosions. (a) Example of a sulcus like pseudoerosion of the capitate bone (black circle) in a left hand of a 52 years old female patient. Referred for suspected scaphoid fracture, which was not verified. (b) Example of a pseudoerosion at the level of the scaphoid waist (black circle) in a right hand of a 66 years old female patient. Referred because of unspecific wrist pain, which afterwards subsided without treatment after one week. (c) Scaphoid rim simulating an erosion in a left hand of a 38 years old male patient (white circle). Referred because of presurgical planning after fracture of the fifth metacarpal and luxation of the fourth and third metacarpal.

Table 1. Pseudoerosions.

| Citation | Type of Article | Imaging Modality | Reported Pseudoerosion | Explanation |
|--|-------------------|---|-------------------------------|--|
| Alasaarela et al., 1998 [58] | Original research | Magnetic resonance imaging (MRI) (1.0T T1, T2 and proton density, PD) | False positive interpretation | Pre-erosive oedematous changes in subchondral bone in MRI |
| Barnabe et al., 2016 [11] | Original research | High-resolution peripheral quantitative computed tomography (HRpQCT) | Carpal pseudoerosions | Arterial foramina |
| Canella Moraes Carmo et al., 2009 [54] | Original research | Computed tomography (CT) | Carpal pseudoerosions | ligament insertions tendinous sulci |
| Dohn et al., 2006 [53] | Original research | Sonography | Erosion-like changes | Metacarpophalangeal (MCP) joints |
| Dohn et al., 2013 [57] | Original research | Sonography | False positive interpretation | Cortical irregularities (osteophytes, notches at the metacarpal neck, subcortical bone cysts) |
| Ejbjerg et al., 2004 [44] | Original research | MRI (1.0 T1 spin echo, STIR, T2 spin echo fat-suppressed) | Erosion-like changes | Capitate, lunate |
| Martel et al., 1965 [3] | Original research | Plain radiography | Carpal pseudoerosion | Normal deep groove in the capitate in about 10% |
| McQueen et al., 2005 [51] | Review article | MRI (T1, T2 fat-saturated) | False positive interpretation | Attachments of interosseous ligaments of the wrist, articular ligaments of the MCP joints, nutrient foramina |
| Peluso et al., 2015 [52] | Original research | 3D sonography | False positive interpretation | Arterial foramina Osteophytes |
| Robertson et al., 2006 [56] | Original research | MRI (1.5T, T1 spin echo, fat-suppressed FSE, fat-suppressed PD-weighted FSE, 3D SPGR) | Carpal pseudoerosions | ligament insertions |
| Torshizy et al., 2008 [55] | Original research | CT | Tarsal pseudoerosions | attachment site of joint capsule ligament insertions tendinous sulci |
| Wawer et al., 2014 [41] | Original research | Plain radiography | Carpal pseudoerosions | ligament insertions |

STIR = Short TI Inversion Recovery, FSE = Fast Spin Echo, SPGR = Spoiled Gradient Recalled Echo.

3.2. Artifact-Related Pseudoerosions

Artifact-related pseudoerosions were mentioned in 18 original papers and reviews and may be caused due to (1) partial volume artifacts of cross-sectional images or other modality-specific artifacts (ultrasound diffraction or reflection, insufficient fat suppression with MRI), or (2) a low signal-to-noise ratio (Table 2) [1,5,52,57–68].

Table 2. Imaging difficulties.

| Citation | Type of Article | Imaging Modality | Reported Problem |
|------------------------------|-------------------|-------------------|---|
| Alasaarela et al., 1998 [58] | Original research | CT | Examination of a curvilinear object—the more the reformat plane parallels the z-axis, the more resolution of multiplanar reformats is impaired. The partial volume effect is harmful. |
| | | Plain radiography | Information dependent on projections used |
| Albrecht et al., 2013 [1] | Original research | Plain radiography | 2D character of radiography |
| | | CT | No simultaneous assessment of inflammatory changes of RA |

Table 2. Cont.

| Citation | Type of Article | Imaging Modality | Reported Problem |
|-------------------------------|-------------------|--|---|
| Amin et al., 2012 [62] | Original research | Plain radiography | Beam has to hit erosion tangentially to show cortical break |
| Aurell et al., 2018 [63] | Original research | Plain radiography | Possibility of false negative evaluation, if the orifice of the erosion is not hit tangentially |
| Cimmino et al., 2002 [60] | Original research | MRI (T2 spin echo or gradient echo) | Failed fat suppression can mimic bone marrow edema |
| Dohn et al., 2013 [57] | Original research | Sonography | Some areas of hand and wrist are inaccessible for ultrasound beam |
| Dohn et al., 2008 [65] | Original research | MRI (0.6T T1 3D fast field echo) | Overestimation of erosion size due to difficult differentiation between cortical bone and erosion |
| Ejbjerg et al., 2006 [64] | Original research | Plain radiography | Up to 30% of an MCP joint bone has to be eroded before detection |
| Emond et al., 2012 [68] | Original research | MRI (1T 3D spoiled gradient echo) | Boundaries of erosions difficult to differentiate |
| Foley-Nolan et al., 1991 [59] | Original research | Plain radiography | Erosions only visible when large percentage of bone thickness has been destroyed |
| Forslind et al., 2003 [61] | Original research | Plain radiography | Delineation of erosions difficult in patients with osteoporosis |
| | | MRI (1.0T 3D T2 gradient echo, T1 spin echo with and without fat-saturation) | False negative interpretation due to contiguous looking erosions |
| Kleyer et al., 2016 [66] | Original research | MRI (1.5T T1) | Small cortical breaks not seen on MRI—validation by HRpQCT |
| McQueen et al., 1998 [69] | Original research | MRI (1.5T T1 and T2 with and without fat suppression) | Partial volume artefacts may lead to false positive indications of erosions |
| McQueen et al., 2001 [70] | Original research | Plain radiography | Identification of erosions hampered by poor visibility at the carpus |
| Peluso et al., 2015 [52] | Original research | Ultrasonography | Due to anatomical structure, multiplanar distribution of bones that restricts the ultrasound beam and alters the correct visualization |
| Ulas et al., 2019 [67] | Original research | MRI (1.5T): Susceptibility-weighted imaging, SWI T1w | False positive identification of erosions due to motion artefacts, strong susceptibility artefacts at tissue intersections Weak differentiation of cortical bone |
| Wakefield et al., 2000 [5] | Original research | Plain radiography | Typical anatomical location of bone erosions difficult to see until it lies in the tangential plane of the radiographic beam. |
| | | Plain radiography | Periarticular osteoporosis |
| Wawer et al., 2014 [41] | Original research | Plain radiography | Less density in subcortical cancellous bone due to synovial and bony hyperemia, overlapping of carpal bones, presence of osteophytes |

4. Discussion

From the viewpoint of imaging anatomy, a misinterpretation of erosions in RA may occur due to (1) anatomic pseudoerosions, or (2) artifact-related pseudoerosions as a result of an inadequate investigation technique. Pseudoerosions and erosions are commonly located at certain areas of the surface outline of the calcified bone, also known as calcified zones. These may therefore, besides cortical bone and trabecular bone, be regarded as a third type of organization of the bone matrix.

The term “calcified zones” (Figure 3) in this context is therefore proposed to describe the borders of the subchondral and enthesial calcified bone with the adjacent underlying trabecular structures. It may be extended for describing all parts of intraarticular bone apart from the cortex. With its overlying tissue of hyaline cartilage, synovium or capsule-ligamentous structures it forms anatomic units. The relationship between these zones and the adjacent tissues is so tight that the fibrous layers

of tendon sheaths, bursae, periosteum or the cartilaginous zones of entheses or hyaline cartilage are in direct continuation with the subjacent bone, thus providing direct contact with synovial tissue. The concept of the subchondral zone was used by Dihlmann [71] to describe the mineralized zone of hyaline cartilage as part of the subchondral bone. It may be extended to describe a subligamentous, subtendinous or subbursal zone of the bone. Utilizing sub-millimeter spatial resolution CT, these calcified zones can be displayed. Differentiating the normal calcified zone from erosional changes, i.e., irregular margins and sclerotic reaction, is the main challenge in differentiating true erosions from pseudoerosions [72].

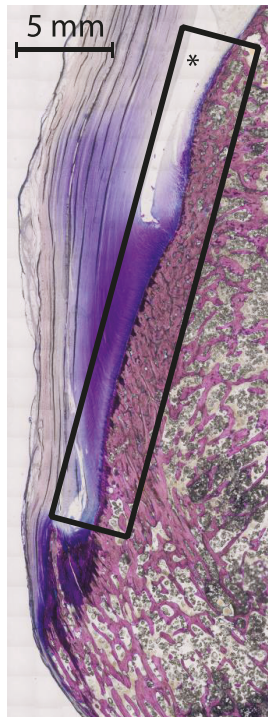


Figure 3. Example of a calcified zone. Thin ground section of the calcaneal tuberosity, the calcaneal tendon and the calcaneal bursa—also a frequent location of bone erosions. The described calcified zone as subchondral and enthesial calcified bone with adjacent underlying trabecular structures including the overlying tissues is marked by the rectangle. The asterisk marks the calcaneal bursa. A 5 mm scale is included, the tissue was stained with Giemsa.

Pseudoerosions have to be differentiated from other pathologies as ganglion cysts, crystal-induced arthropathies, tuberculosis or other infections, and from degenerative lesions in the form of erosions, subchondral (pseudo)cysts or beak-shaped osteophytes as there are so many similarities in location [38,60]. Intraosseous ganglion cysts are common and almost always have a continuity with a ligament which underwent mucous degeneration [73,74]. Especially in the elderly population, the more prevalent degenerative changes of the bone may be difficult to be differentiated from RA-related erosions [38,75]. However, in children interpretational problems may arise. There, normal concavities simulating erosions have been referred to as “bony depressions” at certain locations in the wrist [76–78]. Such pseudoerosions in children may be big, indicating that size is not a reliable feature for differentiating normal variants from true erosions.

4.1. Anatomic Pseudoerosions

An anatomic pseudoerosion can be defined as a normal concavity of a bone outlined by a smooth and thin calcified zone with the potential for a false-positive misinterpretation of an erosion. In this form, the term pseudoerosion is more precise than “notch” or “bony depression” and may be preferred as it contains a prognostic impact for the imaging assessment of arthritis. Such clinically oriented annotations, examples are the scaphoid waist and the metacarpal neck as typical sites for fractures, have been in use in traumatology and may be of help in the assessment of arthritis-related erosions, too (list of described pseudoerosions in Table 3, an overview of anatomical pseudoerosions in the hand may also be found in Figure 4).

Table 3. List of pseudoerosions with anatomic description.

| Location | Name | Description |
|---|-------------------------------------|---|
| Scaphoid waist, palmar aspect | Scaphoid waist | Tendon hood of radial-sided carpal tunnel with radio-scapho-capitate ligament |
| Scaphoid, radial aspect of midpart | | Scapho-capsular ligament or mucosal fold insertions |
| Capitate, distal ulnar portion [41] | Ulnar capitate notch | Intercarpal ligaments |
| Capitate, radial portion | Radial capitate notch | Intercarpal ligaments |
| Lunate, radial aspect | | Scapholunate ligament |
| Hamate, distal radial portion [41] | | Insertion of capitohamate ligament and carpometacarpal ligaments |
| Hamate, distal ulnar portion [41] | | Insertion of carpometacarpal ligaments |
| Triquetrum, radial and dorsal aspect | Radial triquetral notch | Insertion of the radiotriquetral ligament |
| Triquetrum, ulnar and proximal aspect | | Insertion of the ulnotriquetral ligament |
| Metacarpal bases | metacarpal base notches | Insertion of intercarpal ligaments |
| Metacarpal or metatarsal neck and heads | metacarpal or metatarsal head notch | Insertion of metacarpophalangeal ligaments or joint capsule |
| 5th metatarsal head | | Slight normal varus angulation of metatarsal head |
| Achilles tendon insertion | | Insertion jutting |

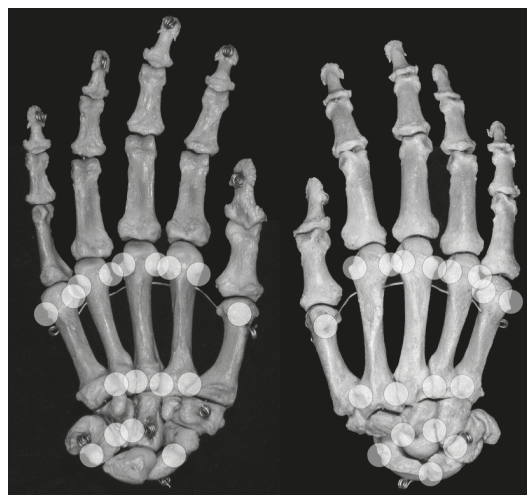


Figure 4. Locations of anatomical pseudoerosions. Overview on possible locations of anatomical pseudoerosions as summarized in Table 3. Right skeletal hand, on the left view from palmar, on the right view from dorsal.

Grooves due to ligament or tendon insertions have a varying appearance as described in the enthesis concept by Benjamin and McGonagle [79]. Such prominent grooves can cause the appearance of a pseudoerosion (Figure 2a). A groove may occur in three forms: (1) at a non-apophyseal direct tendon or ligament attachment where the uncalcified components of the enthesis enters the bone, (2) at an apophysis with overhanging edges, or (3) at an incomplete apophysis, a jutting, at the indirect attachments of a tendon or ligament with a tangential transition into the periosteum. For example, pseudoerosions resulting from the first form are the metacarpal ligament insertions at the bases of the metacarpal bones [80]. At the dorsal aspect of the triquetral bone, such a pseudoerosion may be formed by the distal insertion of the radiotriquetral ligament along with other components of the dorsal radiocarpal ligament. On the capitate, on which several strong carpal ligaments have their insertion, and many other carpal bones, intercarpal ligaments may cause pseudoerosions [51]. Examples for the second form may be the non-spherical form of metacarpal and metatarsal heads, which can be explained by the collateral ligament complexes running laterally and medially with smoothly outlined shallow metacarpal grooves containing these structures. At the metacarpals, these grooves are bordered by little tubercles for the proximal attachment of the collateral ligaments (Figure 4) [81]. Moraes do Carmo et al. [54] identified three concavities in the first metacarpal head (intersesamoid, ulnar, and radial) and two in those of the fingers (ulnar and radial). They described dorsal depressions of the metacarpal heads due to the extensor digitorum tendons in one third of their anatomic specimens which correlated with observations with ultrasound made by Boutry et al. [82,83]. A similar study was done for defining pseudoerosions of the metatarsal heads by Torshizy et al. [55] who described anatomic variations in the normal osseous concavities of the lateral and medial aspects of each metatarsal head. Typical jutties, i.e., examples for the third form of grooves, are the small round or oval subcapsular notches at the proximal phalangeal bases [80,84]. At the Achilles tendon insertion, proximal to its jutting shallow irregularities beneath the calcaneal bursa may represent true erosions [85].

Osseous sulci are commonly roofed with a ligament, fascia or other fibrous tissue, thus forming an osteofibrous channel for a tendon within a synovial tendon sheath. A subcapital neck of the distal metacarpal and the metatarsal bones is a small metaphyseal narrowing that may cause a pseudoerosion on projection radiographs, ultrasound or MRI [86]. At the distal fifth metacarpal bone, due to its slight varus angulation this neck may be more prominent.

Nutritional channels may appear as pseudoerosion on MR if their orifice is displayed as a little T2-weighted hyperintense spot [51]. Their superficial orifice is often located at a roughness of the calcified zones which as a whole may simulate an erosion [11,34,87]. Some of these iuxtaarticular surface roughnesses may be specified as crests or ridges that correspond to attachment sites for redundant joint capsule [55]. Others, especially on carpal bones, may be due to indentations of innominate ligamentous attachments or synovial folds [51]. Such typical structures visible between the radial aspect of the scaphoid and the radial carpal collateral ligament may be called scapho-capsular ligaments (Figure 2C). Roughness of the calcified zones may be visible at various sites and should be differentiated from shallow extensive true erosions and from advanced cartilage degeneration [88].

4.2. Artefact-Related Pseudoerosions

Artefact-related pseudoerosions are defined as an interruption of the sharp outline of the calcified zones. Important causes are a low signal-to-noise ratio, a partial volume artefact, or in case of ultrasound irregular backscattering with artefacts on an incongruent or rough surface. A low signal-to-noise ratio could be caused by over-penetration of the X-ray beam through the bone or due to insufficient spatial or contrast resolution. This effect is more severe in cases with low calcium content in the calcified zones or the subjacent trabecular bone, previously referred to as subchondral osteoporosis or as pre-erosions, and may be enhanced by swelling of the overlying soft tissue. With ultrasound, diffraction or a complex backscattering of the waves on a curved or irregular surface may cause various pseudo-effects on the retrieved image [51,69,89].

Although X-ray is most commonly used in the diagnosis of RA it is CT which can be regarded as the best imaging modality for differentiating pseudoerosions from true erosions [53,62,63,90–94]. Several studies [34,95–98] describe a significant decrease of trabecular volume and number and an increased trabecular heterogeneity in patients with rheumatoid arthritis by using HRpCT. This trabecular bone loss as the intramedullary component of bone erosions may contribute the largest part and may therefore be a reason for misinterpretation of erosions or pseudoerosions in radiographs as this imaging method is relatively insensitive to trabecular bone loss [60,99].

In addition, MRI and US are reported to be more sensitive than plain radiography [53,62,90–94], but this especially seems to be dependent on the location investigated [88,100]. In some cases, radiography may even be superior to MRI in detecting bony erosions despite its lack of three dimensionality [1,3,5,58,99,101,102]. Through its high spatial resolution it can differentiate smaller erosions which otherwise would present themselves as continuous on MRI [61,65].

Thus, it is important to recognize several parameters to achieve a decrease of cognitive diagnostic errors especially in early arthritis. These include slight variations in the respective projection technique and individual ligament laxity or postinflammatory scarring of ligaments. In addition, the roughness of a calcified zone, and the transitional changes between normal bone and true inflammatory erosions are until now not or only scarcely addressed. Even the projection of the joints, even if the relevant anatomic landmarks are displayed according to the standards, is highly variable. One has also to keep in mind that discrete forms of malalignment due to ulnar deviations or other forms of arthritic subluxation, ligament laxity with a slight rotation of bones, and variations in their arrangement may cause a more prominent appearance of a pseudoerosion [3,51,57,102].

4.3. Erosions-in-Pseudoerosions

Both anatomic and artefact-related pseudoerosions are located at sites with direct or indirect contact to inflammatory tissue in arthritis, and therefore, are at higher risk for destruction. Areas of the articular bone without any cartilage covering are more prone to erosive destructions by synovial tissue and effusion [3,32]. Hence, in an anatomically preformed concavity a true inflammatory erosion may develop. McQueen et al. [51] described these erosions-in-pseudoerosions (Figure 5) for the attachment sites of the intercarpal ligaments. It may be observed at the site of ligamentous attachments covered by synovial folds at the metacarpal or metatarsal heads or at the wrist. On the other hand, true erosions may be classified as normal variants. It remains unclear whether these are incidental findings or subclinical erosions [3,57,102].



Figure 5. Erosions within pseudoerosions. Example of the development of an erosion within a pseudoerosion (black/white circle) in a left hand of a 65 years old female patient with longstanding mild seropositive rheumatoid arthritis. Left: radiograph, right: MRI.

4.4. Limitations

A limitation of this study was that the defined search terms resulted in a large quantity of papers, which had to be screened. However, generally accepted terms for mimickers of true erosions do not exist, are described in various forms and additionally with more equivocal definitions than expected at the beginning of this project. Nonetheless, this wide search net allowed for the inclusion of all relevant sources describing the phenomenon of pseudoerosions and minimized the possibility of excluding the respective publications.

5. Conclusions

In conclusion, a pseudoerosion is more than just a negative definition of an erosion. It can be defined as a normal osseous concavity (anatomic pseudoerosion) and/or an artefactual interruption of the calcified zones (artefact-related pseudoerosion). It can be classified according to their configuration, shape, content, and can be directly anatomically named. “Calcified zone” is a term to describe the deep components of the subchondral, subligamentous and subtendinous bone and may be applied for all non-cancellous borders of a bone, thus representing a third type of the bone matrix beside the cortical and the trabecular bone. Anatomic pseudoerosions are almost always related to a ligament insertion or the osteo-fibrous channel of a tendon sheath, therefore, being of high risk for microdamage and the development of a “true” arthritic erosion. Understanding these peculiar aspects of the bony surface with relation to ligament insertions and osteofibrous channels may be of help in improving the assessment of erosions and for reducing over- and underdiagnosis of true erosions.

6. Take Home Message

1. Pseudoerosions may be subclassified into anatomic (normal osseous cavity) and artefact-related (artefactual interruption of the calcified zone).
2. The term “calcified zone” describes the deep components of the subchondral, subligamentous and subtendinous bone and may be applied for all non-cancellous borders of a bone.
3. Pseudoerosions can be regarded as anatomic sites at risk for the development of “true” arthritic erosions.

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References

1. Albrecht, A.; Finzel, S.; Englbrecht, M.; Rech, J.; Hueber, A.; Schlechtweg, P.; Uder, M.; Schett, G. The structural basis of MRI bone erosions: An assessment by microCT. *Ann. Rheum. Dis.* **2013**, *72*, 1351–1357. [[CrossRef](#)] [[PubMed](#)]
2. Hetland, M.L.; Ejbjerg, B.; Horslev-Petersen, K.; Jacobsen, S.; Vestergaard, A.; Jurik, A.G.; Stengaard-Pedersen, K.; Junker, P.; Lottenburger, T.; Hansen, I.; et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann. Rheum. Dis.* **2009**, *68*, 384–390. [[CrossRef](#)] [[PubMed](#)]
3. Martel, W.; Hayes, J.T.; Duff, I.F. The Pattern of Bone Erosion in the Hand and Wrist in Rheumatoid Arthritis. *Radiology* **1965**, *84*, 204–214. [[CrossRef](#)] [[PubMed](#)]
4. Buckland-Wright, J.C. Microfocal radiographic examination of erosions in the wrist and hand of patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **1984**, *43*, 160–171. [[CrossRef](#)]
5. Wakefield, R.J.; Gibbon, W.W.; Conaghan, P.G.; O'Connor, P.; McGonagle, D.; Pease, C.; Green, M.J.; Veale, D.J.; Isaacs, J.D.; Emery, P. The value of sonography in the detection of bone erosions in patients with rheumatoid arthritis: A comparison with conventional radiography. *Arthritis Rheum.* **2000**, *43*, 2762–2770. [[CrossRef](#)]

6. Kellgren, J.H.; Lawrence, J.S. Radiological assessment of rheumatoid arthritis. *Ann. Rheum. Dis.* **1957**, *16*, 485–493. [[CrossRef](#)]
7. Larsen, A.; Dale, K.; Eek, M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. *Acta Radiol. Diagn.* **1977**, *18*, 481–491. [[CrossRef](#)]
8. Larsen, A. Radiological grading of rheumatoid arthritis. An interobserver study. *Scand. J. Rheumatol.* **1973**, *2*, 136–138. [[CrossRef](#)]
9. van der Heijde, D.M. Plain X-rays in rheumatoid arthritis: Overview of scoring methods, their reliability and applicability. *Baillieres Clin. Rheumatol.* **1996**, *10*, 435–453. [[CrossRef](#)]
10. van der Heijde, D.; Dankert, T.; Nieman, F.; Rau, R.; Boers, M. Reliability and sensitivity to change of a simplification of the Sharp/van der Heijde radiological assessment in rheumatoid arthritis. *Rheumatology* **1999**, *38*, 941–947. [[CrossRef](#)]
11. Barnabe, C.; Toepfer, D.; Marotte, H.; Hauge, E.M.; Scharmg, A.; Kocijan, R.; Kraus, S.; Boutroy, S.; Schett, G.; Keller, K.K.; et al. Definition for Rheumatoid Arthritis Erosions Imaged with High Resolution Peripheral Quantitative Computed Tomography and Interreader Reliability for Detection and Measurement. *J. Rheumatol.* **2016**, *43*, 1935–1940. [[CrossRef](#)] [[PubMed](#)]
12. Ibrahim-Nasser, N.; Marotte, H.; Valery, A.; Salliot, C.; Toumi, H.; Lespessailles, E. Precision and sources of variability in the assessment of rheumatoid arthritis erosions by HRpQCT. *Jt. Bone Spine* **2018**, *85*, 211–217. [[CrossRef](#)] [[PubMed](#)]
13. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O.; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **2010**, *69*, 1580–1588. [[CrossRef](#)] [[PubMed](#)]
14. Funovits, J.; Aletaha, D.; Bykerk, V.; Combe, B.; Dougados, M.; Emery, P.; Felson, D.; Hawker, G.; Hazes, J.M.; Huizinga, T.; et al. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis: Methodological report phase I. *Ann. Rheum. Dis.* **2010**, *69*, 1589–1595. [[CrossRef](#)]
15. van der Heijde, D.; van der Helm-van Mil, A.H.; Aletaha, D.; Bingham, C.O.; Burmester, G.R.; Dougados, M.; Emery, P.; Felson, D.; Knevel, R.; Kvien, T.K. EULAR definition of erosive disease in light of the 2010 ACR/EULAR rheumatoid arthritis classification criteria. *Ann. Rheum. Dis.* **2013**, *72*, 479–481. [[CrossRef](#)]
16. Siddle, H.J.; Hensor, E.M.; Hodgson, R.J.; Grainger, A.J.; Redmond, A.C.; Wakefield, R.J.; Helliwell, P.S. Anatomical location of erosions at the metatarsophalangeal joints in patients with rheumatoid arthritis. *Rheumatology* **2014**, *53*, 932–936. [[CrossRef](#)]
17. Alcalde, M.; D'Agostino, M.A.; Bruyn, G.A.; Moller, I.; Iagnocco, A.; Wakefield, R.J.; Naredo, E.; Force, O.U.T. A systematic literature review of US definitions, scoring systems and validity according to the OMERACT filter for tendon lesion in RA and other inflammatory joint diseases. *Rheumatology* **2012**, *51*, 1246–1260. [[CrossRef](#)]
18. Ostergaard, M.; Peterfy, C.; Conaghan, P.; McQueen, F.; Bird, P.; Ejlberg, B.; Shnier, R.; O'Connor, P.; Klarlund, M.; Emery, P.; et al. OMERACT Rheumatoid Arthritis Magnetic Resonance Imaging Studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. *J. Rheumatol.* **2003**, *30*, 1385–1386.
19. Ostergaard, M.; Moller Dohn, U.; Duer-Jensen, A.; Hetland, M.L.; Horslev-Petersen, K.; Stengaard-Pedersen, K.; Junker, P.; Podenphant, J.; Ejlberg, B. Patterns of magnetic resonance imaging bone erosion in rheumatoid arthritis—which bones are most frequently involved and show the most change? *J. Rheumatol.* **2011**, *38*, 2014–2017. [[CrossRef](#)]
20. Hammer, H.B.; Haavardsholm, E.A.; Boyesen, P.; Kvien, T.K. Bone erosions at the distal ulna detected by ultrasonography are associated with structural damage assessed by conventional radiography and MRI: A study of patients with recent onset rheumatoid arthritis. *Rheumatology* **2009**, *48*, 1530–1532. [[CrossRef](#)]
21. Resnick, D.; Gmelich, J.T. Bone fragmentation in the rheumatoid wrist: Radiographic and pathologic considerations. *Radiology* **1975**, *114*, 315–321. [[CrossRef](#)]
22. Boutry, N.; Morel, M.; Flipo, R.M.; Demondion, X.; Cotten, A. Early rheumatoid arthritis: A review of MRI and sonographic findings. *AJR Am. J. Roentgenol.* **2007**, *189*, 1502–1509. [[CrossRef](#)]
23. Leak, R.S.; Rayan, G.M.; Arthur, R.E. Longitudinal radiographic analysis of rheumatoid arthritis in the hand and wrist. *J. Hand Surg. Am. Vol.* **2003**, *28*, 427–434. [[CrossRef](#)]

24. Lee, K.A.; Min, S.H.; Kim, T.H.; Lee, S.H.; Kim, H.R. Magnetic resonance imaging-assessed synovial and bone changes in hand and wrist joints of rheumatoid arthritis patients. *Korean J. Intern. Med.* **2019**, *34*, 651–659. [[CrossRef](#)]
25. Kitamura, T.; Murase, T.; Hashimoto, J.; Tomita, T.; Arimitsu, S.; Yoshikawa, H.; Sugamoto, K. Radiographic study on the pattern of wrist joint destruction in rheumatoid arthritis. *Clin. Rheumatol.* **2011**, *30*, 353–359. [[CrossRef](#)]
26. Taleisnik, J. Rheumatoid synovitis of the volar compartment of the wrist joint: Its radiological signs and its contribution to wrist and hand deformity. *J. Hand Surg. Am. Vol.* **1979**, *4*, 526–535. [[CrossRef](#)]
27. Karasick, D.; Schweitzer, M.E.; O'Hara, B.J. Distal fibular notch: A frequent manifestation of the rheumatoid ankle. *Skelet. Radiol.* **1997**, *26*, 529–532.
28. Baan, H.; Bezooijen, R.; Avenarius, J.K.; Dubbeldam, R.; Drossaers-Bakker, W.K.; van de Laar, M.A. Magnetic resonance imaging of the rheumatic foot according to the RAMRIS system is reliable. *J. Rheumatol.* **2011**, *38*, 1003–1008. [[CrossRef](#)]
29. Lee, D.M.; Weinblatt, M.E. Rheumatoid arthritis. *Lancet* **2001**, *358*, 903–911. [[CrossRef](#)]
30. Tan, A.L.; Tanner, S.F.; Conaghan, P.G.; Radjenovic, A.; O'Connor, P.; Brown, A.K.; Emery, P.; McGonagle, D. Role of metacarpophalangeal joint anatomic factors in the distribution of synovitis and bone erosion in early rheumatoid arthritis. *Arthritis Rheum.* **2003**, *48*, 1214–1222. [[CrossRef](#)]
31. Schett, G.; Gravallese, E. Bone erosion in rheumatoid arthritis: Mechanisms, diagnosis and treatment. *Nat. Rev. Rheumatol.* **2012**, *8*, 656–664. [[CrossRef](#)]
32. Werner, D.; Simon, D.; Englbrecht, M.; Stemmler, F.; Simon, C.; Berlin, A.; Haschka, J.; Renner, N.; Buder, T.; Engelke, K.; et al. Early Changes of the Cortical Micro-Channel System in the Bare Area of the Joints of Patients with Rheumatoid Arthritis. *Arthritis Rheumatol.* **2017**, *69*, 1580–1587. [[CrossRef](#)]
33. McQueen, F.; Clarke, A.; McHaffie, A.; Reeves, Q.; Williams, M.; Robinson, E.; Dong, J.; Chand, A.; Mulders, D.; Dalbeth, N. Assessment of cartilage loss at the wrist in rheumatoid arthritis using a new MRI scoring system. *Ann. Rheum. Dis.* **2010**, *69*, 1971–1975. [[CrossRef](#)]
34. Peters, M.; van Tubergen, A.; Scharmga, A.; Driessen, A.; van Rietbergen, B.; Loeffen, D.; Weijers, R.; Geusens, P.; van den Bergh, J. Assessment of Cortical Interruptions in the Finger Joints of Patients with Rheumatoid Arthritis Using HR-pQCT, Radiography, and MRI. *J. Bone Miner. Res.* **2018**, *33*, 1676–1685. [[CrossRef](#)]
35. Conaghan, P.G.; O'Connor, P.; McGonagle, D.; Astin, P.; Wakefield, R.J.; Gibbon, W.W.; Quinn, M.; Karim, Z.; Green, M.J.; Proudman, S.; et al. Elucidation of the relationship between synovitis and bone damage: A randomized magnetic resonance imaging study of individual joints in patients with early rheumatoid arthritis. *Arthritis Rheum.* **2003**, *48*, 64–71. [[CrossRef](#)]
36. Schleich, C.; Muller-Lutz, A.; Sewerin, P.; Ostendorf, B.; Buchbender, C.; Schneider, M.; Antoch, G.; Miese, F. Intra-individual assessment of inflammatory severity and cartilage composition of finger joints in rheumatoid arthritis. *Skelet. Radiol.* **2015**, *44*, 513–518. [[CrossRef](#)]
37. Favalli, E.G.; Becciolini, A.; Biggoggero, M. Structural integrity versus radiographic progression in rheumatoid arthritis. *RMD Open* **2015**, *1*, e000064. [[CrossRef](#)]
38. Boeters, D.M.; Nieuwenhuis, W.P.; van Steenberghe, H.W.; Reijnierse, M.; Landewe, R.B.M.; van der Helm-van Mil, A.H.M. Are MRI-detected erosions specific for RA? A large explorative cross-sectional study. *Ann. Rheum. Dis.* **2018**, *77*, 861–868. [[CrossRef](#)]
39. Felloni, P.; Larkman, N.; Dunca, R.; Cotton, A. Inflammatory Arthritides: Imaging Pitfalls. In *Pitfalls in Musculoskeletal Radiology*; Peh, W., Ed.; Springer: Berlin/Heidelberg, Germany, 2017; pp. 697–712.
40. Dihlmann, W.; Bandick, J. *Die Gelenksilhouette: Das Informationspotential der Röntgenstrahlen*; Springer: Berlin/Heidelberg, Germany, 1995.
41. Wawer, R.; Budzik, J.F.; Demondion, X.; Forzy, G.; Cotten, A. Carpal pseudoerosions: A plain X-ray interpretation pitfall. *Skelet. Radiol.* **2014**, *43*, 1377–1385. [[CrossRef](#)]
42. Sharp, J.T.; Wolfe, F.; Lassere, M.; Boers, M.; Van Der Heijde, D.; Larsen, A.; Paulus, H.; Rau, R.; Strand, V. Variability of precision in scoring radiographic abnormalities in rheumatoid arthritis by experienced readers. *J. Rheumatol.* **2004**, *31*, 1062–1072.

43. McQueen, F.M.; Benton, N.; Perry, D.; Crabbe, J.; Robinson, E.; Yeoman, S.; McLean, L.; Stewart, N. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum.* **2003**, *48*, 1814–1827. [[CrossRef](#)]
44. Ejbjerg, B.; Narvestad, E.; Rostrup, E.; Szkudlarek, M.; Jacobsen, S.; Thomsen, H.S.; Ostergaard, M. Magnetic resonance imaging of wrist and finger joints in healthy subjects occasionally shows changes resembling erosions and synovitis as seen in rheumatoid arthritis. *Arthritis Rheum.* **2004**, *50*, 1097–1106. [[CrossRef](#)] [[PubMed](#)]
45. Olech, E.; Crues, J.V.; Yocum, D.E.; Merrill, J.T. Bone marrow edema is the most specific finding for rheumatoid arthritis (RA) on noncontrast magnetic resonance imaging of the hands and wrists: A comparison of patients with RA and healthy controls. *J. Rheumatol.* **2010**, *37*, 265–274. [[CrossRef](#)] [[PubMed](#)]
46. Rothschild, B.M. Significance of “Erosion-like Lesions” in “Healthy Controls”. *J. Rheumatol.* **2010**, *37*, 1964. [[CrossRef](#)] [[PubMed](#)]
47. Finzel, S.; Ohrndorf, S.; Englbrecht, M.; Stach, C.; Messerschmidt, J.; Schett, M.; Backhaus, G. A detailed comparative study of high-resolution ultrasound and micro-computed tomography for detection of arthritic bone erosions. *Arthritis Rheum.* **2011**, *63*, 1231–1236. [[CrossRef](#)]
48. Langs, G.; Peloschek, P.; Bischof, H.; Kainberger, F. Model-based erosion spotting and visualization in rheumatoid arthritis. *Acad. Radiol.* **2007**, *14*, 1179–1188. [[CrossRef](#)]
49. Yang, H.; Rivoire, J.; Hoppe, M.; Srikhum, W.; Imboden, J.; Link, T.M.; Li, X. Computer-aided and manual quantifications of MRI synovitis, bone marrow edema-like lesions, erosion and cartilage loss in rheumatoid arthritis of the wrist. *Skelet. Radiol.* **2015**, *44*, 539–547. [[CrossRef](#)]
50. Liberati, A.; Altman, D.G.; Tetzlaff, J.; Mulrow, C.; Gotzsche, P.C.; Ioannidis, J.P.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *J. Clin. Epidemiol.* **2009**, *62*, e1–34. [[CrossRef](#)]
51. McQueen, F.; Ostergaard, M.; Peterfy, C.; Lassere, M.; Ejbjerg, B.; Bird, P.; O'Connor, P.; Genant, H.; Shnier, R.; Emery, P.; et al. Pitfalls in scoring MR images of rheumatoid arthritis wrist and metacarpophalangeal joints. *Ann. Rheum. Dis.* **2005**, *64*, i48–i55. [[CrossRef](#)]
52. Peluso, G.; Bosello, S.L.; Gremese, E.; Mirone, L.; Di Gregorio, F.; Di Molfetta, V.; Pirroni, T.; Ferraccioli, G. Detection of bone erosions in early rheumatoid arthritis: 3D ultrasonography versus computed tomography. *Clin. Rheumatol.* **2015**, *34*, 1181–1186. [[CrossRef](#)]
53. Dohn, U.M.; Ejbjerg, B.J.; Court-Payen, M.; Hasselquist, M.; Narvestad, E.; Szkudlarek, M.; Moller, J.M.; Thomsen, H.S.; Ostergaard, M. Are bone erosions detected by magnetic resonance imaging and ultrasonography true erosions? A comparison with computed tomography in rheumatoid arthritis metacarpophalangeal joints. *Arthritis Res. Ther.* **2006**, *8*, R110. [[CrossRef](#)]
54. Canella Moraes Carmo, C.; Cruz, G.P.; Trudell, D.; Hughes, T.; Chung, C.; Resnick, D. Anatomical features of metacarpal heads that simulate bone erosions: Cadaveric study using computed tomography scanning and sectional radiography. *J. Comput. Assist. Tomogr.* **2009**, *33*, 573–578. [[CrossRef](#)]
55. Torshizy, H.; Hughes, T.H.; Trudell, D.; Resnick, D. Anatomic features of metatarsal heads that simulate erosive disease: Cadaveric study using CT, radiography, and dissection with special emphasis on cross-sectional characterization of osseous anatomy. *AJR Am. J. Roentgenol.* **2008**, *190*, W175–W181. [[CrossRef](#)]
56. Robertson, P.L.; Page, P.J.; McColl, G.J. Inflammatory arthritis-like and other MR findings in wrists of asymptomatic subjects. *Skelet. Radiol.* **2006**, *35*, 754–764. [[CrossRef](#)]
57. Dohn, U.M.; Terslev, L.; Szkudlarek, M.; Hansen, M.S.; Hetland, M.L.; Hansen, A.; Madsen, O.R.; Hasselquist, M.; Moller, J.; Ostergaard, M. Detection, scoring and volume assessment of bone erosions by ultrasonography in rheumatoid arthritis: Comparison with CT. *Ann. Rheum. Dis.* **2013**, *72*, 530–534. [[CrossRef](#)]
58. Alasaarela, E.; Suramo, I.; Tervonen, O.; Lahde, S.; Takalo, R.; Hakala, M. Evaluation of humeral head erosions in rheumatoid arthritis: A comparison of ultrasonography, magnetic resonance imaging, computed tomography and plain radiography. *Br. J. Rheumatol.* **1998**, *37*, 1152–1156. [[CrossRef](#)]
59. Foley-Nolan, D.; Stack, J.P.; Ryan, M.; Redmond, U.; Barry, C.; Ennis, J.; Coughlan, R.J. Magnetic resonance imaging in the assessment of rheumatoid arthritis—A comparison with plain film radiographs. *Br. J. Rheumatol.* **1991**, *30*, 101–106. [[CrossRef](#)]

60. Cimmino, M.A.; Bountis, C.; Silvestri, E.; Garlaschi, G.; Accardo, S. An appraisal of magnetic resonance imaging of the wrist in rheumatoid arthritis. *Semin. Arthritis Rheum.* **2000**, *30*, 180–195. [[CrossRef](#)]
61. Forslind, K.; Johanson, A.; Larsson, E.M.; Svensson, B. Magnetic resonance imaging of the fifth metatarsophalangeal joint compared with conventional radiography in patients with early rheumatoid arthritis. *Scand. J. Rheumatol.* **2003**, *32*, 131–137. [[CrossRef](#)]
62. Amin, M.F.; Ismail, F.M.; el Shereef, R.R. The role of ultrasonography in early detection and monitoring of shoulder erosions, and disease activity in rheumatoid arthritis patients; comparison with MRI examination. *Acad. Radiol.* **2012**, *19*, 693–700. [[CrossRef](#)]
63. Aurell, Y.; Andersson, M.; Forslind, K. Cone-beam computed tomography, a new low-dose three-dimensional imaging technique for assessment of bone erosions in rheumatoid arthritis: Reliability assessment and comparison with conventional radiography—A BARFOT study. *Scand. J. Rheumatol.* **2018**, *47*, 173–177. [[CrossRef](#)]
64. Ejbjerg, B.J.; Vestergaard, A.; Jacobsen, S.; Thomsen, H.; Ostergaard, M. Conventional radiography requires a MRI-estimated bone volume loss of 20% to 30% to allow certain detection of bone erosions in rheumatoid arthritis metacarpophalangeal joints. *Arthritis Res. Ther.* **2006**, *8*, R59. [[CrossRef](#)]
65. Dohn, U.M.; Ejbjerg, B.J.; Hasselquist, M.; Narvestad, E.; Moller, J.; Thomsen, H.S.; Ostergaard, M. Detection of bone erosions in rheumatoid arthritis wrist joints with magnetic resonance imaging, computed tomography and radiography. *Arthritis Res. Ther.* **2008**, *10*, R25. [[CrossRef](#)]
66. Kleyer, A.; Krieter, M.; Oliveira, I.; Faustini, F.; Simon, D.; Kaemmerer, N.; Cavalcante, A.; Tabosa, T.; Rech, J.; Hueber, A.; et al. High prevalence of tenosynovial inflammation before onset of rheumatoid arthritis and its link to progression to RA-A combined MRI/CT study. *Semin. Arthritis Rheum.* **2016**, *46*, 143–150. [[CrossRef](#)]
67. Ulas, S.T.; Diekhoff, T.; Hermann, K.G.A.; Poddubnyy, D.; Hamm, B.; Makowski, M.R. Susceptibility-weighted MR imaging to improve the specificity of erosion detection: A prospective feasibility study in hand arthritis. *Skelet. Radiol.* **2019**, *48*, 721–728. [[CrossRef](#)]
68. Emond, P.D.; Inglis, D.; Choi, A.; Tricta, J.; Adachi, J.D.; Gordon, C.L. Volume measurement of bone erosions in magnetic resonance images of patients with rheumatoid arthritis. *Magn. Reson. Med.* **2012**, *67*, 814–823. [[CrossRef](#)]
69. McQueen, F.M.; Stewart, N.; Crabbe, J.; Robinson, E.; Yeoman, S.; Tan, P.L.; McLean, L. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals a high prevalence of erosions at four months after symptom onset. *Ann. Rheum. Dis.* **1998**, *57*, 350–356. [[CrossRef](#)]
70. McQueen, F.M.; Benton, N.; Crabbe, J.; Robinson, E.; Yeoman, S.; McLean, L.; Stewart, N. What is the fate of erosions in early rheumatoid arthritis? Tracking individual lesions using X rays and magnetic resonance imaging over the first two years of disease. *Ann. Rheum. Dis.* **2001**, *60*, 859–868.
71. Dihlmann, W. A early sign of arthritis. The loss of subchondral marginal lamellae. *Z. Rheumaforsch.* **1968**, *27*, 129–132.
72. Regensburger, A.; Rech, J.; Englbrecht, M.; Finzel, S.; Kraus, S.; Hecht, K.; Kleyer, A.; Haschka, J.; Hueber, A.J.; Cavallaro, A.; et al. A comparative analysis of magnetic resonance imaging and high-resolution peripheral quantitative computed tomography of the hand for the detection of erosion repair in rheumatoid arthritis. *Rheumatology* **2015**, *54*, 1573–1581. [[CrossRef](#)]
73. Schrank, C.; Meirer, R.; Stabler, A.; Nerlich, A.; Reiser, M.; Putz, R. Morphology and topography of intraosseous ganglion cysts in the carpus: An anatomic, histopathologic, and magnetic resonance imaging correlation study. *J. Hand Surg. Am.* **2003**, *28*, 52–61. [[CrossRef](#)]
74. Paparo, F.; Fabbro, E.; Piccazzo, R.; Revelli, M.; Ferrero, G.; Muda, A.; Cimmino, M.A.; Garlaschi, G. Multimodality imaging of intraosseous ganglia of the wrist and their differential diagnosis. *Radiol. Med.* **2012**, *117*, 1355–1373. [[CrossRef](#)]
75. Mangnus, L.; van Steenbergen, H.W.; Lindqvist, E.; Brouwer, E.; Reijnierse, M.; Huizinga, T.W.; Gregersen, P.K.; Berglin, E.; Rantapaa-Dahlqvist, S.; van der Heijde, D.; et al. Studies on ageing and the severity of radiographic joint damage in rheumatoid arthritis. *Arthritis Res. Ther.* **2015**, *17*, 222. [[CrossRef](#)]
76. Avenarius, D.M.; Ording Muller, L.S.; Eldevik, P.; Owens, C.M.; Rosendahl, K. The paediatric wrist revisited—Findings of bony depressions in healthy children on radiographs compared to MRI. *Pediatr. Radiol.* **2012**, *42*, 791–798. [[CrossRef](#)]

77. Boavida, P.; Hargunani, R.; Owens, C.M.; Rosendahl, K. Magnetic resonance imaging and radiographic assessment of carpal depressions in children with juvenile idiopathic arthritis: Normal variants or erosions? *J. Rheumatol.* **2012**, *39*, 645–650. [[CrossRef](#)]
78. Boavida, P.; Lambot-Juhan, K.; Muller, L.S.; Damasio, B.; de Horatio, L.T.; Malattia, C.; Owens, C.M.; Rosendahl, K. Carpal erosions in children with juvenile idiopathic arthritis: Repeatability of a newly devised MR-scoring system. *Pediatr. Radiol.* **2015**, *45*, 1972–1980. [[CrossRef](#)]
79. Benjamin, M.; McGonagle, D. The anatomical basis for disease localisation in seronegative spondyloarthropathy at entheses and related sites. *J. Anat.* **2001**, *199*, 503–526. [[CrossRef](#)]
80. Platzer, W. *Taschenatlas Anatomie 01. Bewegungsapparat*; Thieme Georg Verlag: New York, NY, USA, 2009.
81. Standring, S. *Gray's Anatomy: The Anatomical Basis of Clinical Practice*; Elsevier: Amsterdam, The Netherlands, 2008.
82. Boutry, N.; Larde, A.; Demondion, X.; Cortet, B.; Cotten, H.; Cotten, A. Metacarpophalangeal joints at US in asymptomatic volunteers and cadaveric specimens. *Radiology* **2004**, *232*, 716–724. [[CrossRef](#)]
83. Fanghänel, J.; Pera, F.; Anderhuber, F.; Nitsch, R. *Waldeyer—Anatomie des Menschen*, 18th ed.; Walter de Gruyter: Berlin, Germany, 2009.
84. Manaster, B.J.; Crim, J.R. *Imaging Anatomy: Musculoskeletal*; Elsevier: Amsterdam, The Netherlands, 2016.
85. Eshed, I.; Bollow, M.; McGonagle, D.G.; Tan, A.L.; Althoff, C.E.; Asbach, P.; Hermann, K.G. MRI of enthesitis of the appendicular skeleton in spondyloarthritis. *Ann. Rheum. Dis.* **2007**, *66*, 1553–1559. [[CrossRef](#)]
86. Boutry, N.; Hachulla, E.; Flipo, R.M.; Cortet, B.; Cotten, A. MR imaging findings in hands in early rheumatoid arthritis: Comparison with those in systemic lupus erythematosus and primary Sjogren syndrome. *Radiology* **2005**, *236*, 593–600. [[CrossRef](#)]
87. Boutroy, S.; Chapurlat, R.; Vanden-Bossche, A.; Locrelle, H.; Thomas, T.; Marotte, H. Erosion or vascular channel? *Arthritis Rheum.* **2015**, *67*, 2956. [[CrossRef](#)]
88. Hoving, J.L.; Buchbinder, R.; Hall, S.; Lawler, G.; Coombs, P.; McNealy, S.; Bird, P.; Connell, D. A comparison of magnetic resonance imaging, sonography, and radiography of the hand in patients with early rheumatoid arthritis. *J. Rheumatol.* **2004**, *31*, 663–675.
89. Wang, B.; Overgaard, S.; Chemnitz, J.; Ding, M. Cancellous and Cortical Bone Microarchitectures of Femoral Neck in Rheumatoid Arthritis and Osteoarthritis Compared with Donor Controls. *Calcif. Tissue Int.* **2016**, *98*, 456–464. [[CrossRef](#)] [[PubMed](#)]
90. Pery, D.; Stewart, N.; Benton, N.; Robinson, E.; Yeoman, S.; Crabbe, J.; McQueen, F. Detection of erosions in the rheumatoid hand; a comparative study of multidetector computerized tomography versus magnetic resonance scanning. *J. Rheumatol.* **2005**, *32*, 256–267. [[PubMed](#)]
91. Dohn, U.M.; Ejbjerg, B.J.; Hasselquist, M.; Narvestad, E.; Court-Payen, M.; Szkudlarek, M.; Moller, J.; Thomsen, H.S.; Ostergaard, M. Rheumatoid arthritis bone erosion volumes on CT and MRI: Reliability and correlations with erosion scores on CT, MRI and radiography. *Ann. Rheum. Dis.* **2007**, *66*, 1388–1392. [[CrossRef](#)] [[PubMed](#)]
92. Hermann, K.G.; Backhaus, M.; Schneider, U.; Labs, K.; Loreck, D.; Zuhlsdorf, S.; Schink, T.; Fischer, T.; Hamm, B.; Bollow, M. Rheumatoid arthritis of the shoulder joint: Comparison of conventional radiography, ultrasound, and dynamic contrast-enhanced magnetic resonance imaging. *Arthritis Rheum.* **2003**, *48*, 3338–3349. [[CrossRef](#)]
93. Hodler, J.; Terrier, B.; von Schulthess, G.K.; Fuchs, W.A. MRI and sonography of the shoulder. *Clin. Radiol.* **1991**, *43*, 323–327. [[CrossRef](#)]
94. Klarlund, M.; Ostergaard, M.; Jensen, K.E.; Madsen, J.L.; Skjold, H.; Lorenzen, I. Magnetic resonance imaging, radiography, and scintigraphy of the finger joints: One year follow up of patients with early arthritis. The TIRA Group. *Ann. Rheum. Dis.* **2000**, *59*, 521–528. [[CrossRef](#)]
95. Yang, H.; Yu, A.; Burghardt, A.J.; Virayavanich, W.; Link, T.M.; Imboden, J.B.; Li, X. Quantitative characterization of metacarpal and radial bone in rheumatoid arthritis using high resolution-peripheral quantitative computed tomography. *Int. J. Rheum. Dis.* **2017**, *20*, 353–362. [[CrossRef](#)]
96. Fouque-Aubert, A.; Boutroy, S.; Marotte, H.; Vilayphiou, N.; Bacchetta, J.; Miossec, P.; Delmas, P.D.; Chapurlat, R.D. Assessment of hand bone loss in rheumatoid arthritis by high-resolution peripheral quantitative CT. *Ann. Rheum. Dis.* **2010**, *69*, 1671–1676. [[CrossRef](#)]

97. Feehan, L.; Buie, H.; Li, L.; McKay, H. A customized protocol to assess bone quality in the metacarpal head, metacarpal shaft and distal radius: A high resolution peripheral quantitative computed tomography precision study. *BMC Musculoskelet. Disord.* **2013**, *14*, 367. [[CrossRef](#)] [[PubMed](#)]
98. Kocijan, R.; Finzel, S.; Englbrecht, M.; Engelke, K.; Rech, J.; Schett, G. Decreased quantity and quality of the periarticular and nonperiarticular bone in patients with rheumatoid arthritis: A cross-sectional HR-pQCT study. *J. Bone Miner. Res.* **2014**, *29*, 1005–1014. [[CrossRef](#)] [[PubMed](#)]
99. Guerhazi, A.; Taouli, B.; Lynch, J.A.; Peterfy, C.G. Imaging of bone erosion in rheumatoid arthritis. *Semin. Musculoskelet. Radiol.* **2004**, *8*, 269–285. [[CrossRef](#)] [[PubMed](#)]
100. Zayat, A.S.; Ellegaard, K.; Conaghan, P.G.; Terslev, L.; Hensor, E.M.; Freeston, J.E.; Emery, P.; Wakefield, R.J. The specificity of ultrasound-detected bone erosions for rheumatoid arthritis. *Ann. Rheum. Dis.* **2015**, *74*, 897–903. [[CrossRef](#)] [[PubMed](#)]
101. Buckland-Wright, J.C. X-ray assessment of activity in rheumatoid disease. *Br. J. Rheumatol.* **1983**, *22*, 3–10. [[CrossRef](#)]
102. Canella, C.; Philippe, P.; Pansini, V.; Salleron, J.; Flipo, R.M.; Cotten, A. Use of tomosynthesis for erosion evaluation in rheumatoid arthritic hands and wrists. *Radiology* **2011**, *258*, 199–205. [[CrossRef](#)]



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Article

Methotrexate Restores CD73 Expression on Th1.17 in Rheumatoid Arthritis and Psoriatic Arthritis Patients and May Contribute to Its Anti-Inflammatory Effect through Ado Production

Marion Bossennec ^{1,2}, Céline Rodriguez ^{1,2}, Margaux Hubert ^{1,2}, Anthony Di-Roio ^{1,2},
Christelle Machon ^{3,4}, Jérôme Guitton ^{4,5}, Priscilla Battiston-Montagne ^{1,6}, Mathilde Couturier ⁷,
Hubert Marotte ^{8,9}, Christophe Caux ^{1,2}, Fabienne Coury ^{7,10,†} and
Christine Ménétrier-Caux ^{1,2,*,†}

- ¹ Immunology Department, University of Lyon, Claude Bernard Lyon 1 University, INSERM 1052, CNRS 5286, Cancer Research Center of Lyon (CRCL), Centre Léon Bérard, 69008 Lyon, France; marion.bossennec@gmail.com (M.B.); celine.rodriguez@lyon.unicancer.fr (C.R.); margaux.hubert@lyon.unicancer.fr (M.H.); anthony.diroio@lyon.unicancer.fr (A.D.-R.); priscillia.battiston-montagne@lyon.unicancer.fr (P.B.-M.); christophe.caux@lyon.unicancer.fr (C.C.)
- ² Team “Therapeutic Targeting of the Tumor Cells and Their Immune Stroma”, INSERM U1052, CRCL, 69008 Lyon, France
- ³ Analytical Chemistry Department, ISPB Faculty of Pharmacy, University of Lyon, Claude Bernard Lyon 1 University, 69008 Lyon, France; christelle.machon@chu-lyon.fr
- ⁴ Biochemistry and Toxicology Laboratory, Lyon Sud Hospital, 69310 Pierre-Bénite, France; jerome.guitton@univ-lyon1.fr
- ⁵ Toxicology Department, ISPB Faculty of Pharmacy, University of Lyon, Claude Bernard Lyon 1 University, 69008 Lyon, France
- ⁶ Cytometry Platform, CRCL, 69008 Lyon, France
- ⁷ Rheumatology Department, Lyon Sud Hospital, 69310 Pierre-Bénite, France; docteurcouthuriersrhumato@gmail.com (M.C.); fabienne.coury-lucas@chu-lyon.fr (F.C.)
- ⁸ Rheumatology Department, CHU Saint-Etienne, 42100 Saint-Etienne, France; hubert.marotte@chu-st-etienne.fr
- ⁹ INSERM U1059, Jean Monnet University, 42100 Saint-Etienne, France
- ¹⁰ INSERM, UMR 1033, Claude Bernard Lyon 1 University, 69008 Lyon, France
- * Correspondence: christine.caux@lyon.unicancer.fr; Tel.: +33-4-78-78-27-50
- † Co-last authorship.

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Abstract: Objectives: Th1.17 are highly polyfunctional, potentially harmful CD4⁺ effector T cells (Teff) through IFN- γ and IL-17A coproduction. Th1.17 take part in the pathophysiology of rheumatoid arthritis (RA) and psoriatic arthritis (PsA), in which their hyper activation results in part from defects in negative regulation mechanisms. We recently demonstrated that the ecto-nucleotidase CD73 delineates a Th1.17-enriched Teff population and acts as an endogenous regulatory mechanism. Because Methotrexate (MTX), used as first line treatment of RA and PsA, increases extracellular concentrations of AMP and immunosuppressive adenosine, we investigated the modulation of CD73 by MTX treatment on Teff in RA/PsA patients. Methods: In a prospective cohort of 26 RA and 15 PsA patients before or under MTX treatment, we evaluated CD73 expression on blood Teff subsets, their cytokine production and AMPase functions. Results: We showed a decreased CD73 expression on Th1.17 and Th1 in untreated patients compared to healthy donors that was partly restored under MTX. This decrease in untreated patients leads to a halved Ado production by Th1.17 cells. CD73⁺ Teff remained functional under MTX treatment, but their CD73 re-expression may contribute to control their activation. Conclusion: Our study unveils uncovered mode of action of MTX on Teff subsets modulation and in the adenosine-dependent termination of inflammation in RA and PsA.

Keywords: Th1.17; IL-17A; IFN- γ , CD73; adenosine; rheumatoid arthritis; psoriatic arthritis; methotrexate; regulation

1. Introduction

Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA) are chronic inflammatory disorders characterized by tenderness and swelling of the joints. If not treated, both can lead to bone erosion, resulting in joint destruction due to osteoclasts activation. RA is an autoimmune systemic disease characterized by the presence of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) [1]. PsA is usually associated with the skin condition psoriasis (Pso) [2]. In both cases, study of the immune infiltrate of inflamed joints has shed light on particularly deleterious memory CD4⁺ T helper lymphocytes (Th): Th17 and Th1.17 [3–5]. These cells, found enriched at the inflamed site, secrete high amounts of the pro-inflammatory cytokine IL-17A, responsible for many features of both RA and PsA. IL-17A indeed contributes to the recruitment of pro-inflammatory monocytes and neutrophils at the site of inflammation, and promotes the transformation of the fibroblast-like synoviocytes present in the synovial lining toward a pro-inflammatory phenotype [6–8]. In the presence of IL-17A, these cells secrete IL-6 and TNF [9] and high levels of RANKL, which in turn stimulate osteoclast differentiation [10]. Therefore, IL-17A is implicated in the positive feedback loop at the roots of the chronic inflammation and bone destruction observed in joints. Moreover, in RA and PsA, T cells display reduced sensitivity to the diverse immune suppressive functions exerted by regulatory T cells (Treg) [11]. Treg are involved in the modulation of extracellular purine derivatives levels, through their membrane expression of the ecto-enzyme CD39, which degrades pro-inflammatory extracellular ATP into AMP. AMP can, in turn, be degraded into immunosuppressive adenosine (Ado) by the ecto-nucleotidase CD73. We recently demonstrated that in human, Treg express CD39, but no CD73; therefore, third-party CD73 expressing cells are required for the degradation of AMP into Ado [12]. CD73 is in particular expressed by a fraction of non-regulatory CD4⁺ memory T cells (Teff) strongly enriched in Th1.17 and Th17 [12]. We showed that CD73 expression by Teff renders them selectively sensitive to Ado through their cooperation with CD39⁺ Treg for extracellular ATP degradation. In the context of chronic inflammation, modulation of expression of these ecto-enzymes on Teff, and thus of Ado production, may regulate T cell activation.

RA and PsA treatment options are mainly composed of an arsenal of disease modifying anti rheumatic drugs (DMARDs), among which Methotrexate (MTX) is still largely used due to its efficiency, low toxicity and cost effectiveness [13,14]. Its mode of action at low doses (5–20 mg/week) for RA and PsA treatment remains incompletely understood. Apart from its role as anti-folate agent, other mechanisms have been proposed to explain clinical improvement upon MTX treatment (reviewed in [13]). Robust evidence suggests that MTX acts through the potentiation of Ado signaling. Indeed, MTX inhibits the 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase enzyme (ATIC) [13–15] resulting in increase in both intracellular AICAR levels and extracellular AMP and Ado release. However, the majority of extracellular Ado is in fact generated from extracellular transport of ATP and AMP degraded by CD39/CD73 that are required for MTX-induced immune suppression in mouse models [16]. Extracellular Ado inhibits T cell activation and proliferation in a paracrine manner, through the engagement of its A2A and A2B receptors which expression is upregulated on activated Teff [12,17,18], and through the amplification of Treg expressing A2AR [19].

In this study, we evaluated the presence and distribution of CD73⁺ Teff among Th populations in the blood of untreated RA and PsA patients. We also assessed the functionality of these cells in patients in comparison to healthy donors' blood. We demonstrated that loss of CD73 membrane expression in untreated patients resulted in a lowered Ado production, potentially contributing to impaired control of the inflammation. Under MTX treatment, CD73 was partially recovered on Teff, which was associated with a restored capacity to degrade AMP into Ado.

Therefore, CD73 expression level on Teff may represent a therapeutic target worth considering in the treatment of RA by restoring and stabilizing it through an immunosuppressive feedback loop enabling Ado production.

2. Experimental Section

2.1. Patients

Patients aged ≥ 18 years, naive of biologics, with RA fulfilling the American College of Rheumatology and European League Against Rheumatism 2009 criteria [20] or with PsA fulfilling the Classification of PsA (CASPAR) criteria [21] were enrolled in the LADORIC study (NCT03953378) after written informed consent, in accordance with the Declaration of Helsinki. The approval of the ethics committee was not required in accordance with our institution's policy. Clinical and biological information was collected prospectively (Table 1).

Table 1. Characteristics of RA and PsA patients enrolled in the study.

| | RA Patients <i>n</i> = 26 | PsA Patients <i>n</i> = 15 | Healthy Controls <i>n</i> = 12 |
|--------------------------------------|------------------------------|-------------------------------|-----------------------------------|
| Gender ratio (f/m, <i>n</i>) | 19/7 | 9/6 | 8/4 |
| Age (years) | 56 (22–82) | 53 (31–79) | 49 (25–69) |
| DAS28 Untreated | 4.6 (1.6–6.4) | - | - |
| MTX-treated | 3.4 (1.8–7.2) | - | - |
| CRP (mg/mL) Untreated | 17.9 (0.6–60) | 16.4 (2.6–88) | - |
| MTX-treated | 5.2 (0.5–29) | 4.3 (1.3–10) | - |
| Mean MTX treatment duration (months) | 9.3 (3–22) | 6 (3–22) | - |
| MTX doses (mg/week) | (15–20) | (15–20) | - |
| RF detection (yes/no, <i>n</i>) | 22/4 | Not detected | - |
| ACPA detection (yes/no, <i>n</i>) | 24/2 | Not detected | - |

Data presented as (range). RA: Rheumatoid Arthritis; PsA: Psoriatic Arthritis; DAS28: 28 joints activity score; RF: Rheumatoid Factor; ACPA: antibodies against cyclic citrullinated peptides. Two RA patients under MTX treatment also received corticosteroids (7.5 mg).

Severity of the disease was assessed using the Disease Activity Score in 28 joints (DAS28-CRP) [22] for RA and CRP for PsA at baseline visit and 3 or 6 months after MTX treatment onset. DAS28-CRP ≥ 3.2 in untreated RA patients and CRP ≥ 6 mg/mL in untreated PsA patients were set as thresholds for active disease. Samples of venous blood and, when available, synovial fluid (SF) were collected for each patient before the onset of MTX (untreated patients) or during the course of MTX treatment (MTX-treated patients). Blood samples from age- and sex-matched anonymous healthy donors (HD) were obtained from the Etablissement Français du Sang.

2.2. Peripheral Blood Mononuclear Cells (PBMC) and Synovial Fluid Mononuclear Cells (SFMC) Isolation

Blood samples were centrifuged on a Ficoll (Eurobio, Les Ullis, France) density gradient to purify PBMC. SF samples were diluted in HBSS (Life Technologies, Cailloux sur Fontaines, France) containing 10 mM EDTA (Sigma, Saint Quentin-Fallavier, France), and processed as blood samples to isolate SFMC.

2.3. Flow Cytometry Analyses

Multi-parametric Flow Cytometry (FC) staining were performed on PBMC or SFMC from RA and PsA patients using different panels described in Table S1 (Panels A and B) to assess CD73 expression on total memory T cells and within Th subsets. When specified, proliferation was assessed using an anti-human anti-Ki67 antibody (Ki-67, Biolegend, Saint-Cyr-l'École, CA, France). For FoxP3 intracellular

staining to characterize Treg, cells were treated using the FoxP3 Fixation and Permeabilization kit (Life Technologies), according to manufacturer instructions. Samples were analyzed on a LSR-Fortessa (BD Biosciences, Pont de Claix, France) with conserved settings throughout the entire study. Data were analyzed using FlowJo Software (Tree Star v10.4, Franklin Lakes, NJ, USA).

2.4. Analysis of Cytokines Production Capacity after Reactivation

PBMC were activated with PMA and Ionomycin (Sigma-Aldrich) as previously described [12] and intracellular cytokines (IL-17A, TNF- α , IFN- γ , IL-22) produced by CD73⁺ and CD73^{neg} Teff were analyzed using the specific panel described in Table S1 (Panel C). Stainings were analyzed on a LSR-Fortessa and Teff polyfunctionality was evaluated using the Boolean method (FlowJo software) and then processed using Pestre and SPICE v5.3 softwares, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA.

2.5. CD73⁺ Teff Sorting and In Vitro Activation to Assess CD73 Dynamic Expression

Memory CD4⁺ T cells were purified from HD PBMCs using MagniSort™ Human CD4⁺ Memory T Cell Enrichment Kit (Life Technologies). CD73⁺CD4⁺ Teff (CD4⁺CD45RA^{neg}CD127⁺CD25^{neg}CD39^{neg}CD73⁺) and CD73^{neg}CD4⁺ Teff (CD4⁺CD45RA^{neg}CD127⁺CD25^{neg}CD39^{neg}CD73^{neg}) were sorted from purified memory CD4⁺ T cells by multi-parametric FC (FACS Aria III, BD Biosciences) using antibodies against CD25 (2A3, BD-Biosciences), CD45RA (2H4LDH11LDB9, Beckman-Coulter, Brea, CA, USA), as well as CD127 (eBioRDR5), CD39 (eBioA1) and CD73 (AD2) (all from e-Bioscience), alongside a viability marker (DAPI).

Sorted populations were stained with CellTrace Violet (CTV) (20 μ M, Life Technologies) proliferation markers before incubation with Expand beads (Life Technologies) (ratio 1:4) in 96-round-bottomed-well plates in 200 μ L of complete RPMI medium for 4 days at 37 °C under 5% CO₂.

After 4 days' proliferation, part of the cells was stained for viability and CD73 expression with anti-human anti-CD73 and cells were fixed with 2% Formaldehyde solution (Sigma) and stored at 4 °C. The rest of the cells were washed and removed from Expand beads using a magnet. They were then incubated for a 2-day period without TCR signal in 200 μ L of complete RPMI medium containing IL-2 (50 UI, Chiron). Cells were then stained for viability and CD73 expression as well. All time points were analyzed concomitantly by FC for viability, proliferation and CD73 expression (LSR Fortessa, BD Biosciences).

2.6. Th1.17, Th1 and Th17 Sorting for In Vitro AMP Degradation Assay

Memory CD4⁺ T cells were purified using MojoSort Human CD4⁺ Memory T Cell Isolation Kit (Biolegend). Th1, Th17 and Th1.17 cells were sorted from purified memory CD4⁺ T cells by multi-parametric FC (FACS Aria III, BD Biosciences) based on chemokine receptors expression [23] (Figure S1) using the combination of antibodies described in Table S1 (Panel D).

The capacity of 5×10^4 cells of each Th subpopulation of HD, RA untreated or MTX-treated patients to degrade AMP was analyzed after 2 h incubation at 37 °C under 5% CO₂ with labeled AMP (AMP_{13C,15N}, 37.5 μ M, Sigma-Aldrich) in 200 μ L of serum-free RPMI medium supplemented with antibiotics and L-glutamine (Life Technologies). As a control, cells were pre-incubated with a CD73 chemical inhibitor (APCP, 50 μ M, Sigma) for 30 min before adding AMP_{13C,15N} to assess CD73-specific degradation.

2.7. Nucleotides and Nucleosides Quantification by Liquid Chromatography Coupled with Tandem Mass Spectrometry (LC-MS/MS)

Cell supernatants were harvested, boiled at 65 °C for 5 s and frozen at -20 °C. AMP_{13C,15N} and Ado_{13C,15N} were quantified in 50 μ L of supernatant, after solid phase extraction, using a LC-MS/MS method as described [12,24]. Concentrations of nucleotides in the supernatants were calculated using

calibration curves of the corresponding labeled nucleotides (AMP_{15N} and Ado_{13C}). AMP_{13C} and Ado_{15N} were used as internal standards. We also verified that APCP did not interfere with AMP and Ado quantification.

2.8. Statistical Analysis

Data formatting and statistical tests were performed using Prism software (Graphpad Inc., San Diego, CA, USA). Kruskal-Wallis test, Mann-Whitney test and ANOVA2 were used when comparing unpaired data according to the parameters considered. Wilcoxon test and ANOVA2 with paired values were performed to analyze patients' data follow up. We evaluated correlation between biological parameters and activity of the disease (DAS28-CRP) using Spearman correlation tests.

3. Results

3.1. Activated Th1.17 from Peripheral Blood of Untreated RA and PsA Patients Express Low Levels of CD73

Total memory CD4⁺ and CD8⁺ T cells frequencies were not modified in peripheral blood of untreated RA and PsA patients compared to HD (Figure S2A), neither were frequencies of Th subpopulations based on their CCR6 and CXCR3 expression [23] (Figure 1A). Treg frequency was unaltered in untreated RA and PsA patients' blood (Figure 1B). However, in RA patients, Treg displayed a higher frequency of CD39 expression compared to HD and PsA patients, although the mean fluorescence intensity (MFI) of CD39 was not different (Figure 1C). Interestingly, we found a significantly lower CD73 expression on total Teff population from RA and PsA patients compared to HD (Figure 1D). Since we established that, in HD, CD73 is enriched in Th1.17 and Th17 phenotype [12], we stratified our analysis on Th subpopulations based on CXCR3 and CCR6 expression that allows to distinguish Th subsets with different cytokine pattern (Figure S1). CD73 expression was significantly decreased on Th1.17 (by 1.5 and 1.4 fold on RA and PsA patients respectively) and Th1 (by 1.9 fold on both RA and PsA patients) (Figure 1E).

Using CD39 [25,26], we demonstrated a significantly increased activation of blood-associated Th1.17 and Th17 in RA and PsA patients compared to HD ones (Figure 1F). In parallel, we observed that in vitro TCR stimulation was sufficient to decrease CD73 expression in sorted CD73⁺ Teff from HD (Figure S2B). Taken together, these results indicate that loss of CD73 expression on Th1.17 associated to high CD39 expression in untreated RA and PsA patients' blood could reflect the activated state of this population. The access to paired samples of PBMC and SFMC for RA and PsA patients before treatment enabled us to show that the decrease of CD73 expression and CD39 up-regulation on total Teff was even more dramatic at the site of inflammation both in RA (Figure 1G) and in PsA (Figure S2C). In addition, proliferating cells (identified as Ki67⁺ cells) were included within CD39⁺CD73^{neg} Teff in RA (Figure 1H and Figure S2E) and PsA (Figure S2D,F) SF, further highlighting the absence of CD73 expression by proliferating Teff.

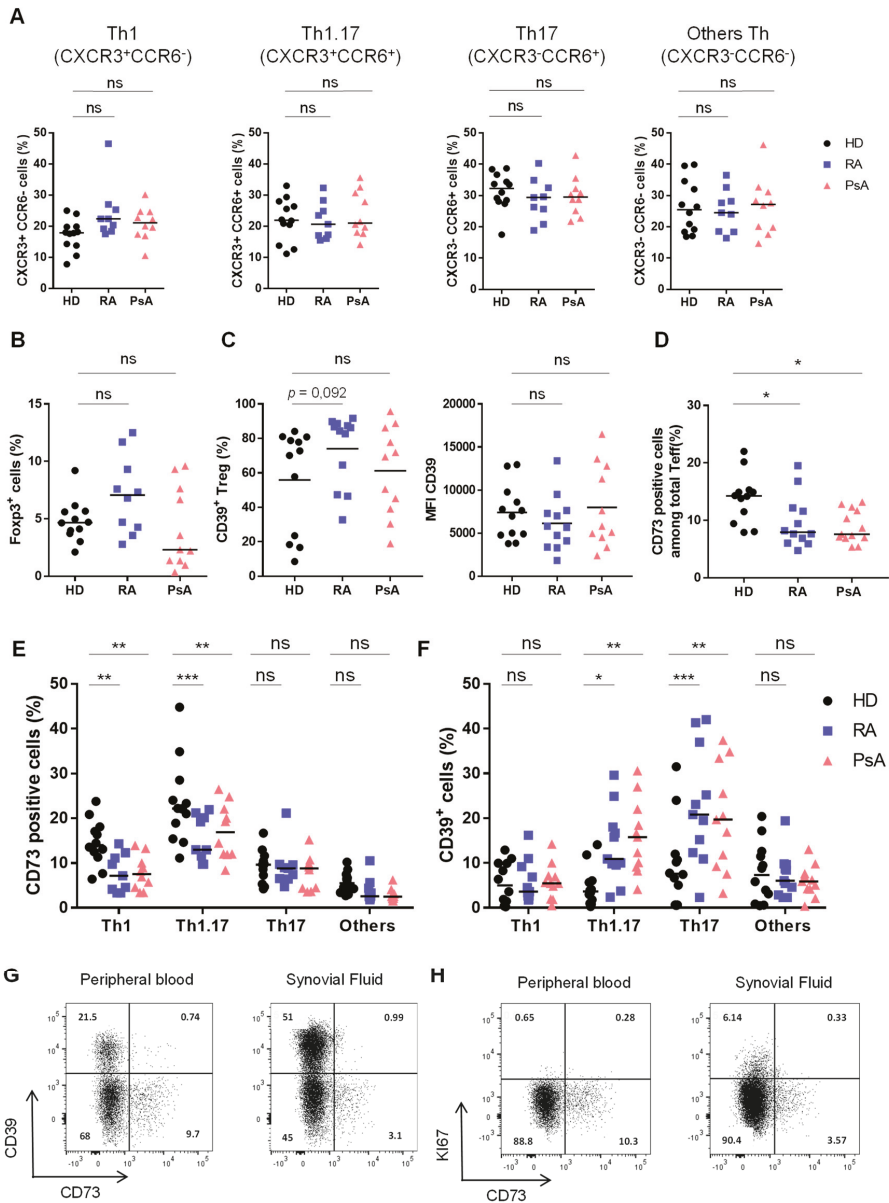


Figure 1. Unchanged Th populations but impaired CD73 expression on Th1 and Th1.17 from RA and PsA patients. (A). Th subsets frequencies based on phenotypic analysis (CXCR3/CCR6 staining) among memory CD4⁺ T cells. (B). Frequencies of FoxP3⁺ Treg among memory CD4⁺ T cells. (C). Frequencies (left) and MFI (right) of CD39⁺ Treg among memory CD4⁺ T cells. (D). CD73 expression on total Teff. (E). CD73 expression by Th subsets. (F). CD39 expression by Th subsets. (from A to F: analyses performed on peripheral blood). (G,H). Flow cytometry plots on total Teff in peripheral blood and SF of an untreated RA patient showing CD73/CD39 (G) and CD73/Ki67 (H) staining. (A–D): Kruskal-Wallis test, (E,F): ANOVA-2. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

In untreated RA patients, percentage of CD39⁺ Treg positively correlated ($r = 0.68, p = 0.03$) with the severity of the disease evaluated through DAS28-CRP score. In contrast, no correlation was noticed for CD39 percentage on Th1, Th1.17 and Th17 subsets (not shown). No correlation with the severity of the disease was noticed for global CD73 expression on Teff nor global Th1, Th1.17 and Th17 proportions in blood. Moreover, the proportion of CD73 expressed by Th1 and Th1.17 populations did not correlate with disease severity despite they were strongly reduced in untreated RA patients compared to HD. In contrast expression of CD73 on Th17 and IL-17A production by CD73⁺ Teff tend to inversely correlate with disease severity ($r = -0.65, p = 0.06$ and $r = -0.63, p = 0.07$, respectively) (Figure S3). For PsA untreated patients no correlation was observed probably because either the heterogeneity of the disease or too small cohort size to achieve robust correlations.

3.2. Untreated RA and PsA Patients' Blood Teff are Polyfunctional but Express Lower Levels of CD73 among IFN- γ /IL-17A Expressing Cells

We previously showed that CD73 marks polyfunctional Teff in blood but also in healthy (tonsil, colon) and tumor (breast and ovarian) tissues [12]. In our setting, no striking modifications of the polyfunctionality (IFN- γ , IL-17A, and TNF- α) of CD73⁺ and CD73^{neg} Teff was noticed in untreated RA and PsA patients compared to HD (Figure 2A) and global levels of each cytokine were unchanged (Figure S4A). Of note, CD73⁺ Teff, which were higher single IL-22 producers in HD [12] were an even more important source of IL-22 in PsA ($9.1 \pm 2.6\%$ versus $4.2 \pm 1.3\%$ of CD73⁺ Teff) (Figure S4B). In line with phenotypic analyses (Figure 1E), the IFN- γ /IL-17A coproducing cells, corresponding to Th1.17 (Figure S1), expressed significantly less CD73 (Figure 2B). Finally, Teff from untreated RA and PsA patients according to their DAS28-CRP or CRP level, respectively, showed enhanced IFN- γ and IL-17A production by CD73⁺ Teff in patients with active RA (DAS28-CRP ≥ 3.2), although not reaching statistical significance (Figure 2C).

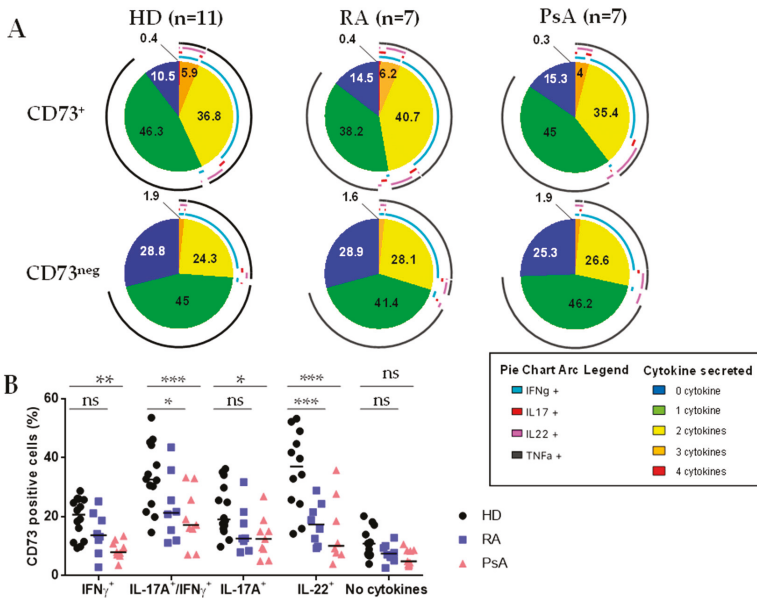


Figure 2. Cont.

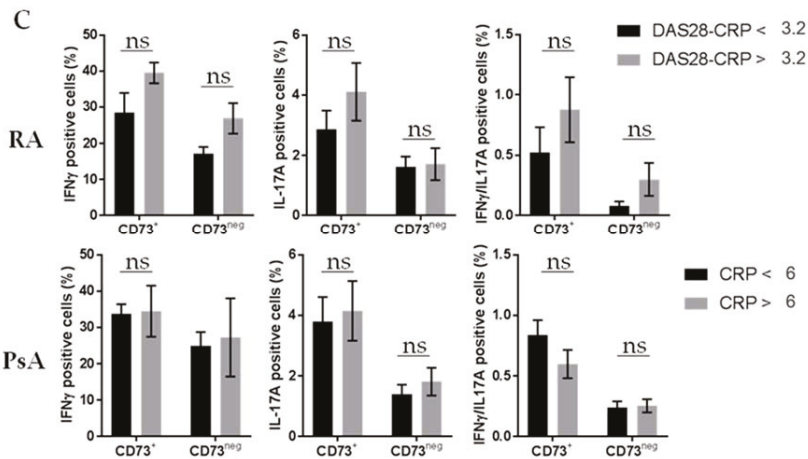


Figure 2. Polyfunctionality of Teff is not altered in RA and PsA patients but there are less CD73⁺ cells identified in IL-17A/IFN- γ secreting Teff. (A): SPICE[®] representation of the cytokines secreted by either CD73⁺ or CD73^{neg} Teff from peripheral blood of HD, or untreated RA and PsA patients. (B): CD73 expression on Teff according to their secretion of IFN- γ , IL-17A and IL-22 in peripheral blood of HD or untreated RA and PsA patients. (C): IFN- γ and IL-17A mono- or co-production by Teff according to their CD73 expression in untreated RA and PsA patients stratified on DAS28-CRP score and CRP seric level respectively. (B,C): ANOVA-2. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

3.3. Treatment of Patients with MTX Partially Restores CD73 Expression on Teff Resulting in an Enhanced Ado Production

Due to the described MTX involvement in the regulation of purine metabolism [13,26], we also analyzed the impact of MTX treatment on CD73 expression by Th subpopulations. We recently demonstrated that exogenously added Ado on CD73⁺ Teff blocked their proliferation and cytokine secretion [12]. Here, we evidenced that CD73⁺ Teff re-acquired CD73 expression after two days in resting condition (no TCR signaling), demonstrating dynamic CD73 protein expression at the surface of Teff (Figure S2B). In this context, we analyzed CD73 expression on Th subsets in MTX-treated RA and PsA patients, considering that a gain of CD73 expression could reflect drug efficiency on these populations and could contribute to its anti-inflammatory effects. MTX did not impact Th subsets frequencies compared to untreated RA and PsA patients (Figure 3A). However, CD73 expression on Teff was increased in MTX-treated RA patients compared to untreated ones while no significant variation was observed in PsA patients (Figure 3B). Interestingly, in RA patients, CD73 expression was especially increased within Th1.17 (Figure 3C) and overall CD73 expression level tended towards those observed in HD. Finally, the analysis of paired patients' blood samples (for, respectively, four and seven patients for RA and PsA) before and under MTX treatment showed significantly increased CD73 levels on Th1 and Th1.17 subsets (Figure 3D), the two populations with significantly altered CD73 expression in untreated patients compared to HD (Figure 1E). This impact of MTX was restricted to CD4⁺CD73⁺ Teff, as we did not notice striking variations in memory CD8⁺ T cell frequencies (Figure S5A) or in Treg or CD39⁺ Treg frequencies (Figure S5B,C) even in paired samples (Figure S5D–F). Of note, CD39 levels did not seem to be modulated by MTX treatment on Teff either (Figure S5G,H).

Among HD donors' Th subsets, Th1.17 was the main subset able to degrade AMP into Ado (Figure S6), in line with its strongest expression of CD73 (Figure 1E). This generation of Ado was dependent on CD73, as demonstrated by the loss of Ado in the presence of APCP (Figure S6). In comparison the quantities of Ado generated was four-fold lower for the Th1 subset. Interestingly, we evidenced that reduced CD73 expression on Th1.17 and Th1 of untreated RA patients was associated

with 50% decrease in Ado production (Figure 3E). Strikingly, upon MTX treatment, the level of Ado production was restored to HD donor levels (Figure 3E).

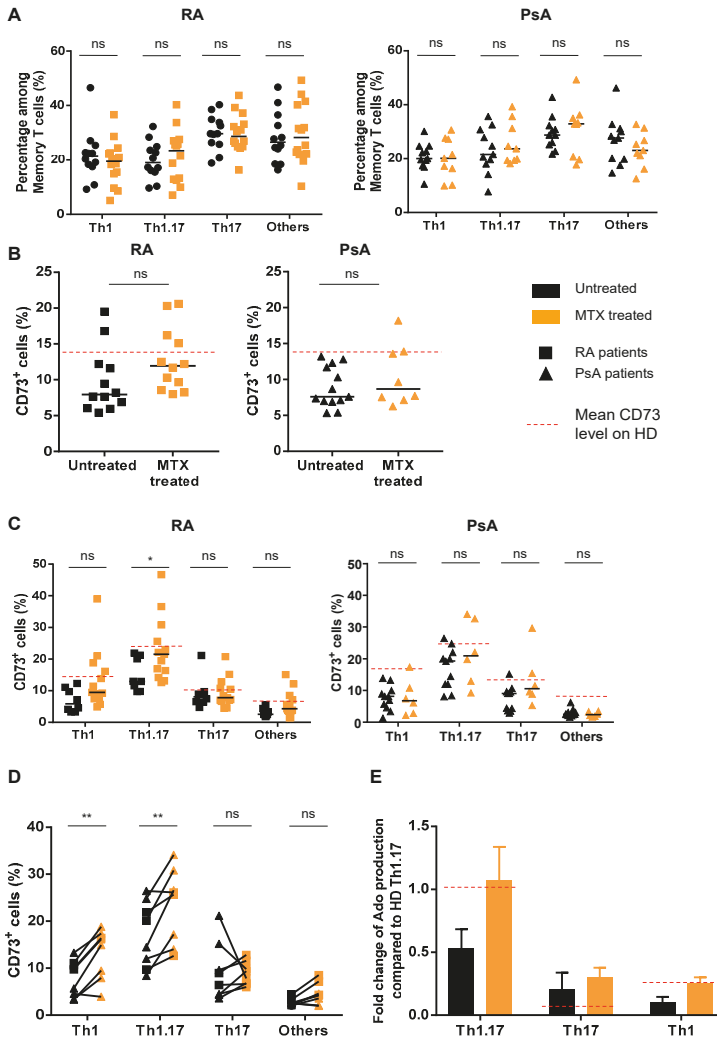


Figure 3. MTX treatment restores CD73 levels on Teff populations in RA patients but not in PsA patients. (A) Frequencies of Th subsets in peripheral blood of untreated versus MTX-treated RA (left) and PsA (right) patients. (B) CD73 percentages on total Teff in peripheral blood of untreated versus MTX treated RA (left) and PsA (right) patients. (C) CD73 percentages on Th subsets of untreated versus MTX-treated RA (up) and PsA (down) patients. (D) Paired samples showing CD73 percentages modulation in Th subsets upon MTX treatment in RA ($n = 4$) and PsA ($n = 7$) patients. (E) Fold change of Ado produced by sorted Th1.17, Th17 and Th1 subsets from untreated ($n = 3$) and MTX-treated RA ($n = 3$) patients. Cells were incubated for 2 h with AMP_{13C15N} isotope (37.5 μ M) +/- APCP (50 μ M) before Ado quantification in supernatants by LC-MS/MS. Ado production was normalized to the production by Th1.17 from HD's blood used as reference. A and C: ANOVA-2, B: Mann-Whitney test, D: ANOVA-2 with paired values. * $p < 0.05$, ** $p < 0.001$.

3.4. MTX Treatment Impacts Teff Polyfunctionality

Interestingly, we showed that CD73 expression was increased on cells coproducing IFN- γ and IL-17A, corresponding to Th1.17, from MTX-treated RA patients (Figure 4A,B). These results mirror the phenotypic analysis obtained for the Th1.17 subset (Figure 3C). In addition, we evidenced that MTX treatment significantly increased CD73 frequency among IL-22 producing cells in RA patients (Figure 4A). Of note, no major variation on global pattern of cytokines production was detected in RA patients when comparing untreated and MTX-treated samples (Figure S7A) and a rather modest increase of IL-17A⁺ and IFN- γ ⁺/IL-17A⁺ coproducing cells was observed in MTX-treated PsA patients compared to untreated ones (Figure S7B). CD73^{neg} Teff functionality was not altered by MTX neither in RA nor in PsA patients (Figure 4C,D). Surprisingly, CD73⁺ Teff displayed increased capacity to produce IFN- γ in RA patients (Figure 4C) and to co-produce IFN- γ and IL-17A after MTX treatment in both RA (Figure 4C) and PsA patients (Figure 4D).

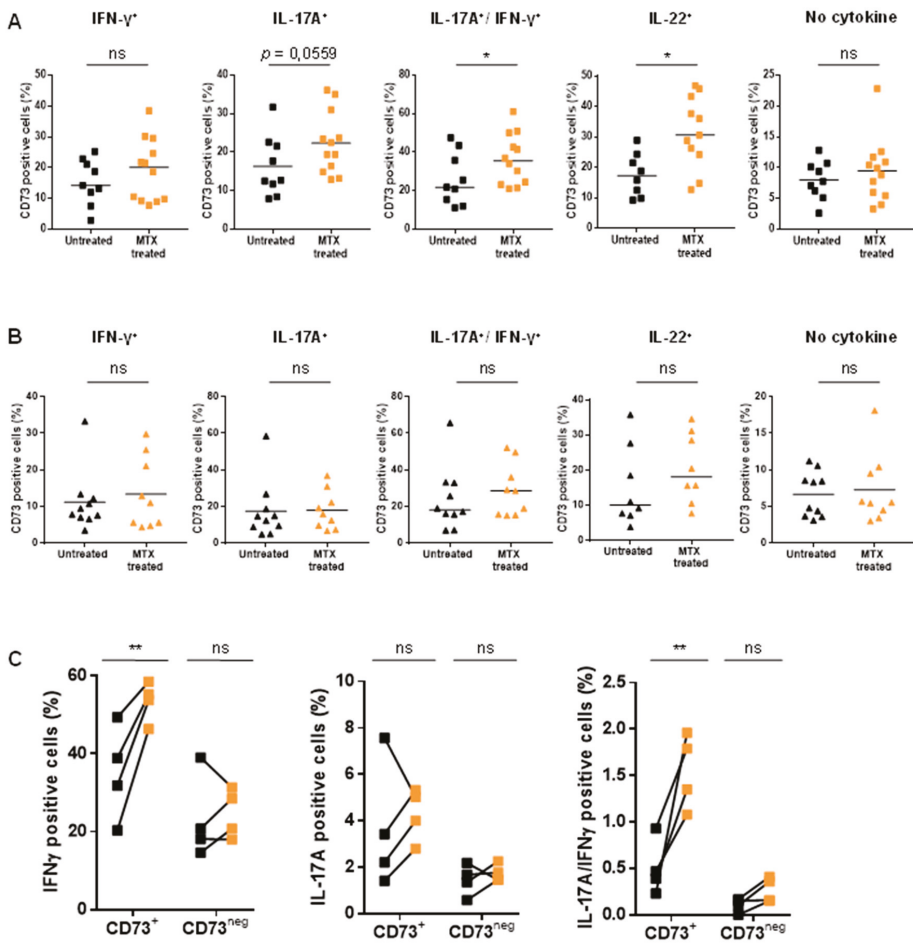


Figure 4. Cont.

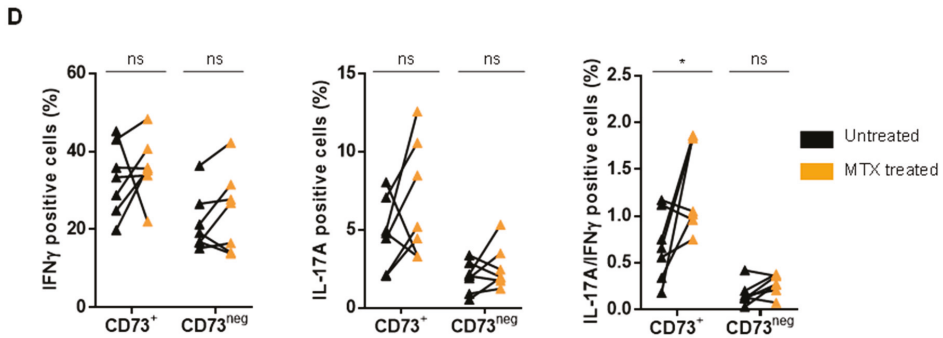


Figure 4. Impact of MTX treatment on T effector polyfunctionality. (A,B). Analysis of CD73 expression on cytokine secreting T effector mirrors phenotypic analysis in RA (A) and PsA (B) patients. (C,D). IFN- γ , IL-17A producing and IFN- γ /IL-17A co-producers in RA ($n = 4$) (C) and PsA ($n = 7$) (D) patients before initiation of MTX treatment and under MTX treatment. A,B: Mann-Whitney tests, C,D: ANOVA-2 paired samples * $p < 0.05$, ** $p < 0.001$.

4. Discussion

In this study, we demonstrated a dynamic expression of CD73 on Th populations in peripheral blood of RA and PsA patients modulated by MTX treatment. Decreased CD73 levels were detected on Th1, Th1.17, Th22 and to a lesser extent on Th17 cells in untreated patients compared to HD. The partial restoration of CD73 levels on these Th effectors upon MTX treatment reveals a novel regulatory action of this immunosuppressive drug.

The development of new therapeutic approaches for the treatment of RA and PsA is an ongoing challenge that requires to decipher the pathologic modifications of immune cells at the roots of the establishment of the chronic inflammatory state that characterizes these pathologies.

The diverse pro-inflammatory Th populations appear as good targets to breakdown the chronic inflammation [27]. In the context of systemic inflammation, the monitoring of CD39/CD73 expression levels in the peripheral blood of patients may therefore be a marker of T effector inflammatory potential, an important parameter in the evaluation of disease activity. We focused our work on the polyfunctional CD73⁺ T effector population that we recently described in human as enriched in Th1.17 and Th17 [12]. Since we previously showed that CD73 expression renders them selectively sensitive to the inhibition by CD39⁺ Treg through autocrine Ado activity, we aimed at better understanding the dynamic of CD73 expression on Th1.17 and Th17 that play a central role in RA and PsA [3,4,28]. FC analyses on PBMC from untreated RA and PsA patients did not highlight any modification in Treg frequencies, consistent with previous data [29]. However, we noticed up-regulated expression of CD39 on these Treg in untreated RA, which was correlated to disease activity at odds to other studies focusing on RA [3,28,30]. CD73 expression was significantly diminished on blood T effector in untreated RA and PsA patients compared to HD. This decrease was even stronger in SFMC, indicating that blood CD73 levels might mirror the extent of the inflammation in the affected joints as previously suggested in juvenile idiopathic arthritis [31]. Dampened or inefficient Treg suppressive functions described by others in RA and PsA patients [32] could partly result from this decreased expression of CD73 on activated T effector, as it may impair CD39⁺ Treg and CD73⁺ T effector cooperation for self-inhibition through autocrine Ado production that will act only in a nearby environment due its very short half-life. In line with this, CD73 expression level on synovial lymphocytes was proposed as a marker of disease activity in idiopathic juvenile arthritis [31] and could also be monitored in adults.

This decreased CD73 expression was particularly pronounced on Th1 and Th1.17 subsets compared to HD. Considering the strong plasticity of Th differentiation and recent data showing that Th1.17 can shift to unconventional Th1 cells when exposed to inflammatory cytokines [3,33], we suggest

this decreased CD73 expression observed on Th1 might comprise activated non-classical Th1 cells phenotypically characterized by CXCR3 expression. Interestingly, this loss of CD73 expression was associated with a decreased production of Ado by Th1.17 and Th1 cells from untreated patients. Therefore, we propose that loss of CD73 expression on Teff might be a mechanism of escape to the suppression exerted by Treg, thereby enabling them with uncontrolled proliferation and secretion of pro-inflammatory cytokines. Although not reaching statistical significance, CD73 downregulation was also observed on Th17 cells that highly express CD39, suggesting an activated state. In addition, the only parameter that negatively correlated to disease activity in RA was CD73 expression by Th17 cells. This suggests that CD73 expression on Th17 cells could contribute in RA to limit the aggressiveness of the disease through the generation of Ado that could impair their own expansion but not IL-17A production as we previously demonstrated the inability of Ado to alter IL-17A secretion in contrast to other cytokines (IFN- γ , GM-CSF, IL-22, IL-10, IL-13) [12,30].

Considering the polyfunctionality of Teff in untreated patients, we did not notice significant variations compared to HD in contrast to other studies reporting higher frequency of IL-17A producing cells in peripheral blood of PsA patients [34,35]. These discrepancies may rely on the methodology used to investigate IL-17A production, and also from patients' medical history and sampling criteria. These results remain however to be confirmed in a bigger cohort of untreated RA and PsA patients. It would also be interesting to confirm that Teff from untreated patients display low or no alteration of their cytokine production in presence of exogenous AMP because of their low expression of CD73 contrary to HD [12]. In addition, we evidenced that the frequency of single IL-22 producing cells is higher in untreated PsA patients compared to HD and to untreated RA patients (Figure S4B). This is in line with the high IL-22 production by Teff previously reported in blood of PsA patients [36]. CD73 expression could not precisely investigated on Th22 cells using as specific phenotypic markers are lacking so far, but we demonstrated that single IL-22-producing cells were enriched among CD73⁺ Teff compared to CD73^{neg} counterparts, although this population was decreased in untreated patients. While IL-22 has been reported to contribute to Pso pathogenesis [37], there is, however, no clear consensus as to whether this cytokine is deleterious in PsA. In our study, we reported no variation in the proportion of single IL-22 producing cells between untreated and MTX treated PsA patients. From our point of view, it is therefore important to evaluate the CD73 status of these IL-22 secreting cells to determine if they can be regulated by Ado. A recent study on the contrary suggests a regulatory role of IL-22 in PsA patients with a reported decreased IL-22⁺ cells frequency in PsA patients compared to HD [38]. However, in this paper, treated and untreated patients have been pooled together, making the interpretation more difficult. In RA, inflammation in the SF seems mediated by IL-17A independently of IL-22 signaling [39]. Nevertheless, IL-22 can be found in high concentrations in RA patients due to its production by Th17 and Th1.17 cells that are subsets highly active in the pathology.

In our study, MTX treatment of RA and PsA patients did not induce changes on Treg frequency and CD39 MFI (Figure S5C,E,F). This contrasts with another study showing increased CD39⁺ Treg frequencies in MTX responders [40]. However, in this study, the authors suggest the direct production of Ado by Treg expressing both CD39 and CD73 which is in contradiction with observations we and others have reported [12,41]. However, high CD39⁺ Treg frequency at baseline could correlate with good MTX response, and CD39 is therefore suggested as a biomarker of the MTX response in RA [40,42]. Our analysis of untreated and MTX-treated paired samples of RA and PsA patients demonstrated a CD73 frequency in Th subsets closer to HD in MTX-treated patients. This could result from an arrest in cell proliferation due to A2AR engagement by Ado released after MTX-induced AICAR blockade [15]. We hypothesize that restoration of CD73 expression, through degradation of AMP, in turn accentuates the Ado-mediated immunosuppression initiated by MTX. The loss of CD73 expression on Teff could indeed be necessary for their proliferation and pro-inflammatory features to escape CD39⁺ Treg-mediated suppression through cooperative Ado production.

In MTX-treated PsA patients, up-regulation of CD73 on Teff appears less important than in RA patients. This could rely on the fact that PsA is a joint affection mostly developing

subsequently to established PsA, that is more heterogeneous in its characterization and symptoms. The immune-biological regulation of PsA inflammation is therefore different from RA and joint inflammation in PsA might be fueled by specific immune cells active in PsA, possibly interfering with the biological effect of MTX. Indeed, auto-reactive CD8⁺ T cells have been shown to contribute to PsA and should be considered.

We also showed an enhanced cytokine production capacity of CD73⁺ Teff in RA patients after MTX treatment (IFN- γ , IL-17A, IL-17A/IFN- γ , IL-22). However, we can expect these cells to be less pro-inflammatory in vivo, since they express higher level of CD73, favoring Ado production. We and others have indeed previously showed that Ado strongly reduced CD73⁺ Teff cytokine pattern except IL-17A and, to a lesser extent, IL-22 [12,30]. These data suggest a benefit of MTX treatment, which may have synergistic effect with anti-IL-17A in contrast to TNF inhibitors in PsA [43]. Similarly, in RA, although combination of MTX and anti-IL-6R such as Tocilizumab has not shown clinically relevant short-term superiority over Tocilizumab monotherapy [44], concomitant MTX treatment with Tocilizumab may have an interest in low Tocilizumab responders first under monotherapy. Indeed, we evidenced down-regulation of IL-6R on CD73⁺ Teff compared to CD73^{neg} Teff (unpublished results), suggesting a reduced impact of anti-IL-6R on CD73⁺ Teff. Therefore, MTX could better neutralize activated and proliferating CD73⁺ Teff, while anti-IL-6R might target CD73^{neg} Teff; their combined action provide a better regulation of the overall hyper activated Teff population in the contexts of auto-immune disorders.

Purine metabolism and its regulation emerges as a pivotal regulator of immunity and inflammation, Modulation of this pathway in combination with MTX therefore appears as a promising target for new therapeutic strategies.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/8/11/1859/s1>, Figure S1: Co analysis of phenotypic and functional characteristics of Th subsets; Figure S2: Altered frequencies of CD73⁺ T cells in patients blood reflecting their high activation; Figure S3: Correlation between proportion of CD39⁺ Treg, CD73 on Th1, Th17 and Th1.17 and disease activity score (DAS28) in untreated RA patients; Figure S4: Teff from RA and PsA patients evidence the same capacities for cytokine secretion but an increase in Th22 cells is observed in peripheral blood of PsA patients; Figure S5: MTX treatment induces a slight decrease of memory CD8⁺ T cells frequency in PsA patients and does not modify Treg proportion and activation status in RA and PsA patients; Figure S6: Th1.17 cells are the major producers of Ado among Teff in a CD73 dependent manner; Figure S7: MTX treatment moderately increase IL-17A secretion by Teff in PsA but not in RA patients; Table S1: Panels of antibodies used for multi-parametric flow cytometry analysis throughout the study.

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References

1. Firestein, G.S. Evolving concepts of rheumatoid arthritis. *Nature* **2003**, *423*, 356–361. [CrossRef] [PubMed]
2. Gladman, D.D.; Antoni, C.; Mease, P.; Clegg, D.O.; Nash, P. Psoriatic arthritis: Epidemiology, clinical features, course, and outcome. *Ann. Rheum. Dis.* **2005**, *64*, ii14–ii17. [CrossRef] [PubMed]
3. Basdeo, S.A.; Cluxton, D.; Sulaimani, J.; Moran, B.; Canavan, M.; Orr, C.; Veale, D.J.; Fearon, U.; Fletcher, J.M. Ex-Th17 (Nonclassical Th1) Cells Are Functionally Distinct from Classical Th1 and Th17 Cells and Are Not Constrained by Regulatory T Cells. *J. Immunol.* **2017**, *198*, 2249–2259. [CrossRef] [PubMed]
4. Nistala, K.; Adams, S.; Cambrook, H.; Ursu, S.; Olivito, B.; de Jager, W.; Evans, J.G.; Cimaz, R.; Bajaj-Elliott, M.; Wedderburn, L.R. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 14751–14756. [CrossRef]

5. Raychaudhuri, S.P.; Raychaudhuri, S.K.; Genovese, M.C. IL-17 receptor and its functional significance in psoriatic arthritis. *Mol. Cell Biochem.* **2012**, *359*, 419–429. [[CrossRef](#)]
6. Burman, A.; Haworth, O.; Bradfield, P.; Parsonage, G.; Filer, A.; Thomas, A.M.; Amft, N.; Salmon, M.; Buckley, C.D. The role of leukocyte-stromal interactions in chronic inflammatory joint disease. *Jt. Bone Spine* **2005**, *72*, 10–16. [[CrossRef](#)]
7. Hot, A.; Zrioual, S.; Lenief, V.; Miossec, P. IL-17 and tumour necrosis factor α combination induces a HIF-1 α -dependent invasive phenotype in synoviocytes. *Ann. Rheum. Dis.* **2012**, *71*, 1393–1401. [[CrossRef](#)]
8. Raychaudhuri, S.K.; Saxena, A.; Raychaudhuri, S.P. Role of IL-17 in the pathogenesis of psoriatic arthritis and axial spondyloarthritis. *Clin. Rheumatol.* **2015**, *34*, 1019–1023. [[CrossRef](#)]
9. van Hamburg, J.P.; Asmawidjaja, P.S.; Davelaar, N.; Mus, A.M.C.; Colin, E.M.; Hazes, J.M.W.; Dolhain, R.J.; Lubberts, E. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis Rheum.* **2011**, *63*, 73–83. [[CrossRef](#)]
10. Kotake, S.; Udagawa, N.; Takahashi, N.; Matsuzaki, K.; Itoh, K.; Ishiyama, S.; Saito, S.; Inoue, K.; Kamatani, N.; Gillespie, M.T.; et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Investig.* **1999**, *103*, 1345–1352. [[CrossRef](#)]
11. Ménétrier-Caux, C.; Curiel, T.; Faget, J.; Manuel, M.; Caux, C.; Zou, W. Targeting regulatory T cells. *Target. Oncol.* **2012**, *7*, 15–28. [[CrossRef](#)] [[PubMed](#)]
12. Gourdin, N.; Bossennec, M.; Rodriguez, C.; Vigano, S.; Machon, C.; Jandus, C.; Faget, J.; Durand, I.; Chopin, N.; Bauché, D.; et al. Autocrine Adenosine regulates tumor polyfunctional CD73+CD4+ effector T cells devoid of immune checkpoints. *Cancer Res.* **2018**, *78*, 3604–3618. [[CrossRef](#)] [[PubMed](#)]
13. Brown, P.M.; Pratt, A.G.; Isaacs, J.D. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. *Nat. Rev. Rheumatol.* **2016**, *12*, 731–742. [[CrossRef](#)] [[PubMed](#)]
14. Taylor, P.C.; Criado, A.B.; Mongey, A.B.; Avouac, J.; Marotte, H.; Mueller, R.B. How to Get the Most from Methotrexate (MTX) Treatment for Your Rheumatoid Arthritis Patient?-MTX in the Treat-to-Target Strategy. *J. Clin. Med.* **2019**, *8*, 515. [[CrossRef](#)]
15. Montesinos, M.C.; Takedachi, M.; Thompson, L.F.; Wilder, T.F.; Fernández, P.; Cronstein, B.N. The antiinflammatory mechanism of methotrexate depends on extracellular conversion of adenine nucleotides to adenosine by ecto-5'-nucleotidase: Findings in a study of ecto-5'-nucleotidase gene-deficient mice. *Arthritis Rheum.* **2007**, *56*, 1440–1445. [[CrossRef](#)]
16. Mirabet, M.; Herrera, C.; Cordero, O.J.; Mallol, J.; Lluis, C.; Franco, R. Expression of A2B adenosine receptors in human lymphocytes: Their role in T cell activation. *J. Cell Sci.* **1999**, *112*, 491–502.
17. Zarek, P.E.; Huang, C.-T.; Lutz, E.R.; Kowalski, J.; Horton, M.R.; Linden, J.; Drake, C.G.; Powell, J.D. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. *Blood* **2008**, *111*, 251–259. [[CrossRef](#)]
18. Ohta, A.; Kini, R.; Ohta, A.; Subramanian, M.; Madasu, M.; Sitkovsky, M. The development and immunosuppressive functions of CD4+ CD25+ FoxP3+ regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway. *Front. Immunol.* **2012**, *3*, 190. [[CrossRef](#)]
19. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O.; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **2010**, *69*, 1580–1588. [[CrossRef](#)]
20. Taylor, W.; Gladman, D.; Helliwell, P.; Marchesoni, A.; Mease, P.; Mielants, H.; CASPAR Study Group. Classification criteria for psoriatic arthritis: Development of new criteria from a large international study. *Arthritis Rheum.* **2005**, *54*, 2665–2673. [[CrossRef](#)]
21. Wells, G.; Becker, J.C.; Teng, J.; Dougados, M.; Schiff, M.; Smolen, J.; Aletaha, D.; van Riel, P.L. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann. Rheum. Dis.* **2009**, *68*, 954–960.
22. Sallusto, F.; Lanzavecchia, A. Heterogeneity of CD4+ memory T cells: Functional modules for tailored immunity. *Eur. J. Immunol.* **2009**, *39*, 2076–2082. [[CrossRef](#)] [[PubMed](#)]

23. Machon, C.; Jordheim, L.P.; Puy, J.-Y.; Lefebvre, I.; Dumontet, C.; Guitton, J. Fully validated assay for the quantification of endogenous nucleoside mono- and triphosphates using online extraction coupled with liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 2925–2941. [[CrossRef](#)] [[PubMed](#)]
24. Moncrieffe, H.; Nistala, K.; Kamhieh, Y.; Evans, J.; Eddaoudi, A.; Eaton, S.; Wedderburn, L.R. High Expression of the Ectonucleotidase CD39 on T Cells from the Inflamed Site Identifies Two Distinct Populations, One Regulatory and One Memory T Cell Population. *J. Immunol.* **2010**, *185*, 134–143. [[CrossRef](#)] [[PubMed](#)]
25. Zhou, Q.; Yan, J.; Putheti, P.; Wu, Y.; Sun, X.; Toxavidis, V.; Tigges, J.; Kassam, N.; Enjyoji, K.; Robson, S.C.; et al. Isolated CD39 Expression on CD4+ T Cells Denotes both Regulatory and Memory Populations. *Am. J. Transpl.* **2009**, *9*, 2303–2311. [[CrossRef](#)] [[PubMed](#)]
26. Cronstein, B.N.; Sitkovsky, M. Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. *Nat. Rev. Rheumatol.* **2017**, *13*, 41–51. [[CrossRef](#)] [[PubMed](#)]
27. Sarkar, S.; Cooney, L.A.; Fox, D.A. The role of T helper type 17 cells in inflammatory arthritis. *Clin. Exp. Immunol.* **2010**, *159*, 225–237. [[CrossRef](#)]
28. Kotake, S.; Yago, T.; Kobashigawa, T.; Nanke, Y. The Plasticity of Th17 Cells in the Pathogenesis of Rheumatoid Arthritis. *J. Clin. Med.* **2017**, *6*, 67. [[CrossRef](#)]
29. Walter, G.J.; Fleskens, V.; Frederiksen, K.S.; Rajasekhar, M.; Menon, B.; Gerwien, J.G.; Evans, H.G.; Taams, L.S. Phenotypic, Functional, and Gene Expression Profiling of Peripheral CD45RA+ and CD45RO+ CD4+CD25+CD127low Treg Cells in Patients with Chronic Rheumatoid Arthritis. *Arthritis Rheum.* **2016**, *68*, 103–116. [[CrossRef](#)]
30. Herrath, J.; Chemin, K.; Albrecht, I.; Catrina, A.I.; Malmström, V. Surface expression of CD39 identifies an enriched Treg-cell subset in the rheumatic joint, which does not suppress IL-17A secretion. *Eur. J. Immunol.* **2014**, *44*, 2979–2989. [[CrossRef](#)]
31. Botta Gordon-Smith, S.; Ursu, S.; Eaton, S.; Moncrieffe, H.; Wedderburn, L.R. Correlation of Low CD73 Expression on Synovial Lymphocytes with Reduced Adenosine Generation and Higher Disease Severity in Juvenile Idiopathic Arthritis. *Arthritis Rheum.* **2015**, *67*, 545–554. [[CrossRef](#)] [[PubMed](#)]
32. Prakken, B.; Wehrens, E.; van Wijk, F. Editorial: Quality or quantity? Unraveling the role of Treg cells in rheumatoid arthritis. *Arthritis Rheum.* **2013**, *65*, 552–554. [[CrossRef](#)] [[PubMed](#)]
33. Maggi, L.; Santarlasci, V.; Capone, M.; Rossi, M.C.; Querci, V.; Mazzoni, A.; Cimaz, R.; De Palma, R.; Liotta, F.; Maggi, E.; et al. Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells. *Eur. J. Immunol.* **2012**, *42*, 3180–3188. [[CrossRef](#)] [[PubMed](#)]
34. Jandus, C.; Bioley, G.; Rivals, J.-P.; Dudler, J.; Speiser, D.; Romero, P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis Rheum.* **2008**, *58*, 2307–2317. [[CrossRef](#)]
35. Zhang, L.; Li, Y.; Li, Y.; Qi, L.; Liu, X.; Yuan, C.; Hu, N.W.; Ma, D.X.; Li, Z.F.; Yang, Q.; et al. Increased Frequencies of Th22 Cells as well as Th17 Cells in the Peripheral Blood of Patients with Ankylosing Spondylitis and Rheumatoid Arthritis. *PLoS ONE* **2012**, *7*, e31000. [[CrossRef](#)]
36. Benham, H.; Norris, P.; Goodall, J.; Wechalekar, M.D.; FitzGerald, O.; Szentpetery, A.; Smith, M.; Thomas, R.; Gaston, H. Th17 and Th22 cells in psoriatic arthritis and psoriasis. *Arthritis Res. Ther.* **2013**, *15*, R136. [[CrossRef](#)]
37. Wolk, K.; Witte, E.; Warszawska, K.; Schulze-Tanzil, G.; Witte, K.; Philipp, S.; Kunz, S.; Döcke, W.D.; Asadullah, K.; Volk, H.D.; et al. The Th17 cytokine IL-22 induces IL-20 production in keratinocytes: A novel immunological cascade with potential relevance in psoriasis. *Eur. J. Immunol.* **2009**, *39*, 3570–3581. [[CrossRef](#)]
38. Ezeonyeji, A.; Baldwin, H.; Vukmanovic-Stejic, M.; Ehrenstein, M.R. CD4 T-Cell Dysregulation in Psoriatic Arthritis Reveals a Regulatory Role for IL-22. *Front. Immunol.* **2017**, *8*, 1403. [[CrossRef](#)]
39. van Hamburg, J.P.; Corneth, O.B.J.; Paulissen, S.M.J.; Davelaar, N.; Asmawidjaja, P.S.; Mus, A.M.; Lubberts, E. IL-17/Th17 mediated synovial inflammation is IL-22 independent. *Ann. Rheum. Dis.* **2013**, *72*, 1700–1707. [[CrossRef](#)]
40. Peres, R.S.; Liew, F.Y.; Talbot, J.; Carregaro, V.; Oliveira, R.D.; Almeida, S.L.; França, R.F.; Donate, P.B.; Pinto, L.G.; Ferreira, F.I.; et al. Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 2509–2514. [[CrossRef](#)]

41. Dwyer, K.M.; Hanidziar, D.; Putheti, P.; Hill, P.A.; Pommey, S.; McRae, J.L.; Winterhalter, A.; Doherty, G.; Deaglio, S.; Koulmanda, M.; et al. Expression of CD39 by human peripheral blood CD4+CD25+ T cells denotes a regulatory memory phenotype. *Am. J. Transplant.* **2010**, *10*, 2410–2420. [[CrossRef](#)] [[PubMed](#)]
42. Gupta, V.; Katiyar, S.; Singh, A.; Misra, R.; Aggarwal, A. CD39 positive regulatory T cell frequency as a biomarker of treatment response to methotrexate in rheumatoid arthritis. *Int. J. Rheum. Dis.* **2018**, *21*, 1548–1556. [[CrossRef](#)] [[PubMed](#)]
43. Fagerli, K.M.; Lie, E.; van der Heijde, D.; Heiberg, M.S.; Lexberg, A.S.; Rødevand, E.; Kalstad, S.; Mikkelsen, K.; Kvien, T.K. The role of methotrexate co-medication in TNF-inhibitor treatment in patients with psoriatic arthritis: Results from 440 patients included in the NOR-DMARD study. *Ann. Rheum. Dis.* **2014**, *73*, 132–137. [[CrossRef](#)] [[PubMed](#)]
44. Dougados, M.; Soubrier, M.; Antunez, A.; Balint, P.; Balsa, A.; Buch, M.; Kalstad, S.; Mikkelsen, K.; Kvien, T.K. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: Results of an international, cross-sectional study (COMORA). *Ann. Rheum. Dis.* **2013**, *73*, 62–68. [[CrossRef](#)]



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Article

Effectiveness, Tolerability, and Safety of Tofacitinib in Rheumatoid Arthritis: A Retrospective Analysis of Real-World Data from the St. Gallen and Aarau Cohorts

Ruediger B. Mueller^{1,2,3,*}, Caroline Hasler², Florian Popp¹, Frederik Mattow¹,
Mirsada Durmisi², Alexander Souza⁴, Paul Hasler², Andrea Rubbert-Roth¹,
Hendrik Schulze-Koops³ and Johannes von Kempis¹

- ¹ Division of Rheumatology and Immunology, Department of Internal Medicine, Kantonsspital St. Gallen, 9007 St. Gallen, Switzerland; fmpopp@gmail.com (F.P.); fmattow@googlemail.com (F.M.); andrea.rubbert-roth@uk-koeln.de (A.R.-R.); johannes.vonkempis@kssg.ch (J.v.K.)
 - ² Division of Rheumatology, Medical University Department, Kantonsspital Aarau, 5001 Aarau, Switzerland; carolinehasler4@gmail.com (C.H.); m.durmisi@stud.unibas.ch (M.D.); paul.hasler@ksa.ch (P.H.)
 - ³ Division of Rheumatology and Clinical Immunology, Department of Internal Medicine IV, Ludwig-Maximilians-University Munich, 80336 Munich, Germany; Hendrik.Schulze-Koops@med.uni-muenchen.de
 - ⁴ Iterata AG, 5722 Gränichen, Switzerland; souza@iterata.ch
- * Correspondence: Ruediger.Mueller@ksa.ch; Tel.: +41-62-838-4688

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Abstract: Introduction: Tofacitinib is an oral JAK inhibitor indicated for the treatment of rheumatoid arthritis (RA). The efficacy and safety of tofacitinib have been shown in several randomized clinical trials. The study presented here aimed to assess the clinical tolerability and effectiveness of tofacitinib among RA patients in real life. **Methods:** Consecutive patients between January 2015 and April 2017 with RA who fulfilled the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 criteria were included in a prospectively designed analysis of retrospective data. Patients were initiated on tofacitinib 5 mg bid. The primary objective was to analyze the safety of tofacitinib in a real-life cohort. Safety was assessed by the reasons to stop tofacitinib during follow up and changes of liver enzymes, hemoglobin, and creatinine. The secondary outcome was to analyze the frequency of and time to achieve low disease activity (LDA) and remission as defined by 28 joint count disease activity score (DAS28). **Results:** A total of 144 patients were treated with tofacitinib. A total of 84.9% of patients were pre-exposed to at least one biological agent. The average DAS28 at the initiation of tofacitinib was 4.43. A total of 50.0% of patients were positive for rheumatoid factor and 49.0% for ACPA. The mean follow up was 1.22 years (range 10d–3.7a) after initiation of tofacitinib treatment. A total of 94 (64.4%) patients remained on tofacitinib during follow-up. The average time to stop tofacitinib was 190.0 days. Reasons to stop tofacitinib were: insufficient response ($n = 23$), gastrointestinal symptoms ($n = 18$), infection ($n = 5$), myalgia ($n = 2$), remission ($n = 2$), headache ($n = 2$), cough, blue finger syndrome, intolerance, heartburn, psoriasis, and increased liver enzymes (all $n = 1$). Increased alanine amino transferase (ALAT) or aspartate amino transferase (ASAT) $> 2 \times$ upper limit of normal (ULN) were detected in 3.3% and 4.4% of patients, respectively. Hemoglobin decrease of $> 10\%$ was detected in 15.1% of the patients and decreased lymphocytes $< 500/\mu\text{L}$ in 3.4%. An increase of creatinine $> 20\%$ was detected in 9.4% of patients. A total of 62.9% and 50.0% of the patients achieved low disease activity (LDA) or remission after a median of 319 and 645 days, respectively. These rates were significantly higher in patients naïve to biologic agents as compared to patients pre-exposed to biologics (LDA: naïve 100% 92 d, pre-exposed 57.0% 434 d, $p \leq 0.001$; remission: naïve 86.7% 132 d, pre-exposed 44.1%, 692 d, $p = 0.001$). **Conclusions:** Tofacitinib is a safe and effective treatment option for patients with RA. Tofacitinib may induce high rates of LDA and

remission in patients with active disease, even after the use of one or more biologics, though the rate appeared higher in patients naïve to biologics. Tofacitinib may be a valuable option in a treat-to-target approach. Our data demonstrate that Janus kinase (JAK) inhibitors are safe and efficacious in real life patients.

Keywords: tofacitinib; rheumatoid arthritis; oral

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the inflammation and destruction of joints. It may result in functional impairment, declining health status and reduced quality of life for affected patients [1–3]. The principal goal in the treatment of RA is to achieve and maintain remission, or, if that is not attainable, low disease activity (LDA) [4,5].

Conventional synthetic (cs) disease-modifying anti-rheumatic drugs (DMARDs), especially methotrexate (MTX), have long been the cornerstones of RA treatment. In the last 20 years, biologic agents have broadened the clinical armamentarium [6]. Though biologics have revolutionized the managing of RA [7–24], their effects are limited. Approximately 50% of RA patients treated with biologics meet the criteria for low disease activity (28 joint count disease activity score (DAS28) ≤ 3.2) or remission (DAS28 < 2.6), while a significant proportion of patients do not achieve an ACR 20 (American College of Rheumatology) response [14,15]. Furthermore, patients on biologics may experience adverse events (AEs) or loss of effectiveness over time [25], e.g., by developing anti-drug antibodies. To quantify the unmet need for additional therapies, Drosos et al. performed a long-term, real-world observational study of their cases with RA treated according to the European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommendations. Approximately one-fifth of their patients did not respond sufficiently to csDMARDs or bDMARDs (biological disease-modifying anti-rheumatic drugs), substantiating the need for alternative treatments [26].

Tofacitinib is a novel, oral Janus kinase (JAK) inhibitor indicated for the treatment of RA. JAK inhibitors are small-molecule drugs that interfere with the activation of JAKs, a family of enzymes implicated in the signaling of leukocytes. JAK signaling has been shown to play an essential role in immune cell generation, differentiation and responses [27–29]. By inhibiting these signaling mechanisms, JAK inhibitors such as tofacitinib have the potential to successfully interfere with immune activation that is critical for RA [30–32] (Koehler, J. Clin. Med. 2019, 8, 938).

Phase II and III clinical trials have shown that the treatment of RA patients with tofacitinib, either as a monotherapy or in combination with csDMARDs, is capable of significantly reducing disease activity, as measured by ACR response rates, EULAR responses and HAQ-DI scores [33–39]. Studies comparing tofacitinib to other therapeutic strategies in the treatment of RA suggest that the effectiveness of tofacitinib is similar to that of biologic agents [40–43]. The safety profile of tofacitinib does not appear to differ significantly from biologics [34,39–44].

In 2012 and 2014, the FDA and the Swissmedic approved tofacitinib for adult patients with moderate to severe RA who had a prior inadequate response to MTX. Approval from the European Medicines Agency (EMA) was granted in 2017. With JAK inhibitors still representing a relatively novel treatment option in the management of RA, there is a demand to use the experience gained through using tofacitinib in a real-life, clinical setting, to further evaluate its safety and utility. In this study, we aimed to analyze real-life data from routine clinical practice to compare our experience with the results of controlled studies.

2. Methods

2.1. Patient Recruitment

For this retrospective analysis of data, patients were recruited through a chart review of all RA patients at the hospitals of St. Gallen and Aarau, Switzerland. Patients with a clinical diagnosis of RA consistent with the current definition in the 2010 ACR/EULAR criteria were required [45] and initiation of oral tofacitinib 5 mg bid followed. Exclusion criteria were ages younger than 18 years or older than 80 years at disease onset. All patient charts of the cohort from Aarau and St. Gallen were screened sequentially for eligibility. Thus, selected patients were followed until tofacitinib administration was terminated or until the last visit entered in the database. The decision to stop tofacitinib and all other decisions concerning treatment were at the discretion of the treating clinician. Ethical approval for the collection of patient data was given by the regional review board.

2.2. Study Design

This was a longitudinal, retrospective chart review conducted between April 2013 and September 2017 within the St. Gallen and Aarau RA cohorts. The pre-defined primary endpoints were the incidence of adverse events, changes in laboratory values (increase in alanine amino transferase (ALAT) or aspartate amino transferase (ASAT) > 1.2 or 2.0 above the upper limits of normal), decrease in hemoglobin of >10%, lymphocytes <500 or <1000/ μ L, increase in creatinine >20%, and adverse events leading to the termination of tofacitinib treatment. The pre-defined secondary clinical endpoint was longitudinal disease activity as measured by DAS28 and the achievement of LDA (DAS28 \leq 3.2) and remission (DAS28 < 2.6). Data were analyzed for the entire cohort of 144 patients, and, as a secondary analysis, separately for patients who had prior exposure to biologic agents and patients who were naïve to biologic agents.

2.3. Statistical Methods

Summary statistics are reported as median (range) or *n* (%). Kaplan–Meier curves were plotted, and Kaplan–Meier estimates with 95% confidence intervals based on a log–log transformation were computed for the endpoints. Time to LDA and remission was compared between patients with and without prior exposure to biologics with a log-rank test. All analyses were performed in the R programming language (R Foundation, Vienna, Austria, version 3.3.3, R Core Team 2013).

3. Results

3.1. Baseline Demographics

A total of 144 patients from the rheumatology units of the St. Gallen and Aarau rheumatology divisions fulfilled the inclusion criteria and were included in the cohort. The mean age at initiation of tofacitinib was 59.7 years and mean disease duration was 9.1 years. The majority of patients were female (69.4%). A total of 50% were positive for rheumatoid factor (RF), and 48.6% were positive for anti-citrullinated protein antibodies (ACPAs), as described in the records. No additional testing for RF and/or ACPA prior or under tofacitinib treatment was conducted. A total of 56% of the patients were either RF and/or ACPA positive.

Disease activity among the patient cohort was moderate, with a mean DAS28 of 4.43 at the initiation of tofacitinib. A total of 63.3% had a disease classified as erosive. All patients were initiated on a baseline dose of tofacitinib 5 mg bid. Regarding other medications, the mean number of previous csDMARDs was 1.9. A total of 84.7% of patients had been previously exposed to at least one biologic agent; the mean number of previous biologics was 2.2. Mean follow-up was 1.22 years (range 10 days–3.7 years) after initiation of tofacitinib (Table 1).

Table 1. Patient demographics.

| | All | Stopped | Remained on | Naïve to | After |
|---|-----------|-------------|-------------|------------------|-----------|
| | Patients | Tofacitinib | | A Biologic Agent | |
| Number (n) | 144 | 57 | 87 | 22 | 122 |
| Gender (% female) | 69.4 | 64.9 | 72.4 | 72.7 | 68.8 |
| Age at initiation tofacitinib (years, mean) | 59.7 | 59.6 | 59.8 | 58.8 | 59.8 |
| Tofacitinib applied in monotherapy (n, %) | 65 | 22 | 43 | 14 | 51 |
| Concomitant medication | | | | | |
| - Methotrexate | 36 (25.0) | 16 (28.1) | 20 (23.0) | 5 (22.7) | 31 (25.4) |
| - Sulfasalazine | 7 (4.9) | 4 (7.0) | 3 (3.4) | 0 (0) | 7 (5.7) |
| - Leflunomide | 25 (17.3) | 6 (10.5) | 19 (21.8) | 3 (13.6) | 22 (18.0) |
| - Hydroxychloroquine | 11 (7.6) | 5 (8.8) | 6 (6.9) | 0 (0) | 11 (9.0) |
| - Prednisolone or equivalent | 48 (33.3) | 16 (28.1) | 32 (36.8) | 4 (18.2) | 44 (36.1) |
| Disease duration (years, mean) | 9.1 | 9.9 | 8.7 | 2.6 | 10.3 |
| Comorbidities of special interest | | | | | |
| Cardiovascular | | | | | |
| - Coronary heart disease | 10 | 2 | 8 | 1 | 9 |
| - Arterial hypertension | 29 | 13 | 16 | 3 | 26 |
| - Dyslipoproteinemia | 5 | 2 | 3 | 1 | 4 |
| - Valvular heart disease | 2 | 1 | 1 | 1 | 1 |
| - Adipositas | 12 | 6 | 6 | 0 | 2 |
| - PAD | 3 | 1 | 2 | 0 | 3 |
| Osteoporosis | 39 | 15 | 24 | 4 | 25 |
| After a biologic agent (%) | 84.7 | 87.2 | 83.5 | 0 | 100 |
| Previous biologic agents (n, mean) | 2.2 | 2.3 | 2.2 | 0 | 2.6 |
| Previous csDMARDs (n, mean) | 1.9 | 1.9 | 1.8 | 1.4 | 1.9 |
| ACPA pos. (%) | 48.6 | 42.8 | 52.3 | 50.0 | 48.3 |
| Rheumatoid factor pos. (%) | 50.0 | 51.1 | 48.2 | 40.9 | 51.7 |
| Erosive disease (%) | 63.3 | 60.9 | 66.7 | 45.5 | 66.7 |
| DAS28 (mean) | 4.4 | 4.4 | 4.5 | 3.7 | 4.6 |
| ESR (mean) | 17.2 | 18.5 | 16.6 | 18.8 | 16.9 |
| CRP (mean, ULN < 5mg/L) | 8.5 | 8.0 | 8.8 | 8.9 | 8.4 |

n: number. DAS28: 28 joint count disease activity score. DMARDs: disease modifying drugs. ACPA: anti-citrullinated peptide antibody. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. pos.: positive. ULN: upper limit of normal. PAD: peripheral artery disease.

3.2. Disease Activity

For all patients, the mean DAS28 decreased significantly from 4.4 at baseline to 3.59, 3.22, 3.18, and 3.13 at 90, 180, 270, and 360 days (Figure 1). In total, 53% of patients achieved LDA and 48% DAS28 defined remission. The median time to LDA and remission was 319 days and 645 days, respectively.

The rates of LDA and remission under tofacitinib were higher in patients naïve to biologics compared to patients who had been previously exposed: 100% of naïve patients achieved LDA, and 83.3% achieved remission, as compared to 53.3% and 44.9% of pre-exposed to biologics patients. Also, the duration of tofacitinib treatment until LDA or remission was shorter in patients naïve to biologics. Patients in this cohort achieved LDA after a median 92 days and remission after a median 132 days, while medians for achieving LDA and remission among patients pre-exposed to biologic agents amounted to 434 days and 692 days, respectively. In both cases, the difference between naïve and pre-exposed patients was statistically significant (Figure 2, $p < 0.001$).

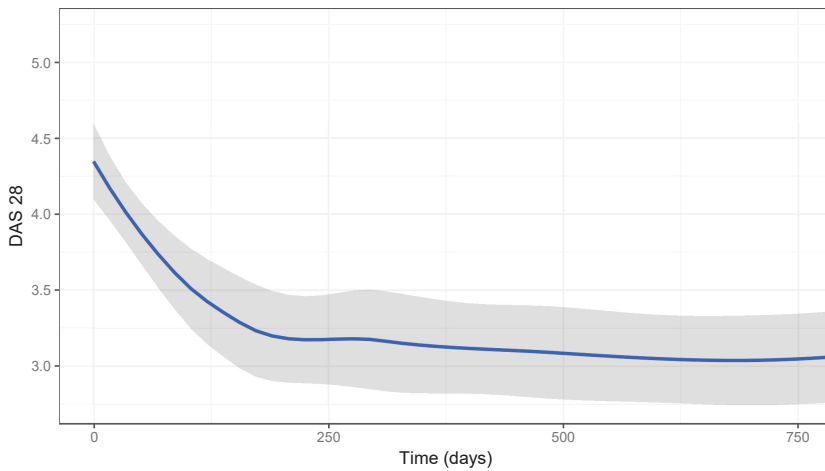


Figure 1. Disease activity: The average disease activity score (DAS28) level is shown for all rheumatoid arthritis (RA) patients treated with tofacitinib with a 95% confidence interval.

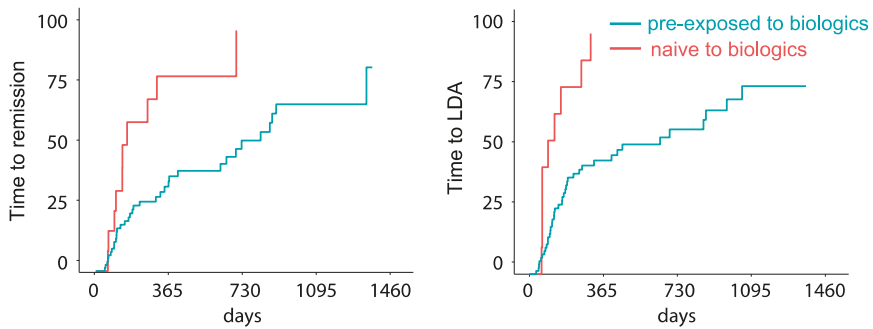


Figure 2. Disease activity: Time to remission (left panel) and low disease activity (LDA, right panel) is shown for all RA patients treated with tofacitinib. Patients previously exposed to biologic agents are shown in green, and patients naïve to biologics in red.

3.3. Discontinuation

A total of 89 (61.8%) patients remained on tofacitinib during follow-up. The median time to stop tofacitinib was 95 days (range: 4–1106). A total of 21 patients (14.6%) stopped tofacitinib due to insufficient responses and 35 patients (23.6%) stopped due to adverse events (AEs, Table 2). Of these, the most frequent reasons for discontinuing tofacitinib were gastrointestinal symptoms ($n = 18$), followed by infection ($n = 5$), myalgia ($n = 2$), remission ($n = 2$), headache, cough, blue finger syndrome, intolerance, heartburn, psoriasis, and increased liver enzymes (all $n = 1$). The median time to stop tofacitinib treatment due to ineffectiveness was 204 days (Figure 3). The median time to stop treatment due to AEs ranged from 10 to 290 days (Figure 3). None of the demographic parameters at baseline was a significant predictor for stopping tofacitinib.

Table 2. Reasons for stopping tofacitinib.

| Reason | Number | Time to Stop Tofa |
|-------------------------|---------------|------------------------------|
| Inefficacy/flare | <i>n</i> = 22 | median d204, range d21–d1106 |
| Gastrointestinal | <i>n</i> = 18 | median d28, range d4–d265d |
| Infection | <i>n</i> = 5 | median d154, range d85–d877 |
| Myalgia | <i>n</i> = 2 | range d92–d171 |
| Remission | <i>n</i> = 2 | range d106–d379 |
| Headache | <i>n</i> = 2 | d30 |
| Cough | <i>n</i> = 1 | d22 |
| Blue finger syndrome | <i>n</i> = 1 | d10 |
| Intolerance | <i>n</i> = 1 | d42 |
| Heartburn | <i>n</i> = 1 | d39 |
| Psoriasis | <i>n</i> = 1 | d287 |
| Increased liver enzymes | <i>n</i> = 1 | d290 |

d: day. *n*: number.

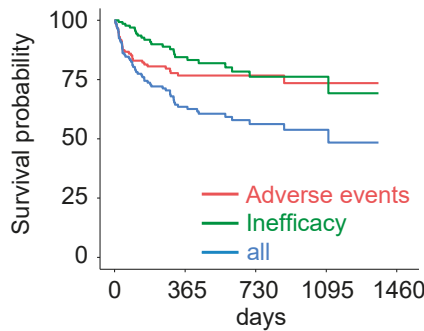


Figure 3. Time to discontinuation of tofacitinib was analyzed for all patients (*n* = 57 out of 144 total patients, blue line). Patients stopping for ineffectiveness (*n* = 22, green line) or adverse events (*n* = 35, red line) are shown separately.

3.4. Laboratory Values

Laboratory values including liver enzymes, creatinine, lymphocyte count, and hemoglobin were followed during tofacitinib treatment. Increased ALAT or ASAT > 2× ULN were detected in 3.3% and 4.4% of patients, respectively. These changes were transient in 50% and 60% of cases, respectively. Hemoglobin decrease of >10% was detected in 15.1% of patients and decreased lymphocytes <500/μL in 3.4%. An increase in creatinine >20% was detected in 9.4% (Figure 4).

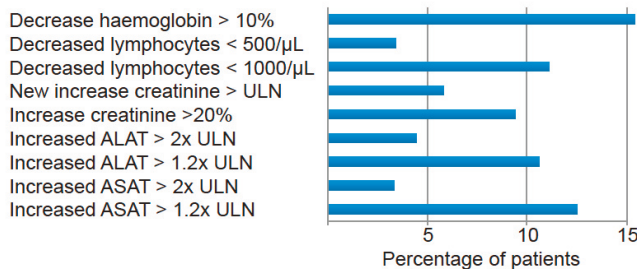


Figure 4. Patients were followed for laboratory changes under treatment with tofacitinib. Data are shown for patients with at least one in- or decrease in one of these parameters during follow-up. Percentages were calculated on patients with available data. ALAT: alanine amino transferase. ASAT: aspartate amino transferase.

4. Discussion

This study retrospectively analyzed real-life data from a cohort of 144 RA patients treated with tofacitinib 5 mg bid, with the aim of assessing the effectiveness and tolerability of tofacitinib in a clinical setting.

4.1. Effectiveness

Among the patient cohort, tofacitinib significantly reduced disease activity, with 58.2% of patients achieving LDA and 49.5% achieving remission at follow-up. This is a little higher than in published phase I–III clinical trials. In these clinical trials, the overall proportion of RA patients achieving DAS28 defined as was LDA 5.7%–47.5% [34,37,43,46,47] and remission 7.2%–23.1% [34,36,37,43,46–49], depending on the exposure to and efficacy of previous treatments. Essentially, our findings corroborate those of previous studies that have shown tofacitinib to be effective in the management of RA [33–37,48,50].

A total of 15.9% of our patients stopped tofacitinib due to ineffectiveness. Percentages of inefficacy were not published in the pivotal clinical trials, especially as this is not a defined outcome. Therefore, the best approximation may be missing an ACR 20 response. The ACR 20 response was not reached in 33.9% of the MTX-IR (methotrexate incomplete responders) patients and 48.2% of the TNF-IR (tumor necrosis factor incomplete responders) patients [51] in the phase II and III program for tofacitinib and 28.7% of naïve patients [47]. In a long-term extension study, 20.4% of patients did not achieve ACR 20 after 24 months and 21.5 after 96 months [52]. Importantly, not achieving ACR 20 does not necessarily mean that a patient or a treating physician considers the therapeutic response to, e.g., tofacitinib, ineffective in a clinical setting. Thus, the rate of 15.9% of patients stopping tofacitinib for ineffectiveness appears to be somewhat lower than observed in the clinical studies and long-term extension studies. However, because, as outlined above, missing an ACR 20 response does not necessarily reflect inefficacy, we think that these rates are comparable.

Although tofacitinib demonstrated effectiveness across all patient demographics, a significant difference was observed between patients naïve to biologic agents and patients who had previously been exposed to biologics: naïve patients had a trend of a higher rate of achieving LDA and remission compared to pre-exposed patients. Also, the duration of tofacitinib treatment until LDA and remission was significantly shorter in patients naïve to biologics. However, the small number of patients naïve to biologics have to be taken into account. These patients naïve to biologics had a shorter mean duration of disease at initiation of tofacitinib and lower mean baseline DAS28, which may have influenced the results. Shorter disease duration [53–55] and lower disease activity at initiation of treatment [56–59] have both been shown to correlate with higher rates of LDA and remission in RA patients. However, the indication that previous biologic therapies are associated with a reduced clinical response to tofacitinib is consistent with recent studies: a meta-analysis of phase II and III clinical trials of tofacitinib in RA patients published in 2016 showed that patients who were naïve to biologics had a numerically better clinical response compared to patients with a prior inadequate response to biologics [60]. This finding was confirmed by a direct comparison study [61]. In the 2015, ACR guidelines for the treatment of RA tofacitinib were still recommended as a second-line drug after treatment with biologic agents resulted in an inadequate response or intolerance [62]. In the EULAR guidelines, tofacitinib is already recommended for RA that has inadequately responded to one or more csDMARDs [63].

This is reflected in the patient cohort of the present analysis, in which only 15.2% of patients were biologic-naïve. However, this and other studies suggest that there is a benefit to be gained from using tofacitinib early in the treatment of RA, before the initiation of biologics, which may call into question the position of tofacitinib as a second-line drug.

4.2. Adverse Events

Few AEs leading to the discontinuation of tofacitinib treatment were observed in this study. Among the AEs that lead to stopping tofacitinib, the most frequent were gastrointestinal AEs, followed by infections. Patients experienced no severe or life-threatening AEs under tofacitinib. The safety profile was comparable to published data, except for a single case of blue finger syndrome [64]. Following initiation, patients developed an increase in LDL cholesterol, which has been established as a side effect of tofacitinib treatment in previous studies [65,66]; however, a tofacitinib-induced increase in cholesterol does not appear to be associated with a higher incidence of cardiovascular AEs in patients [60,65,67].

It is interesting that AEs are the main reason for stopping tofacitinib in the period directly following initiation of treatment, later superseded by insufficient therapeutic responses. Treatment had to be stopped for AEs, if necessary, usually rather early in the course of treatment, considering that the average time of follow up was 1.22 years and the mean time to stop tofacitinib was 183 days. Most patients (23%) discontinued treatment for AEs as early as within the first month (Figure 3). We found no increase in AEs with longer disease duration. Non-tolerability of the drug seems to become apparent rather early after initiation of treatment.

The rate of 24.3% of patients stopping tofacitinib for adverse events in our study is comparable to the rate of 25% published in the 9.5 year long-term extension study published by Wollenhaupt et al. [52].

4.3. Limitations

A significant limitation of this study is that it deals with real-life data. Follow-ups in real-life practice are not as frequent or consistent as in clinical studies. The size of the patient cohort was also limited, and there was a considerable size difference between the sub-cohorts of patients naïve to biologics and patients with prior exposure. However, the design as a retrospective, whole population real-life analysis also constitutes a strength of this study, as its results reflect the variability of patient populations in medical practice more than data on selected patients in controlled clinical trials.

5. Conclusions

The efficacy and safety of tofacitinib have been established in clinical trials. This retrospective analysis of real-life data shows that tofacitinib is also effective and safe in a real-life setting. Over 50% of the patient cohort achieved LDA or remission on a dose of tofacitinib 5 mg bid, with a higher rate of patients naïve to biologic agents achieving either LDA or remission. The safety profile of tofacitinib was generally consistent with previous studies. In conclusion, our results support the use of tofacitinib in the treatment of RA to achieve a more successful clinical outcome.

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Abbreviations

| | |
|---------|---|
| ACPA | anti-citrullinated protein antibody |
| ACR | American College of Rheumatology |
| AE | adverse event |
| AGREE | Abatacept study to gauge remission and joint damage progression in methotrexate-naïve patients with early erosive rheumatoid arthritis |
| CAMERA | Computer Assisted Management in Early Rheumatoid Arthritis |
| COMET | Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis |
| CRP | C-reactive protein |
| Cs | Conventional synthetic |
| DAS | Disease Activity Score |
| DMARD | disease-modifying antirheumatic drug |
| ESR | erythrocyte sedimentation rate |
| EULAR | European League Against Rheumatism |
| GI | gastrointestinal |
| LDA | low disease activity |
| MTX | methotrexate |
| PREMIER | A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment |
| RA | rheumatoid arthritis |
| RF | rheumatoid factor |
| SC | subcutaneous |
| SWEFOT | Swedish Pharmacotherapy Trial |
| TEAR | Treatment of Early Aggressive Rheumatoid Arthritis |
| TEMPO | Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes |

References

1. Heinimann, K.; Von Kempis, J.; Sauter, R.; Schiff, M.; Sokka-Isler, T.; Schulze-Koops, H.; Müller, R. Long-Term Increase of Radiographic Damage and Disability in Patients with RA in Relation to Disease Duration in the Era of Biologics. Results from the SCQM Cohort. *J. Clin. Med.* **2018**, *7*, 57. [[CrossRef](#)] [[PubMed](#)]
2. Drossaers-Bakker, K.W.; Zwinderman, A.H.; Vlieland, T.P.M.V.; Van Zeven, D.; Vos, K.; Breedveld, F.C.; Hazes, J.M.W.; Drossaers-Bakker, K.W. Long-term outcome in rheumatoid arthritis: A simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. *Arthritis Rheum.* **2002**, *47*, 383–390. [[CrossRef](#)] [[PubMed](#)]
3. Eberhardt, K.B.; Fex, E. Functional impairment and disability in early rheumatoid arthritis-development over 5 years. *J. Rheumatol.* **1995**, *22*, 1037–1042. [[PubMed](#)]
4. Furst, D.E.; Keystone, E.C.; Braun, J.; Breedveld, F.C.; Burmester, G.R.; De Benedetti, F.; Dörner, T.; Emery, P.; Fleischmann, R.; Gibofsky, A.; et al. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2012. *Ann. Rheum. Dis.* **2013**, *72* (Suppl. 2), ii2–ii34. [[CrossRef](#)] [[PubMed](#)]
5. Drosos, A.A.; Pelechas, E.; Voulgari, P.V. Rheumatoid Arthritis Treatment. A Back to the Drawing Board Project or High Expectations for Low Unmet Needs? *J. Clin. Med.* **2019**, *8*, 1237. [[CrossRef](#)] [[PubMed](#)]
6. Upchurch, K.S.; Kay, J. Evolution of treatment for rheumatoid arthritis. *Rheumatology* **2012**, *51* (Suppl. 6), vi28–vi36. [[CrossRef](#)] [[PubMed](#)]
7. Lipsky, P.E.; Van Der Heijde, D.M.; Clair, E.W.S.; Furst, D.E.; Breedveld, F.C.; Kalden, J.R.; Smolen, J.S.; Weisman, M.; Emery, P.; Feldmann, M.; et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N. Engl. J. Med.* **2000**, *343*, 1594–1602. [[CrossRef](#)]

8. Breedveld, F.C.; Weisman, M.H.; Kavanaugh, A.F.; Cohen, S.B.; Pavelka, K.; van Vollenhoven, R.; Sharp, J.; Perez, J.L.; Spencer-Green, G.T. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum.* **2006**, *54*, 26–37.
9. Moreland, L.W.; Schiff, M.H.; Baumgartner, S.W.; Tindall, E.A.; Fleischmann, R.M.; Bulpitt, K.J.; Weaver, A.L.; Keystone, E.C.; Furst, D.E.; Mease, P.J.; et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. *Ann. Intern. Med.* **1999**, *130*, 478–486. [[CrossRef](#)]
10. Clair, E.W.S.; Smolen, J.S.; Maini, R.N.; Bathon, J.M.; Emery, P.; Keystone, E.; Schiff, M.; Kalden, J.R.; Wang, B.; DeWoody, K.; et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: A randomized, controlled trial. *Arthritis Rheum.* **2004**, *50*, 3432–3443. [[CrossRef](#)]
11. Weinblatt, M.E.; Keystone, E.C.; Furst, D.E.; Moreland, L.W.; Weisman, M.H.; Birbara, C.A.; Teoh, L.A.; Fischkoff, S.A.; Chartash, E.K. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: The ARMADA trial. *Arthritis Rheum.* **2003**, *48*, 35–45. [[CrossRef](#)] [[PubMed](#)]
12. Maini, R.; Clair, E.W.S.; Breedveld, F.; Furst, D.; Kalden, J.; Weisman, M.; Smolen, J.; Emery, P.; Harriman, G.; Feldmann, M.; et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: A randomised phase III trial. *Lancet* **1999**, *354*, 1932–1939. [[CrossRef](#)]
13. Burmester, G.R.; Mariette, X.; Montecucco, C.; Monteagudo-Saez, I.; Malaise, M.; Tzioufas, A.G.; Bijlsma, J.W.; Unnebrink, K.; Kary, S.; Kupper, H. Adalimumab alone and in combination with disease-modifying antirheumatic drugs for the treatment of rheumatoid arthritis in clinical practice: The Research in Active Rheumatoid Arthritis (ReAct) trial. *Ann. Rheum. Dis.* **2007**, *66*, 732–739. [[CrossRef](#)] [[PubMed](#)]
14. Klareskog, L.; Van Der Heijde, D.; De Jager, J.P.; Gough, A.; Kalden, J.; Malaise, M.; Mola, E.M.; Pavelka, K.; Sany, J.; Settas, L.; et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: Double-blind randomised controlled trial. *Lancet* **2004**, *363*, 675–681.
15. Keystone, E.C.; Kavanaugh, A.F.; Sharp, J.T.; Tannenbaum, H.; Hua, Y.; Teoh, L.S.; Fischkoff, S.A.; Chartash, E.K. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: A randomized, placebo-controlled, 52-week trial. *Arthritis Rheum.* **2004**, *50*, 1400–1411. [[PubMed](#)]
16. Smolen, J.S.; Kay, J.; Doyle, M.K.; Landewe, R.; Matteson, E.L.; Wollenhaupt, J.; Gaylis, N.; Murphy, F.T.; Neal, J.S.; Zhou, Y.; et al. Golimumab in patients with active rheumatoid arthritis after treatment with tumour necrosis factor alpha inhibitors (GO-AFTER study): A multicentre, randomised, double-blind, placebo-controlled, phase III trial. *Lancet* **2009**, *374*, 210–221. [[CrossRef](#)]
17. Fleischmann, R.; Vencovsky, J.; van Vollenhoven, R.F.; Borenstein, D.; Box, J.; Coteur, G.; Goel, N.; Brezinschek, H.P.; Innes, A.; Strand, V. Efficacy and safety of certolizumab pegol monotherapy every 4 weeks in patients with rheumatoid arthritis failing previous disease-modifying antirheumatic therapy: The FAST4WARD study. *Ann. Rheum. Dis.* **2009**, *68*, 805–811. [[CrossRef](#)] [[PubMed](#)]
18. Smolen, J.; Landewe, R.B.; Mease, P.; Brzezicki, J.; Mason, D.; Luijstens, K.; van Vollenhoven, R.F.; Kavanaugh, A.; Schiff, M.; Burmester, G.R.; et al. Efficacy and safety of certolizumab pegol plus methotrexate in active rheumatoid arthritis: The RAPID 2 study. A randomised controlled trial. *Ann. Rheum. Dis.* **2009**, *68*, 797–804. [[CrossRef](#)] [[PubMed](#)]
19. Emery, P.; Deodhar, A.; Rigby, W.F.; Isaacs, J.D.; Combe, B.; Racewicz, A.J.; Latinis, K.; Abud-Mendoza, C.; Szczepański, L.J.; Roschmann, R.A.; et al. Efficacy and safety of different doses and retreatment of rituximab: A randomised, placebo-controlled trial in patients who are biological naive with active rheumatoid arthritis and an inadequate response to methotrexate (Study Evaluating Rituximab's Efficacy in MTX iNadequate rEsponders (SERENE)). *Ann. Rheum. Dis.* **2010**, *69*, 1629–1635. [[PubMed](#)]
20. Keystone, E.C.; Genovese, M.C.; Klareskog, L.; Hsia, E.C.; Hall, S.T.; Miranda, P.C.; Pazdur, J.; Bae, S.C.; Palmer, W.; Zrubek, J.; et al. Golimumab, a human antibody to tumour necrosis factor [alpha] given by monthly subcutaneous injections, in active rheumatoid arthritis despite methotrexate therapy: The GO-FORWARD Study. *Ann. Rheum. Dis.* **2009**, *68*, 789–796. [[PubMed](#)]

21. Schiff, M.; Keiserman, M.; Codding, C.; Songcharoen, S.; Berman, A.; Nayiager, S.; Saldade, C.; Li, T.; Aranda, R.; Becker, J.C.; et al. Efficacy and safety of abatacept or infliximab vs placebo in ATTEST: A phase III, multi-centre, randomised, double-blind, placebo-controlled study in patients with rheumatoid arthritis and an inadequate response to methotrexate. *Ann. Rheum. Dis.* **2008**, *67*, 1096–1103. [[CrossRef](#)] [[PubMed](#)]
22. Jones, G.; Sebba, A.; Gu, J.; Lowenstein, M.B.; Calvo, A.; Gomez-Reino, J.J.; Siri, D.A.; Tomšič, M.; Alecock, E.; Woodworth, T.; et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: The AMBITION study. *Ann. Rheum. Dis.* **2010**, *69*, 88–96. [[CrossRef](#)] [[PubMed](#)]
23. Bykerk, V.P.; Ostor, A.J.K.; Alvaro-Gracia, J.; Pavelka, K.; Ivorra, J.A.R.; Graninger, W.; Bensen, W.; Nurmohamed, M.T.; Krause, A.; Bernasconi, C.; et al. Comparison of tocilizumab as monotherapy or with add-on disease-modifying antirheumatic drugs in patients with rheumatoid arthritis and inadequate responses to previous treatments: An open-label study close to clinical practice. *Clin. Rheumatol.* **2015**, *34*, 563–571. [[CrossRef](#)] [[PubMed](#)]
24. Nam, J.L.; Ramiro, S.; Gaujoux-Viala, C.; Takase, K.; Leon-Garcia, M.; Emery, P.; Gossec, L.; Landewe, R.; Smolen, J.S.; Buch, M.H. Efficacy of biological disease-modifying antirheumatic drugs: A systematic literature review informing the 2013 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann. Rheum. Dis.* **2014**, *73*, 516–528. [[PubMed](#)]
25. Finckh, A.; Simard, J.F.; Duryea, J.; Liang, M.H.; Huang, J.; Daneel, S.; Forster, A.; Gabay, C.; Guerne, P.A. The effectiveness of anti-tumor necrosis factor therapy in preventing progressive radiographic joint damage in rheumatoid arthritis: A population-based study. *Arthritis Rheum.* **2006**, *54*, 54–59. [[CrossRef](#)] [[PubMed](#)]
26. Kaltsonoudis, E.; Pelechas, E.; Voulgari, P.V.; Drosos, A.A. Unmet needs in the treatment of rheumatoid arthritis. An observational study and a real-life experience from a single university center. *Semin. Arthritis Rheum.* **2019**, *48*, 597–602. [[PubMed](#)]
27. Ghoreschi, K.; Laurence, A.; O’Shea, J.J. Janus kinases in immune cell signaling. *Immunol. Rev.* **2009**, *228*, 273–287. [[CrossRef](#)]
28. Meyer, D.M.; Jesson, M.I.; Li, X.; Elrick, M.M.; Funckes-Shippy, C.L.; Warner, J.D.; Gross, C.J.; E Dowty, M.; Ramaiah, S.K.; Hirsch, J.L.; et al. Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550, in rat adjuvant-induced arthritis. *J. Inflamm.* **2010**, *7*, 41.
29. Ortmann, R.A.; Cheng, T.; Visconti, R.; Frucht, D.M.; O’Shea, J.J. Janus kinases and signal transducers and activators of transcription: Their roles in cytokine signaling, development and immunoregulation. *Arthritis Res.* **2000**, *2*, 16–32.
30. Ghoreschi, K.; Jesson, M.I.; Li, X.; Lee, J.L.; Ghosh, S.; Alsup, J.W.; Warner, J.D.; Tanaka, M.; Steward-Tharp, S.M.; Gadina, M.; et al. Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). *J. Immunol.* **2011**, *186*, 4234–4243.
31. O’Shea, J.J.; Laurence, A.; McInnes, I.B. Back to the future: Oral targeted therapy for RA and other autoimmune diseases. *Nat. Rev. Rheumatol.* **2013**, *9*, 173–182. [[CrossRef](#)] [[PubMed](#)]
32. Maeshima, K.; Yamaoka, K.; Kubo, S.; Nakano, K.; Iwata, S.; Saito, K.; Ohishi, M.; Miyahara, H.; Tanaka, S.; Ishii, K.; et al. The JAK inhibitor tofacitinib regulates synovitis through inhibition of interferon-gamma and interleukin-17 production by human CD4⁺ T cells. *Arthritis Rheum.* **2012**, *64*, 1790–1798. [[CrossRef](#)] [[PubMed](#)]
33. Coombs, J.H.; Bloom, B.J.; Breedveld, F.C.; Fletcher, M.P.; Gruben, D.; Kremer, J.M.; Burgos-Vargas, R.; Wilkinson, B.; Zerbin, C.A.; Zwillich, S.H. Improved pain, physical functioning and health status in patients with rheumatoid arthritis treated with CP-690,550, an orally active Janus kinase (JAK) inhibitor: Results from a randomised, double-blind, placebo-controlled trial. *Ann. Rheum. Dis.* **2010**, *69*, 413–416. [[CrossRef](#)] [[PubMed](#)]
34. Burmester, G.R.; Blanco, R.; Charles-Schoeman, C.; Wollenhaupt, J.; Zerbin, C.; Benda, B.; Gruben, D.; Wallenstein, G.; Krishnaswami, S.; Zwillich, S.H.; et al. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: A randomised phase 3 trial. *Lancet* **2013**, *381*, 451–460. [[CrossRef](#)]
35. Fleischmann, R.; Kremer, J.; Cush, J.; Schulze-Koops, H.; Connell, C.A.; Bradley, J.D.; Gruben, D.; Wallenstein, G.V.; Zwillich, S.H.; Kanik, K.S. Placebo-controlled trial of tofacitinib monotherapy in rheumatoid arthritis. *N. Engl. J. Med.* **2012**, *367*, 495–507. [[CrossRef](#)] [[PubMed](#)]

36. Kremer, J.; Li, Z.-G.; Hall, S.; Fleischmann, R.; Genovese, M.; Martín-Mola, E.; Isaacs, J.D.; Gruben, D.; Wallenstein, G.; Krishnaswami, S.; et al. Tofacitinib in combination with nonbiologic disease-modifying antirheumatic drugs in patients with active rheumatoid arthritis: A randomized trial. *Ann. Intern. Med.* **2013**, *159*, 253–261. [[CrossRef](#)] [[PubMed](#)]
37. Wollenhaupt, J.; Silverfield, J.; Lee, E.B.; Curtis, J.R.; Wood, S.P.; Soma, K.; Nduaka, C.I.; Benda, B.; Gruben, D.; Nakamura, H.; et al. Safety and efficacy of tofacitinib, an oral janus kinase inhibitor, for the treatment of rheumatoid arthritis in open-label, longterm extension studies. *J. Rheumatol.* **2014**, *41*, 837–852. [[CrossRef](#)] [[PubMed](#)]
38. Kremer, J.M.; Cohen, S.; Wilkinson, B.E.; Connell, C.A.; French, J.L.; Gómez-Reino, J.; Gruben, D.; Kanik, K.S.; Krishnaswami, S.; Pascual-Ramos, V.; et al. A phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) versus placebo in combination with background methotrexate in patients with active rheumatoid arthritis and an inadequate response to methotrexate alone. *Arthritis Rheum.* **2012**, *64*, 970–981. [[CrossRef](#)]
39. Tanaka, Y.; Suzuki, M.; Nakamura, H.; Toyozumi, S.; Zwillich, S.H. Tofacitinib Study I. Phase II study of tofacitinib (CP-690,550) combined with methotrexate in patients with rheumatoid arthritis and an inadequate response to methotrexate. *Arthritis Care Res.* **2011**, *63*, 1150–1158. [[CrossRef](#)]
40. Wagner, S.; Forejtova, S.; Zwillich, S.H.; Gruben, D.; Koncz, T.; Wallenstein, G.V.; Krishnaswami, S.; Bradley, J.D.; Van Vollenhoven, R.F.; Fleischmann, R.; et al. Tofacitinib or adalimumab versus placebo in rheumatoid arthritis. *N. Engl. J. Med.* **2012**, *367*, 508–519.
41. Vieira, M.C.; Zwillich, S.H.; Jansen, J.P.; Smiechowski, B.; Spurden, D.; Wallenstein, G.V. Tofacitinib Versus Biologic Treatments in Patients With Active Rheumatoid Arthritis Who Have Had an Inadequate Response to Tumor Necrosis Factor Inhibitors: Results From a Network Meta-analysis. *Clin. Ther.* **2016**, *38*, 2628–2641.e5. [[CrossRef](#)] [[PubMed](#)]
42. Bergthaler, E.; Gerber, R.A.; Gruben, D.; Lukic, T.; Makin, C.; Wallenstein, G. Tofacitinib versus Biologic Treatments in Moderate-to-Severe Rheumatoid Arthritis Patients Who Have Had an Inadequate Response to Nonbiologic DMARDs: Systematic Literature Review and Network Meta-Analysis. *Int. J. Rheumatol.* **2017**, *2017*, 8417249. [[CrossRef](#)] [[PubMed](#)]
43. Fleischmann, R.; Mysler, E.; Luo, Z.; Demasi, R.; Soma, K.; Zhang, R.; Takiya, L.; Tatulych, S.; Mojcik, C.; Krishnaswami, S.; et al. Efficacy and safety of tofacitinib monotherapy, tofacitinib with methotrexate, and adalimumab with methotrexate in patients with rheumatoid arthritis (ORAL Strategy): A phase 3b/4, double-blind, head-to-head, randomised controlled trial. *Lancet* **2017**, *390*, 457–468. [[CrossRef](#)]
44. Fleischmann, R.; Cutolo, M.; Genovese, M.C.; Lee, E.B.; Kanik, K.S.; Sadis, S.; Connell, C.A.; Gruben, D.; Krishnaswami, S.; Wallenstein, G.; et al. Phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) or adalimumab monotherapy versus placebo in patients with active rheumatoid arthritis with an inadequate response to disease-modifying antirheumatic drugs. *Arthritis Rheum.* **2012**, *64*, 617–629. [[CrossRef](#)] [[PubMed](#)]
45. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O.; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **2010**, *69*, 1580–1588. [[CrossRef](#)] [[PubMed](#)]
46. Iwamoto, N.; Tsuji, S.; Takatani, A.; Shimizu, T.; Fukui, S.; Umeda, M.; Nishino, A.; Horai, Y.; Koga, T.; Kawashiri, S.-Y.; et al. Efficacy and safety at 24 weeks of daily clinical use of tofacitinib in patients with rheumatoid arthritis. *PLoS ONE.* **2017**, *12*, e0177057. [[CrossRef](#)] [[PubMed](#)]
47. Wilkinson, B.; Krishnaswami, S.; Van Vollenhoven, R.F.; Sexton, D.; Yuan, K.; Chen, J.; Xu, A. Tofacitinib versus methotrexate in rheumatoid arthritis. *N. Engl. J. Med.* **2014**, *370*, 2377–2386.
48. Van Der Heijde, D.; Tanaka, Y.; Fleischmann, R.; Keystone, E.; Kremer, J.; Zerbini, C.; Cardiel, M.H.; Cohen, S.; Nash, P.; Song, Y.-W.; et al. Tofacitinib (CP-690,550) in patients with rheumatoid arthritis receiving methotrexate: Twelve-month data from a twenty-four-month phase III randomized radiographic study. *Arthritis Rheum.* **2013**, *65*, 559–570. [[CrossRef](#)]
49. Smolen, J.S.; Aletaha, D.; Gruben, D.; Zwillich, S.H.; Krishnaswami, S.; Mebus, C. Brief Report: Remission Rates With Tofacitinib Treatment in Rheumatoid Arthritis: A Comparison of Various Remission Criteria. *Arthritis Rheumatol.* **2017**, *69*, 728–734. [[CrossRef](#)]

50. Kremer, J.M.; Bloom, B.J.; Breedveld, F.C.; Coombs, J.H.; Fletcher, M.P.; Gruben, D.; Krishnaswami, S.; Burgos-Vargas, R.; Wilkinson, B.; Zerbini, C.A.F.; et al. The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: Results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo. *Arthritis Rheum.* **2009**, *60*, 1895–1905. [[CrossRef](#)]
51. Charles-Schoeman, C.; Burmester, G.; Nash, P.; Zerbini, C.A.; Soma, K.; Kwok, K.; Hendrikx, T.; Bananis, E.; Fleischmann, R. Efficacy and safety of tofacitinib following inadequate response to conventional synthetic or biological disease-modifying antirheumatic drugs. *Ann. Rheum. Dis.* **2016**, *75*, 1293–1301. [[CrossRef](#)] [[PubMed](#)]
52. Wollenhaupt, J.; Lee, E.-B.; Curtis, J.R.; Silverfield, J.; Terry, K.; Soma, K.; Mojcik, C.; Demasi, R.; Strengholt, S.; Kwok, K.; et al. Safety and efficacy of tofacitinib for up to 9.5 years in the treatment of rheumatoid arthritis: Final results of a global, open-label, long-term extension study. *Arthritis Res. Ther.* **2019**, *21*, 89. [[CrossRef](#)] [[PubMed](#)]
53. Anderson, D.R.; Patil, S.; Kamina, A.; Penson, D.F.; Peduzzi, P.; Concato, J. Validation of a staging system for evaluating prognosis in prostate cancer. *Connect. Med.* **2000**, *64*, 459–464.
54. Harrold, L.R.; Litman, H.J.; Connolly, S.E.; Kelly, S.; Hua, W.; Alemao, E.; Rosenblatt, L.; Rebello, S.; Kremer, J.M. A window of opportunity for abatacept in RA: is disease duration an independent predictor of low disease activity/remission in clinical practice? *Clin. Rheumatol.* **2017**, *36*, 1215–1220. [[CrossRef](#)] [[PubMed](#)]
55. Furst, D.E.; Pangan, A.L.; Harrold, L.R.; Chang, H.; Reed, G.; Kremer, J.M.; Greenberg, J.D. Greater likelihood of remission in rheumatoid arthritis patients treated earlier in the disease course: Results from the Consortium of Rheumatology Researchers of North America registry. *Arthritis Care Res.* **2011**, *63*, 856–864. [[CrossRef](#)]
56. Vastesaeger, N.; Kutzbach, A.G.; Amital, H.; Pavelka, K.; Lazaro, M.A.; Moots, R.J.; Wollenhaupt, J.; Zerbini, C.A.F.; Louw, I.; Combe, B.; et al. Prediction of remission and low disease activity in disease-modifying anti-rheumatic drug-refractory patients with rheumatoid arthritis treated with golimumab. *Rheumatology* **2016**, *55*, 1466–1476. [[CrossRef](#)]
57. Aletaha, D.; Funovits, J.; Keystone, E.C.; Smolen, J.S. Disease activity early in the course of treatment predicts response to therapy after one year in rheumatoid arthritis patients. *Arthritis Rheum.* **2007**, *56*, 3226–3235. [[CrossRef](#)]
58. Smolen, J.S.; Szumski, A.; Koenig, A.S.; Jones, T.V.; Marshall, L. Predictors of remission with etanercept-methotrexate induction therapy and loss of remission with etanercept maintenance, reduction, or withdrawal in moderately active rheumatoid arthritis: Results of the PRESERVE trial. *Arthritis Res. Ther.* **2018**, *20*, 8. [[CrossRef](#)]
59. Kavanaugh, A.; Keystone, E.; Greenberg, J.D.; Reed, G.W.; Griffith, J.M.; Friedman, A.W.; Saunders, K.C.; Ganguli, A. Benefit of biologics initiation in moderate versus severe rheumatoid arthritis: Evidence from a United States registry. *Rheumatology* **2017**, *56*, 1095–1101. [[CrossRef](#)]
60. Charles-Schoeman, C.; Gonzalez-Gay, M.A.; Kaplan, I.; Boy, M.; Geier, J.; Luo, Z.; Zuckerman, A.; Riese, R. Effects of tofacitinib and other DMARDs on lipid profiles in rheumatoid arthritis: Implications for the rheumatologist. *Semin Arthritis Rheum.* **2016**, *46*, 71–80. [[CrossRef](#)]
61. Mori, S.; Yoshitama, T.; Ueki, Y. Tofacitinib Therapy for Rheumatoid Arthritis: A Direct Comparison Study between Biologic-naïve and Experienced Patients. *Intern. Med.* **2018**, *57*, 663–670. [[CrossRef](#)] [[PubMed](#)]
62. Singh, J.A.; Saag, K.G.; Bridges, S.L.; Akl, E.A.; Bannuru, R.R.; Sullivan, M.C.; Vaysbrot, E.; McNaughton, C.; Osani, M.; Shmerling, R.H.; et al. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol.* **2016**, *68*, 1–26. [[CrossRef](#)] [[PubMed](#)]
63. Smolen, J.S.; Breedveld, F.C.; Burmester, G.R.; Bykerk, V.; Dougados, M.; Emery, P.; Kvien, T.K.; Navarro-Compán, M.V.; Oliver, S.; Schoels, M.; et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann. Rheum. Dis.* **2016**, *75*, 3–15. [[CrossRef](#)] [[PubMed](#)]
64. Popp, F.; Semela, D.; von Kempis, J.; Mueller, R.B. Improvement of primary biliary cholangitis (PBC) under treatment with sulfasalazine and abatacept. *BMJ Case Rep.* **2018**. [[CrossRef](#)] [[PubMed](#)]
65. Wu, J.J.; Strober, B.E.; Hansen, P.R.; Ahlehoff, O.; Egeberg, A.; Qureshi, A.A.; Robertson, D.; Valdez, H.; Tan, H.; Wolk, R. Effects of tofacitinib on cardiovascular risk factors and cardiovascular outcomes based on phase III and long-term extension data in patients with plaque psoriasis. *J. Am. Acad. Dermatol.* **2016**, *75*, 897–905. [[CrossRef](#)]

66. Wolk, R.; Armstrong, E.J.; Hansen, P.R.; Thiers, B.; Lan, S.; Tallman, A.M.; Kaur, M.; Tatulych, S. Effect of tofacitinib on lipid levels and lipid-related parameters in patients with moderate to severe psoriasis. *J. Clin. Lipidol.* **2017**, *11*, 1243–1256. [[CrossRef](#)] [[PubMed](#)]
67. Charles-Schoeman, C.; Wicker, P.; Gonzalez-Gay, M.A.; Boy, M.; Zuckerman, A.; Soma, K.; Geier, J.; Kwok, K.; Riese, R. Cardiovascular safety findings in patients with rheumatoid arthritis treated with tofacitinib, an oral Janus kinase inhibitor. *Semin. Arthritis Rheum.* **2016**, *46*, 261–271. [[CrossRef](#)] [[PubMed](#)]



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Article

Effect of Baricitinib and Adalimumab in Reducing Pain and Improving Function in Patients with Rheumatoid Arthritis in Low Disease Activity: Exploratory Analyses from RA-BEAM

Bruno Fautrel ^{1,2,*}, Bruce Kirkham ³, Janet E. Pope ⁴, Tsutomu Takeuchi ⁵, Carol Gaich ⁶, Amanda Quebe ⁶, Baojin Zhu ⁶, Inmaculada de la Torre ⁶, Francesco De Leonardis ⁶ and Peter C. Taylor ⁷

- ¹ Rheumatology Dept, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Sorbonne Université-Assistance Publique Hôpitaux de Paris, 75013 Paris, France
 - ² Department of Rheumatology, Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, 83 bd de l'hôpital, 75013 Paris, France
 - ³ Department of Rheumatology, Guys and St Thomas' NHS Trust, Great Maze Pond, London SE1 9RT, UK
 - ⁴ Department of Medicine, Division of Rheumatology, University of Western Ontario, 1151 Richmond St, London, ON N6A 3K7, Canada
 - ⁵ Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
 - ⁶ Eli Lilly and Company, Indianapolis, IN 46285, USA
 - ⁷ Botnar Research Centre, NDORMS, University of Oxford, Old Road, Oxford OX3 7LD, UK
- * Correspondence: Bruno.fautrel@aphp.fr; Tel.: +33-1421-77620 or +33-1421-77801

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Abstract: Patients with rheumatoid arthritis (RA) may experience residual pain and functional impairment despite good control of disease activity. This study compared improvements in pain and physical function in patients with well-controlled RA after 24 weeks' treatment with baricitinib, adalimumab or placebo in the 52-week RA-BEAM phase III study. Adults with active RA and inadequate response to methotrexate received baricitinib 4 mg once daily, adalimumab 40 mg every two weeks or placebo, with background methotrexate. Patients ($N = 1010$) were categorised as in remission, in remission or low disease activity, or not in remission or low disease activity at week 24. For patients in remission or low disease activity ($n = 310$), improvements in mean pain and physical function scores at week 24 were significantly greater with baricitinib than placebo ($p < 0.001$ and $p < 0.01$, respectively) and adalimumab ($p < 0.05$ for both). For both outcomes, differences between adalimumab and placebo were not significant. The proportions of patients in remission or low disease activity with minimal or no pain and with normalised physical function were numerically greater with baricitinib than placebo. Baricitinib 4 mg once daily provided enhanced improvement in pain and physical function in patients with well-controlled RA, suggesting it may produce effects beyond immunomodulation.

Keywords: rheumatoid arthritis; baricitinib; pain; recovery of function; fatigue; productivity

1. Introduction

A major goal in the contemporary treatment of rheumatoid arthritis (RA) is to achieve remission or low disease activity, with the aim of reducing inflammation to prevent joint damage and physical disability [1,2]. However, achieving this objective target is not always associated with a corresponding improvement in disability (Health Assessment Questionnaire (HAQ)) scores and other patient-reported outcomes (PROs): In a study of the Leiden Early Arthritis Clinic cohort, for example, patients with

RA were now diagnosed after a shorter duration of symptoms and with less inflammation than they were 20 years ago, but HAQ results remained stable over this time while PROs worsened [3]. Indeed, residual pain and functional impairment can persist despite ongoing treatment and can negatively affect quality of life [4,5]. Control of pain and maintenance of physical function are priorities for patients with RA [5–9]. Improvement in PROs should, therefore, be considered an important treatment goal, in addition to reducing inflammation, for improving the health outcomes of such patients [10].

Baricitinib is an oral selective inhibitor of Janus kinase (JAK)1 and JAK2 [11], which are essential for the intracellular signalling of various cytokines associated with inflammation in RA [12,13]. It is approved for the treatment of moderately to severely active RA in adults in over 50 countries, including the USA, Europe and Japan [14–16]. The efficacy and safety of baricitinib as a treatment for RA were established in four phase III, randomised, double-blind, multicentre studies in patients with active disease [17–20].

The objective of these post-hoc analyses was to compare improvements in pain, physical function, fatigue and work productivity/loss between baricitinib, adalimumab and placebo, all given with background methotrexate, in patients with well-controlled RA (in remission or low disease activity) at week 24 in RA-BEAM.

2. Materials and Methods

2.1. RA-BEAM Study Design

RA-BEAM (NCT01710358) was a phase III, double-blind, placebo- and active-controlled study in which patients with active RA were randomised to treatment with baricitinib 4 mg, adalimumab or placebo in addition to background methotrexate for 52 weeks (24 weeks for placebo). All patients had an inadequate response to stable doses of methotrexate before study entry. The study design has been described in detail previously [20]. In brief, 1305 adult patients (aged ≥ 18 years) with moderately to severely active RA were randomised and treated with either baricitinib 4 mg once daily ($N = 487$), adalimumab 40 mg once every two weeks ($N = 330$) or once-daily placebo ($N = 488$) for 52 weeks (24 weeks for placebo) in addition to stable background methotrexate.

RA-BEAM was conducted in accordance with the ethical principles of the 1964 Declaration of Helsinki and its later amendments, and Good Clinical Practice guidelines, and was approved by each centre's institutional review board or ethics committee. Informed consent was obtained from all individual participants included in the study.

2.2. Outcomes Relevant to the Post-Hoc Analyses

Pain was assessed throughout the study, including at week 24, using a pain visual analogue scale (VAS, 0–100 mm), whereas physical function was assessed using the HAQ-Disability Index (HAQ-DI) [21]. Additional PROs assessed at baseline and week 24 included fatigue, measured using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) scale [22], and work absenteeism, presenteeism, productivity loss and activity impairment, measured using the Work Productivity and Activity Impairment Questionnaire-RA [23].

2.3. Statistical Analysis

Patients from all treatment groups were categorised as being in remission, being in remission or low disease activity, or not being in remission or low disease activity at week 24 (Table 1). Remission was defined as Disease Activity Score for 28-joint count with erythrocyte sedimentation rate (DAS28-ESR) < 2.6 and low disease activity as DAS28-ESR ≥ 2.6 and ≤ 3.2 . Not being in remission or low disease activity was defined as DAS28-ESR > 3.2 based on observed data. Changes from baseline to week 24 in pain VAS, HAQ-DI and FACIT-F scores and work-related outcomes were compared between baricitinib, adalimumab and placebo according to remission or low disease activity status. Comparisons were made using analysis of covariance (ANCOVA) adjusted for randomisation variables (region and

baseline joint erosion status (1–2 or ≥ 3) and baseline score. The proportions of patients achieving minimal or no pain and those achieving normalisation of physical function at week 24 were also compared descriptively between treatment groups according to remission status. Minimal or no pain was defined as a VAS score of ≤ 10 mm, and normalisation of physical function was defined as a HAQ-DI score of < 0.5 (including patients with a baseline HAQ-DI score of < 0.5). For all outcome measures, missing values were imputed using modified last observation carried forward (mLOCF) as per the original predefined study analyses [20]. Analyses were not controlled for multiple testing.

For changes from baseline to week 24 in pain and HAQ-DI scores, sensitivity analyses were conducted for patients in remission or low disease activity according to DAS28 with high-sensitivity C-reactive protein (DAS28-hsCRP), Simplified Disease Activity Index (SDAI) or Clinical Disease Activity Index (CDAI) criteria (Table 1).

Table 1. Remission and remission or low disease activity rates at week 24 in patients with moderately to severely active rheumatoid arthritis from RA-BEAM [14,20].

| Treatment [‡] | BARI 4 mg | ADA 40 mg Q2W | Placebo |
|--|-----------|---------------|---------|
| <i>n</i> | 487 | 330 | 488 |
| Remission rates (%) | | | |
| DAS28-ESR < 2.6 | 18 *** | 18 *** | 5 |
| DAS28-hsCRP < 2.6 | 34 *** | 32 *** | 8 |
| SDAI ≤ 3.3 | 16 *** | 14 *** | 3 |
| CDAI ≤ 2.8 | 16 *** | 12 *** | 4 |
| Remission or low disease activity rates (%) | | | |
| DAS28-ESR ≤ 3.2 | 32 *** | 34 *** | 10 |
| DAS28-hsCRP ≤ 3.2 | 52 *** | 48 *** | 19 |
| DAS28-ESR ≥ 2.6 and ≤ 3.2 | 14 | 16 | 5 |
| DAS28-hsCRP ≥ 2.6 and ≤ 3.2 | 18 | 16 | 11 |
| SDAI > 3.3 and ≤ 11 | 35 | 34 | 17 |
| CDAI > 2.8 and ≤ 10 | 34 | 36 | 16 |

[‡] Patients remained on background methotrexate throughout the study; all patients were bDMARD naïve. *** $p < 0.001$ vs. placebo. ADA adalimumab, BARI baricitinib, CDAI Clinical Disease Activity Index, bDMARD biologic disease-modifying antirheumatic drug, DAS28-ESR Disease Activity Score for 28-joint count with erythrocyte sedimentation rate, DAS28-hsCRP Disease Activity Score for 28-joint count with high-sensitivity C-reactive protein, Q2W once every two weeks, SDAI Simplified Disease Activity Index.

3. Results

Baseline characteristics of 1305 randomised and treated patients in RA-BEAM are shown in Table 2. Characteristics were similar across the treatment groups, including the proportions taking steroids and/or concomitant conventional synthetic disease-modifying antirheumatic drugs. Of these patients, 1010 were included in the current analyses—168 (baricitinib, $n = 87$; adalimumab, $n = 57$; placebo, $n = 24$) were in remission, 310 (baricitinib, $n = 154$; adalimumab, $n = 110$; placebo, $n = 46$) were in remission or low disease activity and 700 (baricitinib, $n = 267$; adalimumab, $n = 157$; placebo, $n = 276$) were not in remission or low disease activity at week 24, according to DAS28-ESR criteria.

Table 2. Baseline characteristics of 1305 randomised and treated patients in RA-BEAM, and the 168 patients in remission (DAS28-ESR <2.6) at week 24 [20].

| Characteristic | All Randomised and Treated Patients (N = 1305) | | Patients in Remission at Week 24 (N = 168) | |
|---------------------------------|--|----------------------------|--|---------------------------|
| | Placebo (N = 488) | Baricitinib 4 mg (N = 487) | Placebo (N = 24) | Baricitinib 4 mg (N = 87) |
| Age (years) | 53 ± 12 | 54 ± 12 | 52 ± 12 | 52 ± 13 |
| Female | 382 (78) | 375 (77) | 15 (63) | 62 (71) |
| Time from symptom onset (years) | 10.4 ± 9 | 10.3 ± 9 | 9.1 ± 6 | 8.9 ± 9 |
| ACPA positive | 424 (87) | 427 (88) | 22 (92) | 75 (86) |
| RF positive | 451 (92) | 439 (90) | 22 (92) | 76 (87) |
| ≥3 erosions | 371 (76) | 371 (76) | 17 (71) | 66 (77) |
| mTSS total score | 45 ± 50 | 43 ± 50 | 36 ± 41 | 39 ± 43 |
| Erosion score | 26.8 ± 29 | 25.1 ± 28 | 22.5 ± 24 | 24.4 ± 26 |
| Joint space narrowing score | 18.2 ± 23 | 17.3 ± 23 | 13.1 ± 18 | 14.6 ± 19 |
| Concomitant corticosteroid use | 290 (59) | 275 (56) | 13 (54) | 50 (58) |
| Type of csDMARD currently used | | | | |
| MTX only | 398 (82) | 413 (85) | 20 (83) | 71 (82) |
| MTX + other csDMARD | 89 (18) | 74 (15) | 4 (17) | 16 (18) |
| MTX weekly dose in mg | 15 ± 5 | 15 ± 5 | 15 ± 4 | 14 ± 5 |
| DAS28-hsCRP | 5.7 ± 1.0 | 5.8 ± 0.9 | 5.0 ± 0.8 | 5.3 ± 1.0 |
| DAS28-ESR | 6.4 ± 1.0 | 6.5 ± 0.9 | 5.4 ± 0.8 | 5.9 ± 1.0 |
| CDAI score | 38 ± 13 | 38 ± 12 | 30 ± 10 | 33 ± 12 |
| Pain VAS score (0–100 mm) † | 60 ± 23 | 62 ± 22 | 44 ± 21 | 57 ± 23 |
| HAQ-DI ‡ | 1.6 ± 0.7 | 1.6 ± 0.7 | 1.1 ± 0.5 | 1.3 ± 0.6 |
| FACIT-F § | 28.6 ± 10.7 | 28.1 ± 10.7 | 32.8 ± 7.9 | 32.5 ± 10.0 |
| | | | | 35.7 ± 9.1 |

Data are presented as mean ± standard deviation or n (%). † Higher scores indicate more severe pain. ‡ Score range 0–3, with higher scores indicating greater disability [21]. § Score range 0–52, with lower scores indicating greater fatigue [22]. ACPA anti-citrullinated protein antibody (positivity >10 units/mL), CDAl Clinical Disease Activity Index, csDMARD conventional synthetic disease-modifying antirheumatic drug, DAS28-hsCRP Disease Activity Score in 28 joints using the high-sensitivity C-reactive protein level, DAS28-ESR Disease Activity Score for 28-joint count with erythrocyte sedimentation rate, FACIT-F Functional Assessment of Chronic Illness Therapy-Fatigue, HAQ-DI Health Assessment Questionnaire-Disability Index, mTSS van der Heijde modified total Sharp score, MTX methotrexate, RF rheumatoid factor (positivity >14 units/mL), VAS visual analogue scale.

3.1. Change in Pain VAS Scores

For patients in remission, change from baseline in mean pain VAS score at week 24 with baricitinib was significantly greater than that with placebo ($p < 0.01$) and greater than that achieved with adalimumab, although the difference between baricitinib and adalimumab was not statistically significant (Figure 1). There was no significant difference between adalimumab and placebo. For patients in remission or low disease activity, change from baseline in mean pain VAS score at week 24 was significantly greater with baricitinib than with placebo ($p < 0.001$) and adalimumab ($p < 0.05$). The difference between adalimumab and placebo was not statistically significant. Results of sensitivity analyses using other disease activity measures were consistent with these findings. For patients not in remission or low disease activity, change from baseline in mean pain VAS score at week 24 was significantly greater with baricitinib and adalimumab than with placebo ($p < 0.0001$ and $p = 0.0130$, respectively). There was no significant difference between baricitinib and adalimumab.

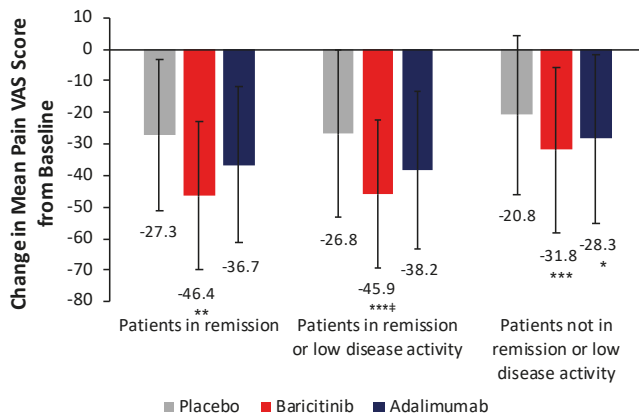


Figure 1. Change from baseline in pain VAS score at week 24 by remission status in patients from RA-BEAM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. placebo; † $p < 0.05$ vs. adalimumab. Error bars indicate standard deviation. Change in pain VAS score based on numbers of patients from RA-BEAM in remission (DAS28-ESR < 2.6): PBO+MTX $n = 24$, BARI+MTX $n = 87$, ADA+MTX $n = 57$; in remission or low disease activity (DAS28-ESR ≥ 2.6 and ≤ 3.2): PBO+MTX $n = 46$, BARI+MTX $n = 154$, ADA+MTX $n = 110$; and not in remission or low disease activity: PBO+MTX $n = 276$, BARI+MTX $n = 266$, ADA+MTX $n = 157$. One patient was missing from the BARI+MTX group for patients not in remission or low disease activity. ADA adalimumab, BARI baricitinib, DAS28-ESR Disease Activity Score for 28-joint count with erythrocyte sedimentation rate, PBO placebo, MTX methotrexate, VAS visual analogue scale.

3.2. Change in HAQ-DI Scores

For patients in remission, change from baseline in mean HAQ-DI score at week 24 was significantly greater with baricitinib and adalimumab than with placebo ($p < 0.01$ and $p < 0.05$, respectively) (Figure 2). The difference between baricitinib and adalimumab was not statistically significant. For patients in remission or low disease activity, change from baseline in HAQ-DI score at week 24 was significantly greater with baricitinib than with placebo ($p < 0.01$) and adalimumab ($p < 0.05$). There was no significant difference between adalimumab and placebo. Results of sensitivity analyses using other disease activity measures were consistent with these findings (data not shown). For patients not in remission or low disease activity, change from baseline in mean HAQ-DI score at week 24 was significantly greater with baricitinib and adalimumab than with placebo ($p < 0.0001$ and $p = 0.0014$, respectively). The difference between baricitinib and adalimumab was not statistically significant.

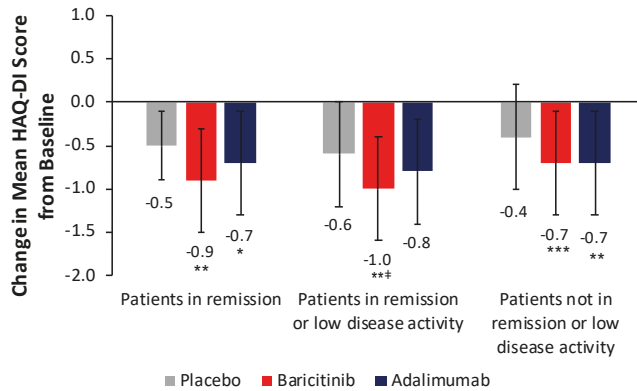


Figure 2. Change from baseline in HAQ-DI score at week 24 by remission status in patients from RA-BEAM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. placebo; † $p < 0.05$ vs. adalimumab. Error bars indicate standard deviation. Change in HAQ-DI score based on numbers of patients from RA-BEAM in remission (DAS28-ESR < 2.6): PBO+MTX $n = 24$, BARI+MTX $n = 87$, ADA+MTX $n = 57$; in remission or low disease activity (DAS28-ESR ≥ 2.6 and ≤ 3.2): PBO+MTX $n = 46$, BARI+MTX $n = 154$, ADA+MTX $n = 110$; and not in remission or low disease activity: PBO+MTX $n = 276$, BARI+MTX $n = 266$, ADA+MTX $n = 156$. One patient was missing from the BARI+MTX group and one from the ADA+MTX group for patients not in remission or low disease activity. ADA adalimumab, BARI baricitinib, DAS28-ESR Disease Activity Score for 28-joint count with erythrocyte sedimentation rate, HAQ-DI Health Assessment Questionnaire-Disability Index, MTX methotrexate, PBO placebo.

3.3. Proportion of Patients Achieving Minimal or No Pain and Proportion Achieving Normalisation of Physical Function

The proportion of patients in remission who achieved minimal or no pain at week 24 was 65.5% (57/87) for baricitinib, 61.4% (35/57) for adalimumab and 41.7% (10/24) for placebo (Figure 3a). The proportion of patients in remission who achieved normalised physical function at week 24 was 75.9% (66/87) for baricitinib, 70.2% (40/57) for adalimumab and 50.0% (12/24) for placebo (Figure 3b). Trends were the same, although proportions achieving these endpoints were slightly lower, for patients in remission or low disease activity.

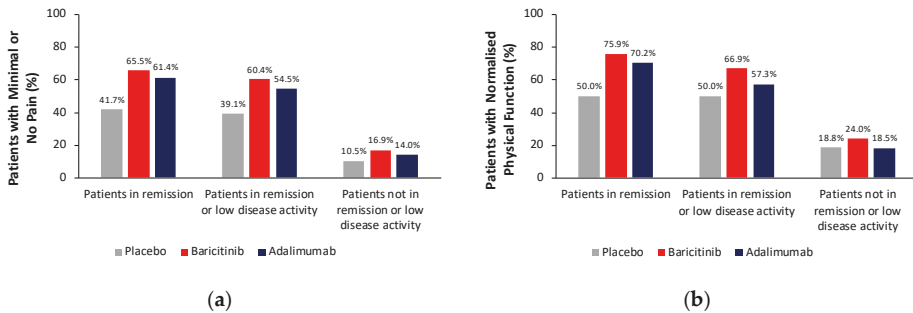


Figure 3. Patients from RA-BEAM with (a) minimal/no pain and (b) normalised physical function at week 24, by remission status. Proportions of patients from RA-BEAM with (a) minimal or no pain (pain VAS ≤ 10 mm) and (b) normalised physical function (HAQ-DI < 0.5) based on numbers of patients in remission (DAS28-ESR < 2.6): PBO+MTX $n = 24$, BARI+MTX $n = 87$, ADA+MTX $n = 57$; in remission or low disease activity (DAS28-ESR ≥ 2.6 and ≤ 3.2): PBO+MTX $n = 46$, BARI+MTX $n = 154$, ADA+MTX $n = 110$; and not in remission or low disease activity (DAS28-ESR > 3.2): PBO+MTX $n = 276$, BARI+MTX $n = 266$, ADA+MTX $n = 156$. One patient was missing from the BARI+MTX group and one from the ADA+MTX group for patients not in remission or low disease activity. For the pain analysis, the number of patients not in remission or low disease activity was PBO+MTX $n = 276$, BARI+MTX $n = 210$, ADA+MTX $n = 120$. ADA adalimumab, BARI baricitinib, DAS28-ESR Disease Activity Score for 28-joint count with erythrocyte sedimentation rate, HAQ-DI Health Assessment Questionnaire-Disability Index, MTX methotrexate, PBO placebo, VAS visual analogue scale.

3.4. Changes in Other Patient-Reported Outcomes

Despite meeting DAS28-ESR criteria for remission or low disease activity, patients continued to experience fatigue, although FACIT-F scores were < 36 in all treatment groups (Figure 4). Work productivity of working patients generally improved in all treatment groups, including those not in remission or low disease activity (Supplementary Table S1). For working patients in remission, improvements in work-related measures were numerically greater with both active treatments than with placebo. For those in remission or low disease activity, improvement in activity impairment was significantly greater with baricitinib than placebo, whereas the difference between adalimumab and placebo was not statistically significant. For working patients not in remission or low disease activity, improvements in the proportion of patients present at work, productivity loss and activity impairment were significantly greater with both active treatments than with placebo.

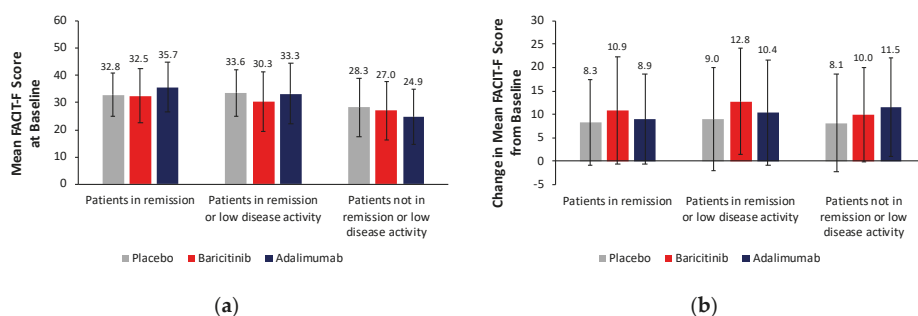


Figure 4. FACIT-F scores in patients from RA-BEAM by remission status (a) at baseline and (b) change at week 24. Error bars indicate standard deviation. FACIT-F scores at baseline and change in FACIT-F scores based on numbers of patients from RA-BEAM in remission (DAS28-ESR <2.6): PBO+MTX $n = 24$, BARI+MTX $n = 87$, ADA+MTX $n = 57$; in remission or low disease activity (DAS28-ESR ≥ 2.6 and ≤ 3.2): PBO+MTX $n = 46$, BARI+MTX $n = 154$, ADA+MTX $n = 110$; and not in remission or low disease activity (DAS28-ESR >3.2): PBO+MTX $n = 276$, BARI+MTX $n = 266$, ADA+MTX $n = 156$. One patient was missing from the BARI+MTX group and one from the ADA+MTX group for patients not in remission or low disease activity. ADA adalimumab, BARI baricitinib, DAS28-ESR Disease Activity Score for 28-joint count with erythrocyte sedimentation rate, FACIT-F Functional Assessment of Chronic Illness Therapy-Fatigue, MTX methotrexate, PBO placebo.

4. Discussion

Residual pain and impaired function persist in many patients with RA despite the achievement of disease control [4,5,24]. This residual pain may be non-inflammatory in origin, caused by sensitisation of nociceptors or peripheral joint damage; or it may be due to central sensitisation [25–27]. Results of the post-hoc analyses reported here suggest that, in patients with moderately to severely active RA and an inadequate response to methotrexate, addition of baricitinib may be more effective than adalimumab and placebo in improving pain and physical function in patients with a good level of disease control (i.e., in remission or low disease activity). Among patients in remission, significantly greater improvements in pain and physical function were observed with baricitinib than with continued methotrexate alone (placebo group). Among patients in remission or low disease activity, greater improvements in pain and physical function were observed with the addition of baricitinib than with the addition of adalimumab or with continued methotrexate alone. Notably, both active treatments were significantly more effective than placebo at improving pain and physical function in patients who did not achieve controlled disease during the study (not in remission or low disease activity).

Another analysis of data from RA-BEAM also showed that, among patients with varying levels of inflammation, patients treated with baricitinib achieved consistent pain relief regardless of the CRP level at week 24 [28]. Furthermore, patients treated with baricitinib achieved greater and more rapid pain relief than those receiving adalimumab or placebo. Using CRP levels as a surrogate for inflammation, baricitinib plus methotrexate was associated with greater relief from the non-inflammatory component of RA-associated pain than adalimumab plus methotrexate or placebo plus methotrexate [28].

The mechanisms underlying the effect of baricitinib on non-inflammatory pain are not understood. As reviewed by Taylor et al. [28], it is possible that inhibition of JAK1 and JAK2 also produces anti-nociceptive effects that are not related to inflammation, such as inhibition of the JAK2-dependent cytokine granulocyte-macrophage colony-stimulating factor, which may be involved in the pathophysiology of pain [29], and/or inhibition of the JAK2-dependent signal transducer and activator of transcription (STAT)3 phosphorylation pathway [30]. Further studies to elucidate the underlying mechanisms are warranted.

Both greater disease activity and longer disease duration have been shown to increase the likelihood of a patient retiring early or stopping work because of RA [31]. In RA-BEAM, work productivity generally improved in all working patients, including those who did not achieve remission or low disease activity, but not all work-related problems were resolved (Supplementary Table S1). Control of all aspects of the disease, including reduction of pain and fatigue and maintenance of function, is an important goal of RA treatment, and patients generally consider these specified outcomes more essential than control of inflammation [10]. Pain and physical function have been identified as key unmet needs, both clinically and for patients themselves, that can adversely affect a patient's ability to function normally and their overall well-being [5].

Control of pain in patients with RA is important, since pain has been shown to contribute to worse long-term outcomes. For example, high pain levels at disease onset have been identified as a risk factor for being in the most disabled tertile of RA patients 5–18 years after disease onset [32]. High baseline pain scores were also shown to predict suboptimal mental health ($p = 0.02$) in a cohort of South African patients with early RA [33].

Maintenance of normal physical function is also important in patients with RA. Data from the Dutch DREAM registry showed that having better physical function (measured using the HAQ-DI) was associated with work participation (odds ratio (OR) 0.32, $p = 0.000$) and with starting work after 2 years of treatment with tumour necrosis factor inhibitors (OR 0.58, $p < 0.1$) [34]. Similarly, a survey of patients with RA from France showed a high correlation between deteriorating function and work capacity, such that only 15% of patients with a HAQ score of ≥ 2 were working, compared with 63% of patients with a HAQ score of < 1 [35]. Poor physical function can also affect health-related quality of life [33].

The assessment of PROs, such as pain and function, in RA should help clinicians to focus more on the impact of the disease on patients themselves and how they are feeling rather than solely on the inflammatory component of the disease [10]. This is likely to aid in shared decision-making discussions between patients and clinicians, enabling clinicians to provide more effective and efficient patient care [25]. Indeed, it has been recommended that assessment of the PROs of pain and physical function (HAQ) be added to the current core set of treatment targets to achieve greater patient involvement in the RA treatment process [10]. Our results suggest that patients who do not respond to treatment, as measured using inflammation-associated endpoints, may still experience treatment benefit, and may, therefore, choose to continue with treatment.

Despite major advances in the treatment of RA, predicting remission a priori at the start of disease-modifying treatment remains a clinical conundrum. Nevertheless, in line with recent treatment recommendations for a treat-to-target approach, the aim of any treating rheumatologist should be to help their patients achieve remission or at least low disease activity [1,2]. With respect to this, it could be of clinical relevance to know if differences exist between agents in treating residual symptoms (such as pain) once inflammation is well controlled. Since baricitinib has demonstrated more effective pain control than adalimumab [28], we were interested in investigating whether this benefit persists even when a good level of disease control has been achieved. The post-hoc analyses reported here are not, on their own, intended to inform clinical practice, but to help towards better defining current evidence for the pain-relieving benefits of baricitinib compared to tumour necrosis factor inhibitors.

Limitations of the current analysis include that post-hoc analyses are exploratory by nature, aimed at creating hypotheses rather than clearly demonstrable facts; the sample sizes for patients achieving remission or low disease activity and working patients were small; and generalisability of the results to patients in routine care who receive baricitinib or adalimumab is uncertain. In addition, analyses were not adjusted for multiple testing.

5. Conclusions

Treatment with baricitinib 4 mg once daily or adalimumab 40 mg every other week resulted in improvements in pain, physical function, fatigue and work productivity/impairment in patients with

RA, independent of the impact on inflammation, measured using DAS28-ESR. Among patients in remission or low disease activity, greater improvements in pain and physical function were observed with baricitinib than with adalimumab and placebo. Research to better understand the role of JAK1/JAK2 pathways in the control of pain beyond the regulation of inflammation is underway to help clarify the differential effects of baricitinib relative to adalimumab on pain.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/8/9/1394/s1>, Table S1: Work-related patient-reported outcomes at week 24 according to remission status in working patients from RA-BEAM.

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References

1. Singh, J.A.; Saag, K.G.; Bridges, S.L., Jr.; Akl, E.A.; Bannuru, R.R.; Sullivan, M.C.; Vaysbrot, E.; McNaughton, C.; Osani, M.; Shmerling, R.H.; et al. 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol.* **2016**, *68*, 1–26. [[CrossRef](#)]
2. Smolen, J.S.; Landewé, R.; Bijlsma, J.; Burmester, G.; Chatzidionysiou, K.; Dougados, M.; Nam, J.; Ramiro, S.; Voshaar, M.; Van Vollenhoven, R.; 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*, 960–977. [[CrossRef](#)]
3. Nieuwenhuis, W.P.; De Wit, M.P.; Boonen, A.; Van Der Helm-Van Mil, A.H. Changes in the clinical presentation of patients with rheumatoid arthritis from the early 1990s to the years 2010: Earlier identification but more severe patient reported outcomes. *Ann. Rheum. Dis.* **2016**, *75*, 2054–2056. [[CrossRef](#)]
4. Ishiguro, N.; Dougados, M.; Cai, Z.; Zhu, B.; Ishida, M.; Sato, M.; Gaich, C.; Quebe, A.; Stoykov, I.; Tanaka, Y. Relationship between disease activity and patient-reported outcomes in rheumatoid arthritis: Post hoc analyses of overall and Japanese results from two phase 3 clinical trials. *Mod. Rheumatol.* **2018**, *28*, 950–959. [[CrossRef](#)]
5. Taylor, P.C.; Moore, A.; Vasilescu, R.; Alvir, J.; Tarallo, M. A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: A current perspective. *Rheumatol. Int.* **2016**, *36*, 685–695. [[CrossRef](#)]
6. Gossec, L.; Dougados, M.; Rincheval, N.; Balanescu, A.; Boumpas, D.T.; Canadello, S.; Carmona, L.; Daurès, J.P.; de Wit, M.; Dijkmans, B.A.; et al. Elaboration of the preliminary Rheumatoid Arthritis Impact of Disease (RAID) score: A EULAR initiative. *Ann. Rheum. Dis.* **2009**, *68*, 1680–1685. [[CrossRef](#)]

7. Khan, N.A.; Spencer, H.J.; Abda, E.; Aggarwal, A.; Alten, R.; Ancuta, C.; Andersone, D.; Bergman, M.; Craig-Muller, J.; Detert, J.; et al. Determinants of discordance in patients' and physicians' rating of rheumatoid arthritis disease activity. *Arthritis Care Res.* **2012**, *64*, 206–214. [CrossRef]
8. Wen, H.; Ralph Schumacher, H.; Li, X.; Gu, J.; Ma, L.; Wei, H.; Yokogawa, N.; Shiroto, K.; Baker, J.F.; Dinnella, J.; et al. Comparison of expectations of physicians and patients with rheumatoid arthritis for rheumatology clinic visits: A pilot, multicenter, international study. *Int. J. Rheum. Dis.* **2012**, *15*, 380–389. [CrossRef]
9. Van Tuyl, L.H.; Sadlonova, M.; Hewlett, S.; Davis, B.; Flurey, C.; Goel, N.; Gossec, L.; Brahe, C.H.; Hill, C.L.; Hoogland, W.; et al. The patient perspective on absence of disease activity in rheumatoid arthritis: A survey to identify key domains of patient-perceived remission. *Ann. Rheum. Dis.* **2017**, *76*, 855–861. [CrossRef]
10. Fautrel, B.; Alten, R.; Kirkham, B.; De La Torre, I.; Durand, F.; Barry, J.; Holzkaemper, T.; Fakhouri WTaylor, P.C. Call for action: How to improve use of patient-reported outcomes to guide clinical decision making in rheumatoid arthritis. *Rheumatol. Int.* **2018**, *38*, 935–947. [CrossRef]
11. Fridman, J.S.; Scherle, P.A.; Collins, R.; Burn, T.C.; Li, Y.; Li, J.; Covington, M.B.; Thomas, B.; Collier, P.; Favata, M.F.; et al. Selective inhibition of JAK1 and JAK2 is efficacious in rodent models of arthritis: Preclinical characterization of INCB028050. *J. Immunol.* **2010**, *184*, 5298–5307. [CrossRef]
12. O'Shea, J.J.; Kontzias, A.; Yamaoka, K.; Tanaka, Y.; Laurence, A. Janus kinase inhibitors in autoimmune diseases. *Ann. Rheum. Dis.* **2013**, *72*, ii111–ii115. [CrossRef]
13. Choy, E.H.S.; Miceli-Richard, C.; González-Gay, M.A.; Sinigaglia, L.; Schlichting, D.E.; Meszaros, G.; de la Torre, I.; Schulze-Koops, H. The effect of JAK/JAK2 inhibition in rheumatoid arthritis: Efficacy and safety of baricitinib. *RMD Open* **2019**, *5*, e000798. [CrossRef]
14. European Medicines Agency. Olumiant 2 mg and 4 mg Film-Coated Tablets. Summary of Product Characteristics. 2018. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004085/WC500223723.pdf (accessed on 11 April 2018).
15. Food and Drug Administration. Olumiant (Baricitinib) Tablets, for Oral Use. Prescribing Information; 2018. Available online: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/207924s0001bl.pdf (accessed on 21 June 2018).
16. Pharmaceutical and Medical Devices Agency. Report on the Deliberation Results. Olumiant Tablets 2 mg, Olumiant Tablets 4 mg. 2017. Available online: <http://www.pmda.go.jp/files/000226301.pdf> (accessed on 21 June 2018).
17. Genovese, M.C.; Kremer, J.; Zamani, O.; Ludivico, C.; Krogulec, M.; Xie, L.; Beattie, S.D.; Koch, A.E.; Cardillo, T.E.; Rooney, T.P.; et al. Baricitinib in patients with refractory rheumatoid arthritis. *N. Engl. J. Med.* **2016**, *374*, 1243–1252. [CrossRef]
18. Dougados, M.; van der Heijde, D.; Chen, Y.C.; Greenwald, M.; Drescher, E.; Liu, J.; Beattie, S.; Witt, S.; de la Torre, I.; Gaich CRoney, T. Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: Results from the RA-BUILD study. *Ann. Rheum. Dis.* **2017**, *76*, 88–95. [CrossRef]
19. Fleischmann, R.; Schiff, M.; van der Heijde, D.; Ramos-Remus, C.; Spindler, A.; Stanislav, M.; Zerbini, C.A.; Gurbuz, S.; Dickson, C.; de Bono, S.; et al. Baricitinib, methotrexate, or combination in patients with rheumatoid arthritis and no or limited prior disease-modifying antirheumatic drug treatment. *Arthritis Rheumatol.* **2017**, *69*, 506–517. [CrossRef]
20. Taylor, P.C.; Keystone, E.C.; van der Heijde, D.; Weinblatt, M.E.; del Carmen Morales, L.; Reyes Gonzaga, J.; Yakushin, S.; Ishii, T.; Emoto, K.; Beattie, S.; et al. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N. Engl. J. Med.* **2017**, *376*, 652–662. [CrossRef]
21. Fries, J.F.; Spitz, P.W.; Young, D.Y. The dimensions of health outcomes: The health assessment questionnaire, disability and pain scales. *J. Rheumatol.* **1982**, *9*, 789–793.
22. Cella, D.; Yount, S.; Sorensen, M.; Chartash, E.; Sengupta, N.; Grober, J. Validation of the Functional Assessment of Chronic Illness Therapy Fatigue Scale relative to other instrumentation in patients with rheumatoid arthritis. *J. Rheumatol.* **2005**, *32*, 811–819.
23. Reilly, M.C.; Zbrozek, A.S.; Dukes, E.M. The validity and reproducibility of a work productivity and activity impairment instrument. *Pharmacoeconomics* **1993**, *4*, 353–365. [CrossRef]
24. Ishida, M.; Kuroiwa, Y.; Yoshida, E.; Sato, M.; Krupa, D.; Henry, N.; Ikeda, K.; Kaneko, Y. Residual symptoms and disease burden among patients with rheumatoid arthritis in remission or low disease activity: A systematic literature review. *Mod. Rheumatol.* **2018**, *28*, 789–799. [CrossRef]

25. Taylor, P.; Manger, B.; Alvaro-Gracia, J.; Johnstone, R.; Gomez-Reino, J.; Eberhardt, E.; Wolfe, F.; Schwartzman, S.; Furfaro, N.; Kavanaugh, A. Patient perceptions concerning pain management in the treatment of rheumatoid arthritis. *J. Int. Med. Res.* **2010**, *38*, 1213–1224. [[CrossRef](#)]
26. Walsh, D.A.; McWilliams, D.F. Mechanisms, impact and management of pain in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **2014**, *10*, 581–592. [[CrossRef](#)]
27. Boyden, S.D.; Hossain, I.N.; Wohlfahrt, A.; Lee, Y.C. Non-inflammatory causes of pain in patients with rheumatoid arthritis. *Curr. Rheumatol. Rep.* **2016**, *18*, 30. [[CrossRef](#)]
28. Taylor, P.C.; Lee, Y.C.; Fleischmann, R.; Takeuchi, T.; Perkins, E.L.; Fautrel, B.; Zhu, B.; Quebe, A.K.; Gaich, C.L.; Zhang, X.; et al. Achieving pain control in rheumatoid arthritis with baricitinib or adalimumab plus methotrexate: Results from the RA-BEAM trial. *J. Clin. Med.* **2019**, *8*, 831. [[CrossRef](#)]
29. Cook, A.D.; Pobjoy, J.; Steidl, S.; Dürr, M.; Braine, E.L.; Turner, A.L.; Lacey, D.C.; Hamilton, J.A. Granulocyte-macrophage colony-stimulating factor is a key mediator in experimental osteoarthritis pain and disease development. *Arthritis Res. Ther.* **2012**, *14*, R199. [[CrossRef](#)]
30. Dominguez, E.; Rivat, C.; Pommier, B.; Mauborgne, A.; Pohl, M. JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat. *J. Neurochem.* **2008**, *107*, 50–60. [[CrossRef](#)]
31. Capron, J.; De Leonardis, F.; Fakhouri, W.; Burke, T.; Rose, A.; Jacob, I. The impact of rheumatoid arthritis (RA) on a patient's ability to stay in work and level of pain experienced. *Value Health* **2017**, *20*, A531–A532. [[CrossRef](#)]
32. Malm, K.; Bergman, S.; Andersson, M.; Bremander, A.; BARFOT Study Group. Predictors of severe self-reported disability in RA in a long-term follow-up study. *Disabil. Rehabil.* **2015**, *37*, 686–691. [[CrossRef](#)]
33. Hodkinson, B.; Musenge, E.; Ally, M.; Meyer, P.W.; Anderson, R.; Tikly, M. Functional disability and health-related quality of life in South Africans with early rheumatoid arthritis. *Scand. J. Rheumatol.* **2012**, *41*, 366–374. [[CrossRef](#)]
34. Manders, S.H.; Kievit, W.; Braakman-Jansen, A.L.; Brus, H.L.; Hendriks, L.; Fransen, J.; van de Laar, M.A.; van Riel, P.L. Determinants associated with work participation in patients with established rheumatoid arthritis taking tumor necrosis factor inhibitors. *J. Rheumatol.* **2014**, *41*, 1263–1269. [[CrossRef](#)]
35. Kobelt, G.; Woronoff, A.S.; Richard, B.; Peeters, P.; Sany, J. Disease status, costs and quality of life of patients with rheumatoid arthritis in France: The ECO-PR study. *Jt. Bone Spine* **2008**, *75*, 408–415. [[CrossRef](#)]



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Article

Next-Generation Sequencing Profiles of the Methylome and Transcriptome in Peripheral Blood Mononuclear Cells of Rheumatoid Arthritis

Chia-Chun Tseng^{1,2}, Yuan-Zhao Lin¹, Chia-Hui Lin¹, Ruei-Nian Li³, Chang-Yi Yen⁴, Hua-Chen Chan⁵, Wen-Chan Tsai⁵, Tsan-Teng Ou⁵, Cheng-Chin Wu⁵, Wan-Yu Sung⁵ and Jeng-Hsien Yen^{1,5,6,7,*}

¹ Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

² Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung 80145, Taiwan

³ Department of Biomedical Science and Environmental Biology, College of Life Science, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

⁴ Department of Internal Medicine, National Cheng Kung University Hospital, Tainan 70403, Taiwan

⁵ Division of Rheumatology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung 80754, Taiwan

⁶ Institute of Medical Science and Technology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan

⁷ Department of Biological Science and Technology, National Chiao-Tung University, Hsinchu 30010, Taiwan

* Correspondence: jehsyeh@kmu.edu.tw; Tel.: +886-7-3121101 (ext. 6088)

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Abstract: Using next-generation sequencing to decipher methylome and transcriptome and underlying molecular mechanisms contributing to rheumatoid arthritis (RA) for improving future therapies, we performed methyl-seq and RNA-seq on peripheral blood mononuclear cells (PBMCs) from RA subjects and normal donors. Principal component analysis and hierarchical clustering revealed distinct methylation signatures in RA with methylation aberrations noted across chromosomes. Methylation alterations varied with CpG features and genic characteristics. Typically, CpG islands and CpG shores were hypermethylated and displayed the greatest methylation variance. Promoters were hypermethylated and enhancers/gene bodies were hypomethylated, with methylation variance associated with expression variance. RA genetically associated genes preferentially displayed differential methylation and differential expression or interacted with differentially methylated and differentially expressed genes. These differentially methylated and differentially expressed genes were enriched with several signaling pathways and disease categories. 10 genes (*CD86*, *RAB20*, *XAF1*, *FOLR3*, *LTBR*, *KCNH8*, *DOK7*, *PDGFA*, *PITPNM2*, *CELSR1*) with concomitantly differential methylation in enhancers/promoters/gene bodies and differential expression in B cells were validated. This integrated analysis of methylome and transcriptome identified novel epigenetic signatures associated with RA and highlighted the interaction between genetics and epigenetics in RA. These findings help our understanding of the pathogenesis of RA and advance epigenetic studies in regards to the disease.

Keywords: rheumatoid arthritis; methylation; next-generation sequencing

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease manifested by sustained chronic inflammation resulting in joint damage and severe disability. Numerous therapies based on our knowledge of RA were developed over the past two decades and helped improve outcomes for those suffering from the disease [1]. Despite the increasing number of treatment strategies, many patients are refractory to their

current treatments, some patients see their clinical response diminish, while others suffer from adverse events from therapy. As such, there is a necessity to develop an improved strategy to treat RA [2].

Engrafting peripheral blood mononuclear cells (PBMCs) of RA patients into severe combined immunodeficient (SCID) mice resulted in a reconstituted synovitis characteristic of human RA [3]. Additionally, these cells secreted numerous inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF- α) [4,5] which orchestrated inflammation, radiographic progression of RA and were, therefore, therapeutic targets of current RA management [6]. These findings highlight the critical role of PBMCs in RA pathogenesis. Therefore, a more comprehensive understanding of PBMCs in RA holds promise in unraveling the complexity of RA and identifying novel therapeutic targets.

In past decades, tremendous efforts have been devoted to exploring RA genetics. However, in recent years, DNA methylation is emerging as one key pathogenic player of RA. DNA methylation acts as a composite measure of environmental exposures [7], making it an intriguing candidate for the investigation of diseases that involve environmental factors, such as RA. Traditionally, DNA methylation has been thought of as being involved in gene silencing but recent work has shown a more complex picture [8]. Most studies investigating the role of DNA methylation in RA utilized the Illumina 450K microarray for methylation profiling and focused on methylation alone. Studies integrating DNA methylation with gene expression at a whole-genome manner to investigate the relationship between methylation, expression and RA, the associations of genomic contexts and DNA methylation and the interaction between differentially methylated genes and genetic at-risk loci in RA remain somewhat limited.

To decipher the methylation signatures involved in RA PBMCs, we performed next generation sequencing to compare the methylome and transcriptome landscape in PBMCs from RA patients and healthy donors, detect changes to the methylome and transcriptome, elucidate the relationship between methylation and expression and interaction between genetically associated genes and epigenetically associated genes. These results offered a map to the PBMCs methylome and shed light on the pathophysiology of RA.

2. Methods

After adjusting for cell types and batch effects (Figure 1), methylome data went through principal component analysis (PCA) and hierarchical clustering (HC) (Figure 1, Step 1), OmicCircos visualization (Figure 1, Step 2), CpG features mapping (Figure 1, Step 3), genic characteristics annotation (Figure 1, Step 4), integration with transcriptome for methylation-expression correlation (Figure 1, Step 5) and identification of concomitantly differentially methylated (false discovery rate (FDR) <0.05) and differentially expressed genes (FDR < 0.05) (Figure 1, Step 6–7). Genes with concomitantly differential methylation and differential expression underwent genetic–epigenetic interaction investigation (Figure 1, Step 8), Ingenuity Pathway Analysis (IPA) (Figure 1, Step 9), and upstream regulator deduction (Figure 1, Step 10). GEO dataset were downloaded for further validation (Figure 1, Step 11). For detailed methods, see Supplementary Files. The study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee of the Kaohsiung Medical University Hospital (KMUHIRB-G(II)-20180031). All subjects gave their informed consent for inclusion before they participated in the study.

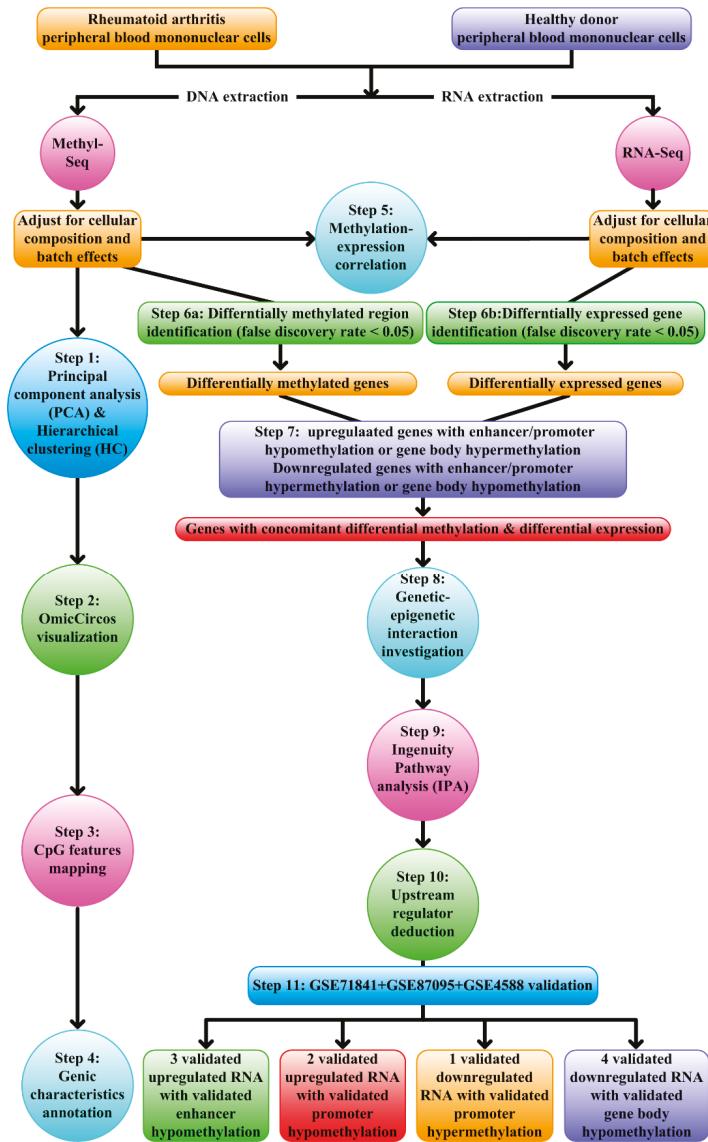


Figure 1. Schematic representation of the next-generation sequencing data analytical workflow. After adjusting for cellular composition and batch effects, methyl-seq data first underwent Principal component analysis (PCA) and hierarchical clustering (HC) (Step 1), OmicCircos visualization (Step 2), CpG features mapping (Step 3), and genic characteristics annotation (Step 4). Methylation and expression profiles were then integrated for methylation-expression correlation (Step 5). Differentially methylated genes (FDR < 0.05) and differentially expressed genes (FDR < 0.05) were identified (Step 6a–6b) and intersected to yield genes with concomitant expression and methylation changes in enhancer/promoter/gene body (Step 7). These differentially methylated and differentially expressed genes underwent genetic–epigenetic interaction investigation (Step 8), IPA (Step 9), and upstream regulator deduction (Step 10). GEO dataset validation (Step 11) confirmed concomitant differential methylation and expression of 10 genes.

3. Results

3.1. Differential Methylation of PBMCs in RA

After adjusting for cellular composition and batch effects, we first profiled DNA methylation alterations between RA and healthy donors with PCA and HC (Figure 1, Step 1). As shown in Figure S2a, RA samples were characterized by distinct methylation profiles compared with healthy donors. We also performed molecular stratification of samples using HC of methylation profiles (Figure S2b). Based on methylation profiles, two distinct groups were identified, with the results reaffirming the classification of RA and healthy donors.

3.2. Distribution of Methylation According to Genome Locations

For a visual representation of the analysis results, the R package Omicircos was used to draw the circos-plot. Supplementary Figure S3 depicted the methylation differences between RA and healthy donors according to chromosome locations. Generally, methylation alterations were scattered across nuclear genomes. No clear concentration of methylation changes was identified.

3.3. CpG Features Mapping

Past studies suggest methylation alterations depended on CpG features [9]. However, whether similar phenomena existed in RA remained unexplored. Traditionally, CpG sites are classified into four classes according to their CpG features. CpG islands are genomic regions of >200 bp with a CG content of >50% and an observed/expected CpG ratio of >60%. CpG shores are located within 2 kb from CpG island). CpG shelves include regions 2–4 kb from CpG island. The remaining regions >4 kb from CpG island are defined as open seas [10]. To clarify whether methylation variations differed with respective CpG features, we classified CpG into CpG islands, CpG shores, CpG shelves, and open seas adopting similar classification schemes (Figure 1, Step 3). On average, CpG islands and CpG shores were hypermethylated in RA, and CpG shelves and open seas were hypomethylated in RA and methylation difference differed with respect to CpG features (Figure 2a). Overall, the methylation variance was most pronounced in CpG islands, CpG shores, followed by open seas and CpG shelves ($p < 0.001$) (Figure 2b).

3.4. Genic Characteristics Annotation

In addition to CpG features, evidence suggested methylation alterations differed with respect to genic characteristics [9]. To test these possibilities in RA, we annotated every CpG to enhancers, promoters, gene bodies, and intergenic regions (Figure 1, Step 4). Generally, CpG in promoters were hypermethylated and CpG in enhancers, gene bodies and intergenic regions were hypomethylated in RA, with significant methylation differences between different genic characteristics (Figure 2c). Furthermore, the methylation variance was most striking in enhancers, followed by promoters and intergenic regions, decreased in gene bodies ($p < 0.001$) (Figure 2d). When we further stratified CpG located in promoters according to their distance to transcription start sites, the results showed preferential methylation alterations near the transcription start sites (Figure S4).

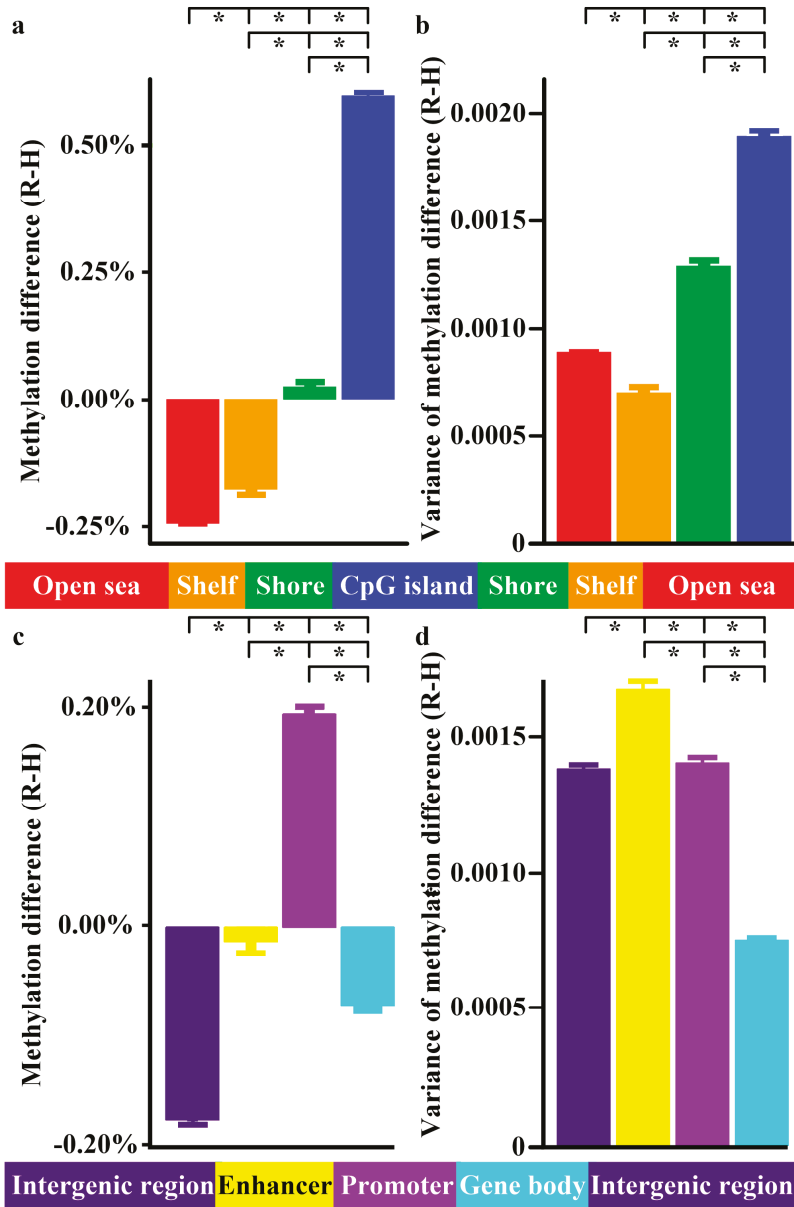


Figure 2. Methylation differences according to CpG features and genic characteristics. The bar charts showed the methylation difference (rheumatoid arthritis (R) minus healthy donor (H)) in CpG island, CpG shore, CpG shelf, open sea (a) and variance of methylation according to respective CpG features (b). Methylation difference in intergenic region, enhancer, promoter, gene body (c) and variance of methylation in respective genic characteristics (d) were also presented. * $p < 0.001$ for methylation difference and variance of methylation between different CpG features and genic characteristics.

3.5. Methylation Variation Linked to Transcription Variation

Since transcription is regulated through epigenetic marks, we subsequently set upon determining whether the presence of methylation alterations was linked to alterations in gene expression (Figure 1, Step 5). We divided CpG into high variance (methylation variance above mean methylation variance) and low variance (methylation variance below mean methylation variance). Enhancer CpG with high methylation variance was associated with greater variation in transcript abundance compared with enhancer CpG with low methylation variance ($p < 0.001$, Figure S5a,b). Promoter CpG with high methylation variance was associated with greater variation in transcript abundance compared with promoter CpG with low methylation variance ($p < 0.001$, Figure S5c,d). We next focused our analysis on CpG located in gene bodies. Again, a higher variance of gene expression was significantly associated with gene body CpG with higher methylation variance ($p < 0.001$, Supplementary Figure S5e,f).

3.6. Integration of Methylation and Expression Profiles

After confirming the association between methylation variation and expression variation, we interrogated methylation and expression profiles to identify differentially methylated genes and differentially expressed genes. We first identify genes with differentially-methylated regions (FDR < 0.05) (Figure 1, Step 6a). In the same time, differentially expressed genes (FDR < 0.05) were found (Figure 1, Step 6b). Since enhancer/promoter methylation was associated with decreased gene expression and gene body methylation was associated with increased gene expression [8,11], we intersected differentially methylated genes and differentially expressed genes to obtain genes with concomitant expression and methylation changes in enhancer/promoter/gene body (Step 7) for following analysis.

3.7. RA Genetically Associated Genes and Their Targets Preferentially Displaying Differential Methylation and Differential Expression

A growing body of literature suggested interaction of genetic loci and differentially methylated loci in phenotype determination [12]. To examine whether there was similar genetic–epigenetic interaction in RA, we utilized GWAS results on RA and non-RA traits and protein–protein interaction information from BioGRID to characterize genetic–epigenetic interaction in RA (Figure 1, Step 8; Figure S1). RA genetically associated genes and their interacting targets are more likely to exhibit differential methylation and differential expression than non-RA genetically associated genes and their interacting targets (Figure S6). This finding highlighted interaction of genetically associated genes and epigenetically associated genes in RA pathogenesis.

3.8. Ingenuity Pathway Analysis

To identify pathways and diseases associated with the differential methylation and differential expression in RA compared with healthy donors, we performed a pathway analysis using IPA. Dendritic cell maturation, inflammasome pathway, iNOS signaling, LPS/IL-1 mediated inhibition of RXR function, neuroinflammation signaling pathway, NF- κ B signaling, PPAR signaling, Toll-like receptor signaling, TREM1 signaling and type 1 diabetes mellitus signaling were identified as enriched pathways (Figure S7, Table S3). Differentially methylated and differentially expressed genes were enriched for genes of atherosclerosis, atopic dermatitis, hematopoietic neoplasm, inflammation of joint, juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, rheumatic disease, rheumatoid arthritis, systemic autoimmune syndrome and viral infection as disease annotation (Figure S8, Table S4).

3.9. Upstream Regulator Deduction

Since altered DNA methylation in differentially methylated regions may contribute to transcriptional dysregulation through altered transcription factor binding [13], to gain insight into involved transcription factors, a network of transcription factors and their targets was constructed using iRegulon (Figure 1, Step 10). iRegulon revealed 13 transcription factors (CEBPA, CEBPB, ETS2, FOS, FOSL2, FOXM1, HLCS, NAP1L1, NFIC, NFKBI, NXP3, RXRA, SNAI1) with significant enrichment of target genes in the network of genes with concomitant differential methylation and differential expression (Figure 3). These transcription factors had well-established roles in inflammation and immune cells development (Table S5).

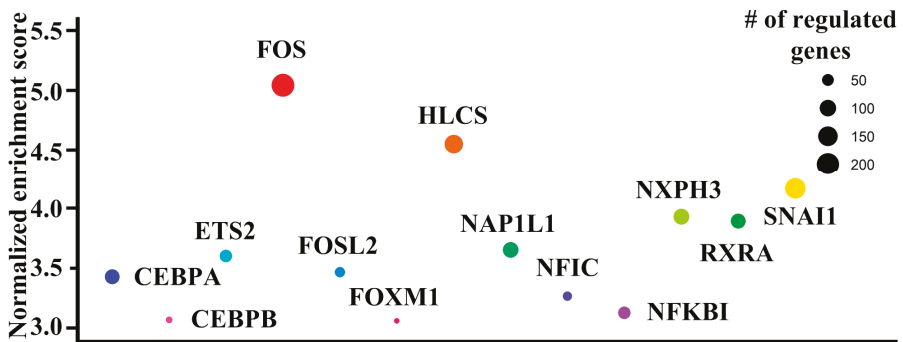


Figure 3. Transcription factors identified through iRegulon analysis. The bubble chart showed the transcription factors associated with differentially methylated and differentially expressed genes identified by iRegulon. Y-axis label represented normalized enrichment score. The sizes of the bubbles were proportional to the number of regulated genes with concomitant differential methylation and differential expression for each transcription factor.

3.10. Validation of Differential Methylation and Differential Expression in RA

To validate the results from next-generation sequencing, we retrieved previously reported methylation and expression patterns of RA CD4 T cells and B cells, both of which were major cellular subsets of PBMCs, from GEO (Figure 1, Step 11) (methylation and expression profiles of CD8 and monocyte unavailable). The magnitude of methylation aberrations across all validated genes was similar to previous studies [14] (Figures 4–6). 10 genes with methylation alteration and expression deregulation were validated in B cells, including three (*CD86*, *RAB20*, *XAF1*) with enhancer hypomethylation and expression upregulation (Figure 4), one (*KCNH8*) with promoter hypermethylation and expression downregulation, two (*FOLR3*, *LTBR*) with promoter hypomethylation and expression upregulation (Figure 5), and four (*DOK7*, *PDGFA*, *PITPNM2*, *CELSR1*) with gene body hypomethylation and expression downregulation (Figure 6).

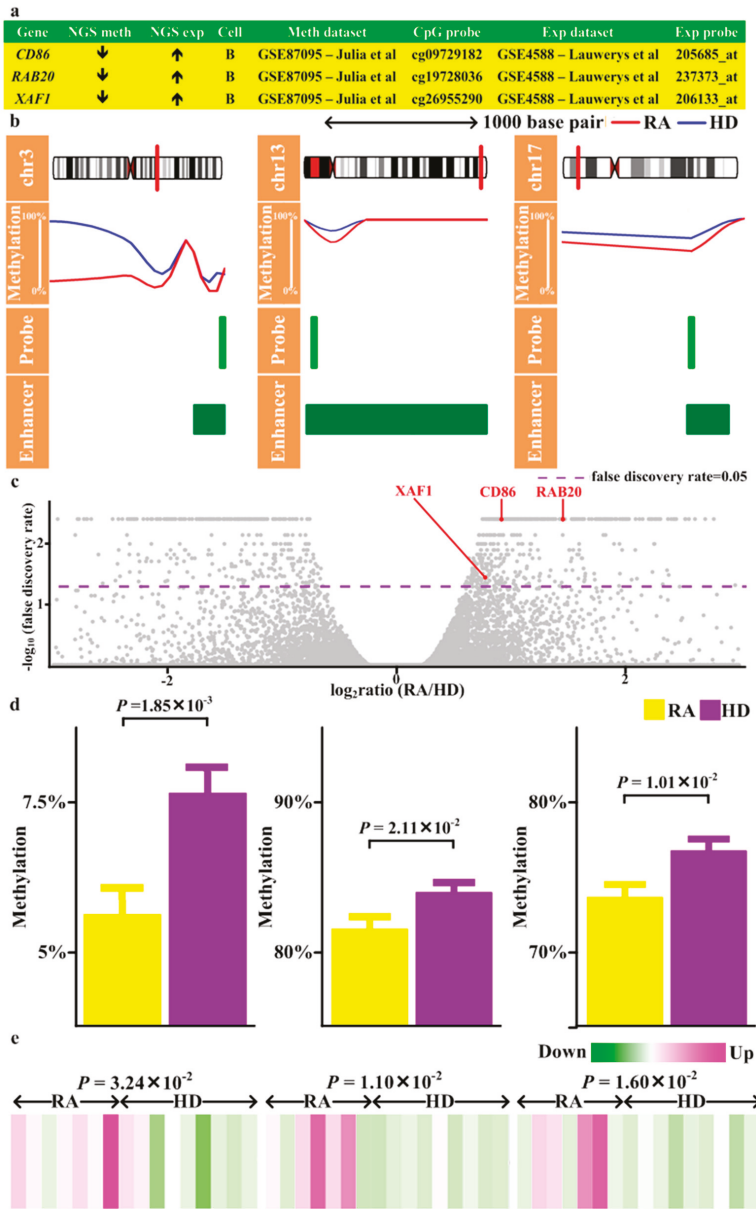


Figure 4. Validation of genes with differential methylation in enhancer and differential expression. (a) The results of methylation and expression obtained from next-generation sequencing (NGS meth, NGS exp), the cell subsets of validation dataset (Cell), the dataset of validation (Meth dataset, Exp dataset), and the probes of validation dataset (CpG probe, Exp probe). (b) Visualization of the methylation levels obtained from NGS in rheumatoid arthritis (RA) and healthy donors (HD) and location of validated CpG probe and enhancers. (c) Volcano plot of the $-\log_{10}(\text{false discovery rate})$ on the Y-axis versus expression change ($\log_2(\text{ratio})$) on the X-axis. Of validated genes, (d) Methylation and (e) Expression levels of corresponding probes in the validation dataset.

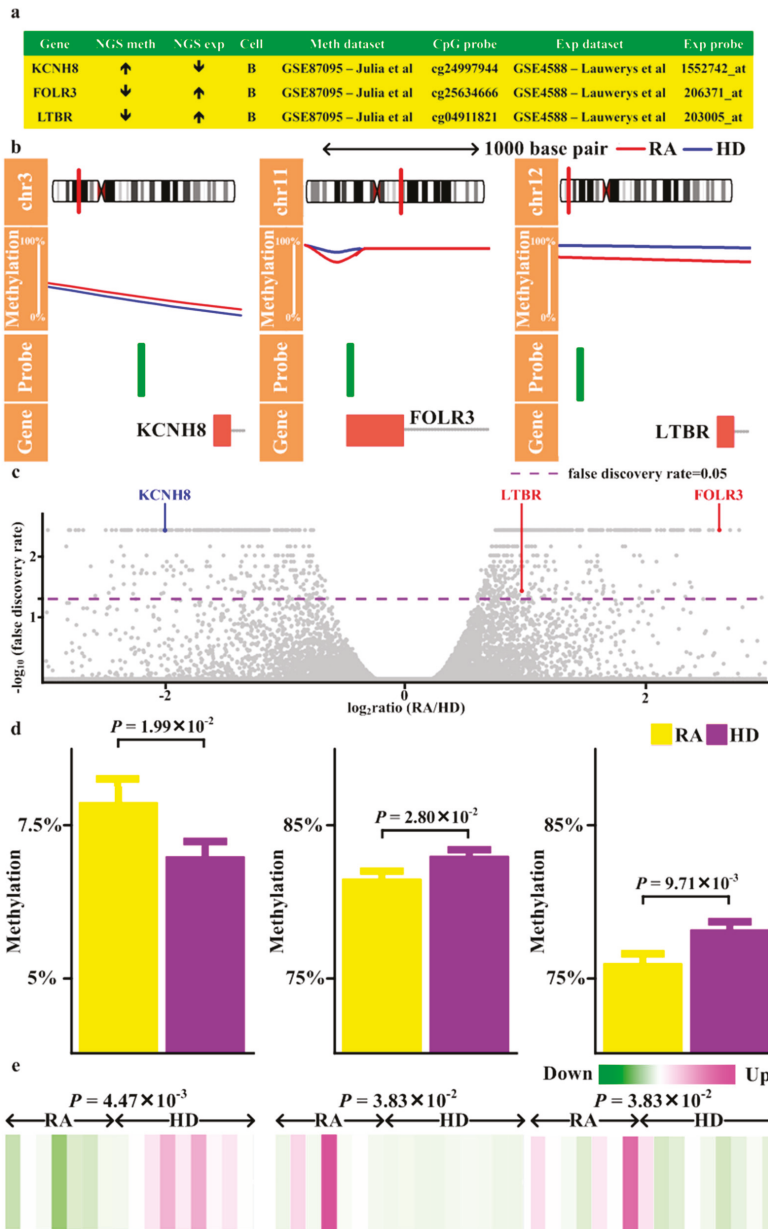


Figure 5. Validation of genes with differential methylation in promoter and differential expression. (a) The results of methylation and expression obtained from next-generation sequencing (NGS meth, NGS exp), the cell subsets of validation dataset (Cell), the dataset of validation (Meth dataset, Exp dataset), and the probes of validation dataset (CpG probe, Exp probe). (b) Visualization of the methylation levels obtained from NGS in RA and healthy donors (HD) and location of validated CpG probe superposed onto the genomic locations of genes. (c) Volcano plot of the $-\log_{10}(\text{false discovery rate})$ on the Y-axis versus expression change (\log_2 ratio) on the X-axis. Of validated genes, (d) Methylation and (e) Expression levels of corresponding probes in the validation dataset.

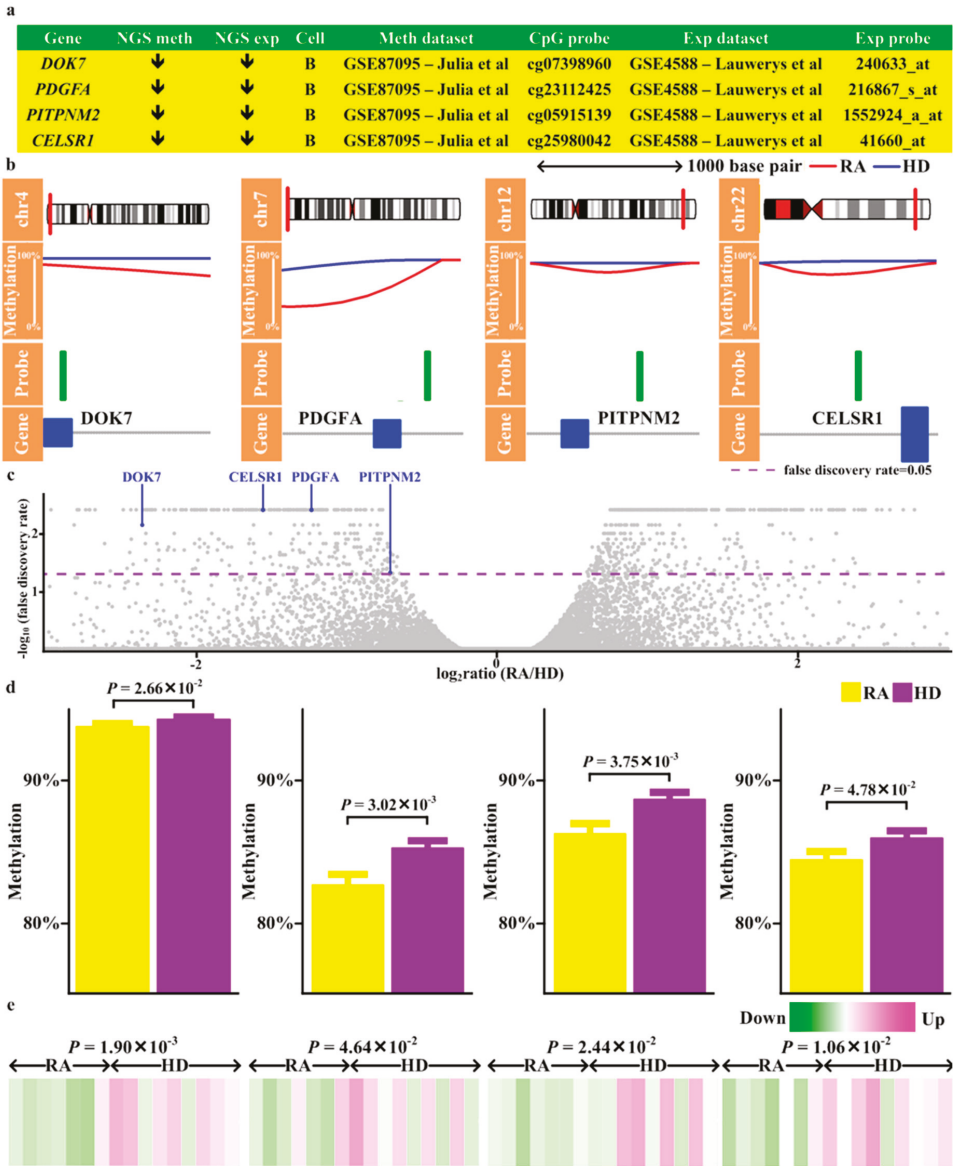


Figure 6. Validation of genes with differential methylation in gene body and differential expression. (a) The results of methylation and expression obtained from next-generation sequencing (NGS meth, NGS exp), the cell subsets of validation dataset (Cell), the dataset of validation (Meth dataset, Exp dataset), and the probes of validation dataset (CpG probe, Exp probe). (b) Visualization of the methylation levels obtained from NGS in RA and healthy donors (HD) and location of validated CpG probe superposed onto the genomic locations of genes. (c) Volcano plot of the $-\log_{10}$ (false discovery rate) on the Y-axis versus expression change (\log_2 ratio) on the X-axis. Of validated genes, (d) Methylation and (e) Expression levels of corresponding probes in the validation dataset.

4. Discussion

Here we reported a comprehensive analysis of methylome and transcriptome in RA. By combining methyl-seq and RNA-seq data, this study provided a global map of the methylation profile in RA. Methylation alterations occurred across human genomes (Figure S3), varied with CpG features and genic characteristics (Figure 2) and associated with gene expression (Figure S5). RA genetically associated genes and their interacting targets preferentially displayed differential methylation and differential expression compared with non-RA genetically associated genes (Supplementary Figure S6). These methylation and transcription aberration associated with several autoimmune and infectious diseases (Figure S8, Table S4). Additionally, we identified several transcription factors as potential regulators (Figure 3). Moreover, 10 genes (*CD86*, *RAB20*, *XAF1*, *KCNH8*, *FOLR3*, *LTBR*, *DOK7*, *PDGFA*, *PITPNM2*, and *CELSR1*) with concomitant methylation and expression alterations in B cells were validated (Figures 4–6). These results highlighted potential roles played by these genes in RA.

The scale of methylation differences across all validated genes was not large, similar to those reported in previous studies [14–16]. Studies suggested that traits-associated methylation changes were predominantly of small magnitude [16,17], tended to be subtle and long-lasting, with stronger but short-lived gene expression alterations [15]. Accumulating evidence further suggested functional consequences of such subtle methylation changes, with halving or doubling of gene transcription accompanying every 1% change in methylation [17]. These collectively supported the biological relevance of methylation alterations validated in this study.

Generally, PCA and HC based on the methylation levels revealed a clear phenotype-driven distinction between RA and healthy donors (Figure S2a,b), supportive of the potential of methylation as diagnostic marker. Similar conclusions were made in other autoimmune diseases, including SLE [18]. However, difference of methylation profiles between RA patients was also noted (Figure S2a), suggesting epigenetic heterogeneity of RA patients. Epigenetic alteration varies with different manifestations of autoimmune disease [18]. Evidence suggests genetic and clinical heterogeneity of RA [19], though it has not been fully defined and warrants further study. Since different serology status implied contrasting genetic architecture and transcriptome changes [20,21], it is tempting to speculate on the roles of autoantibodies in the difference of methylome, as one of patients was positive for anti-citrullinated protein antibodies (ACPA) and the other negative for ACPA. Large scales of studies combining clinical status, immunopathology, methylomics and transcriptomics analysis from ACPA+ vs ACPA- patients will provide valuable insight into the relationship between autoantibodies and epigenetic subsets of RA. This needed to be explored in future studies.

When we characterized methylation variation according to CpG features, CpG islands displayed the highest methylation variation compared with other CpG features (Figure 2b). Since CpG islands had the most pronounced correlation with gene expression level [22], this suggested that despite small methylation differences, there may be more biologically relevant regions of the genome.

In this study, we observed differential enhancer, promoter and gene body methylation between RA and healthy donors (Figure 2c). On average, promoter hypermethylation and enhancer and gene body hypomethylation were noted. Promoter hypermethylation has also been demonstrated in other autoimmune diseases, such psoriasis [23]. Interestingly, promoter hypermethylation was often correlated with gene downregulation [8]. These changes in promoter and gene body DNA methylation might be related to inadequate immune regulation [24] and exemplified by polycyclic forms of RA-asymptomatic during interepisodic period but flare-up intermittently [25].

With regards to methylation variation within promoters, increased methylation variation was noted in the vicinity of transcription start site (Figure S4). Since transcription factor binding sites were enriched in transcription start site [26], the presence of more dynamic DNA methylation in the vicinity of transcription start site provided higher flexibility for different transcription factor bindings under different conditions and thus transcription plasticity.

Our study highlighted that RA genetically associated genes and their interacting targets are more likely to exhibit differential methylation and differential expression than non-RA genetically associated

genes (Figure S6). Although evidence of genetic–epigenetic interaction existed in past literature [12], interaction of genetically associated genes and epigenetically associated genes in autoimmune diseases such as RA was largely uncharacterized. Furthermore, interaction of genetically associated genes and epigenetically associated genes in RA raised the possibility of cooperation of these two interacting systems to facilitate gene regulation in RA pathogenesis. It is possible that both methylation and genetic alterations were necessary for RA development and altered DNA methylation may be a second hit contributing to penetrance as demonstrated by complex multifactorial traits [27] and supported by past studies of autoimmune diseases [28].

Pathway analysis of methylation and expression alterations suggested significantly multiple upregulated inflammatory pathway (including TREM1 signaling) and one downregulated pathway (PPAR signaling) (Figure S7). *TREM1* was expressed in monocyte and amplified production of IL-6 and TNF- α , both critical players in RA pathogenesis [29]. With regards to PPAR, PPAR activation downregulated NF- κ B signaling, primed monocytes into anti-inflammatory properties, and exerted therapeutic effects on RA [30–32]. These findings support the importance of DNA methylation on the regulation of implicated pathways in RA.

In the disease analysis, genes with significantly different DNA methylation and expression alterations were associated with several diseases, including atherosclerosis, atopic dermatitis, hematopoietic neoplasm, inflammation of joint, juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, rheumatic disease, rheumatoid arthritis, systemic autoimmune syndrome and viral infection (Figure S8). Numerous reports linked RA with juvenile idiopathic arthritis, atopic dermatitis, atherosclerosis and lymphoma [33–36]. Viral infection also associated with RA in past epidemiology study [37]. However, it is previously unknown whether these diseases are also linked to RA epigenetically. This study was the first to suggest an epigenetic relationship between these diseases and RA.

When we applied iRegulon to decipher potential upstream regulator, several transcription factors were singled out (Figure 3). All these transcription factors were involved in various aspects of immunological responses (Table S5). Thus, it was conceivable that they participated in regulation of differentially methylated and differentially expressed genes in RA.

During validation with B cell microarray profiles, upregulated *CD86*, *RAB20*, *XAF1*, *FOLR3*, *LTBR* and downregulated *KCNH8*, *DOK7*, *PDGFA*, *PITPNM2*, *CELSR1* with corresponding methylation changes in enhancers/promoters/gene bodies were identified (Figures 4–6). *CD86* activated B cell proliferation and immunoglobulin secretion [38]. Moreover, *CD86* was increased in RA B cells and correlated with disease activity [39,40]. Thus, upregulated *CD86* may contribute to immune activation in RA (Figure S9). Considering *RAB20*, *RAB20* was upregulated by Crohn’s disease-associated polymorphism and vaccination and increases during B cell transformation (Table S3 of [41], Supplementary material S1 of [42,43]). It may be possible that increased *RAB20* contributes to B cell activation and facilitates RA development (Figure S9). Regarding *XAF1*, *XAF1* was one risk gene of sarcoidosis which implicated dysregulated immune responses [44]. Moreover, *XAF1* was downregulated during lymphocyte immortalization and sensitized lymphocyte to apoptosis [45,46]. As a result, *XAF1* has the potential to be involved in RA pathogenesis (Figure S9).

KCNH8 was almost exclusively expressed in B cells [47] and *KCNH8* region was associated with susceptibility to autoimmune diseases, including Crohn’s disease and psoriasis [48]. However, its function in B cells remained unexplored. *FOLR3* was a member of folate receptor family. *FOLR3* was associated with hepatitis C virus clearance [49] and folate receptor-mediated STAT3 activation [50]. Therefore, upregulated *FOLR3* may contribute to immune activation (Figure S9). With regards to *LTBR*, *LTBR* activated NF κ B and blockade of *LTBR* impaired humoral immune response and ameliorated arthritis in the animal model [51–53]. Thus, upregulated *LTBR* potentially activates humoral immunity and facilitated arthritis development (Figure S9).

DOK7 belonged to a family of docking protein and *DOK7* inhibited malignant cell proliferation and increased leukemia patient survival [54,55]. *DOK7* downregulation may lead to increased B cell

proliferation and aggravated RA (Figure S9). *PDGFA* was part of *PDGF* family and *PDGF* family members stimulated B cell growth [56]. Whether downregulated *PDGFA* represents one mechanism to counteract excessive inflammation is unknown (Figure S9). *PITPNM2* was implicated as a risk locus of multiple sclerosis [57] and allergic diseases [58] which were all linked to RA [34,59]. Furthermore, risk protective alleles of allergic disease and drug with anti-inflammatory effects in autoimmune diseases both increased *PITPNM2* expression (Supplementary Table 27 of [58,60]). As a result, decreased *PITPNM2* might enhance RA pathogenesis (Figure S9). *CELSR1* was part of the apoptosis network [61], inhibited proliferation of neural progenitor [62] and decreased in non-nodal mantle cell lymphoma [63]. Therefore, decreased *CELSR1* might facilitate B cell proliferation and therefore sustain immune responses in RA (Figure S9).

In this study, we detected methylation and transcription perturbations in *CD86*. Notably, abatacept, one approved treatment option for RA, decreased *CD86* expression in B cells [64]. It was possible that genes with differential methylation and differential expression identified in this study hold therapeutic promises for RA in the future. These should be addressed by further studies.

Limitations of this work include the relatively small sample size due to the high cost of next-generation sequencing and failure to validate methylation results in CD4 T cells. This may be a result of potentially more aberrant methylation of B cells than CD4 T cells, as demonstrated in another autoimmune disease, Sjogren's syndrome [65]. Future work was needed to fully characterize additional RA samples by next-generation sequencing with additional cell types.

In past decades, progress in understanding the molecular bases of disease pathogenesis and the application of new technologies greatly transformed our treatment of diseases [1]. Integration with multiomic data identified several novel genes and pathways as potential relevant therapeutic avenues that may be important dysregulated mediators at the interface of genetics, epigenetics, and RA pathogenesis. These results may be useful for the development of new, more effective biomarkers and therapeutics. With that goal in mind, future studies are necessary in order to characterize precisely the molecular mechanisms, the functional consequences, and the interactions between differential methylation and genetic risk factors in RA pathogenesis.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/8/9/1284/s1>, Figure S1: Flowcharts of genetic-epigenetic interaction investigation, Figure S2: Principal component analysis and hierarchical clustering results, Figure S3: Circular representation of the methylome of rheumatoid arthritis peripheral blood mononuclear cells in different chromosomes, Figure S4: Distribution of methylation variance relative to transcription start sites in promoters, Figure S5: Transcriptional variance was associated with methylation variance at enhancers, promoters and gene bodies, Figure S6: Graphical representation of enrichment of RA genetically associated genes with differential methylation and differential expression or interacting with differentially methylated and differentially expressed genes, Figure S7: Top 10 significantly perturbed canonical pathways revealed by Ingenuity Pathway Analysis, Figure S8: Top 10 diseases associated with genes having concomitant differential methylation and differential expression in rheumatoid arthritis, Figure S9: Proposed mechanism of *CD86*, *RAB20*, *XAF1*, *FOLR3*, *LTBR*, *PDGFA*, *DOK7*, *PITPNM2*, *CELSR1* in B cells of rheumatoid arthritis, Table S1: Next-generation sequencing RNA quality of rheumatoid arthritis and healthy donor peripheral blood mononuclear cells, Table S2: HACER source cells utilized in enhancer annotation, Table S3: Top ten pathways identified by Ingenuity Pathway Analysis from differentially methylated and differentially expressed genes, Table S4: Top ten diseases identified by Ingenuity Pathway Analysis (IPA) from differentially methylated and differentially expressed genes, Table S5: Roles of identified transcription factors in immunity.

Author Contributions: C.-C.T. designed, analysed experiments, and wrote the manuscript; Y.-Z.L., C.-H.L., R.-N.L., and H.-C.C. coordinated patient samples and performed the experiment; C.-Y.Y., W.-C.T., T.-T.O., C.-C.W., W.-Y.S. helped interpret the data and write the manuscript; J.-H.Y. provided patient samples, designed and analysed experiments, and revised the manuscript.

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References

1. Feldmann, M.; Maini, R.N. Perspectives from Masters in Rheumatology and Autoimmunity: Can We Get Closer to a Cure for Rheumatoid Arthritis? *Arthritis Rheumatol.* **2015**, *67*, 2283–2291. [[CrossRef](#)] [[PubMed](#)]
2. Kawalec, P.; Śladowska, K.; Malinowska-Lipień, I.; Brzostek, T.; Kózka, M. European perspective on the management of rheumatoid arthritis: Clinical utility of tofacitinib. *Ther. Clin. Risk Manag.* **2017**, *14*, 15–29. [[CrossRef](#)] [[PubMed](#)]
3. Kaul, R.; Sharma, A.; Lisse, J.R.; Christadoss, P. Human recombinant IL-2 augments immunoglobulin and induces rheumatoid factor production by rheumatoid arthritis lymphocytes engrafted into severe combined immunodeficient mice. *Clin. Immunol. Immunopathol.* **1995**, *74*, 271–282. [[CrossRef](#)] [[PubMed](#)]
4. Davis, M.C.; Zautra, A.J.; Younger, J.; Motivala, S.J.; Attrep, J.; Irwin, M.R. Chronic stress and regulation of cellular markers of inflammation in rheumatoid arthritis: Implications for fatigue. *Brain Behav. Immun.* **2008**, *22*, 24–32. [[CrossRef](#)] [[PubMed](#)]
5. Katevas, P.; Andonopoulos, A.P.; Kourakli-Symeonidis, A.; Manopoulou, E.; Lafi, T.; Makri, M.; Zombos, N.C. Peripheral blood mononuclear cells from patients with rheumatoid arthritis suppress erythropoiesis in vitro via the production of tumor necrosis factor alpha. *Eur. J. Haematol.* **1994**, *53*, 26–30. [[CrossRef](#)]
6. Ridgley, L.A.; Anderson, A.E.; Pratt, A.G. What are the dominant cytokines in early rheumatoid arthritis? *Curr. Opin. Rheumatol.* **2018**, *30*, 207–214. [[CrossRef](#)]
7. Ye, J.; Richardson, T.G.; McArdle, W.L.; Relton, C.L.; Gillespie, K.M.; Suderman, M.; Hemani, G. Identification of loci where DNA methylation potentially mediates genetic risk of type 1 diabetes. *J. Autoimmun.* **2018**, *93*, 66–75. [[CrossRef](#)]
8. Maunakea, A.K.; Nagarajan, R.P.; Bilenky, M.; Ballinger, T.J.; D'Souza, C.; Fouse, S.D.; Johnson, B.E.; Hong, C.; Nielsen, C.; Zhao, Y.; et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* **2010**, *66*, 253–257. [[CrossRef](#)]
9. Lee, E.J.; Rath, P.; Liu, J.; Ryu, D.; Pei, L.; Noonepalle, S.K.; Shull, A.Y.; Feng, Q.; Litofsky, N.S.; Miller, D.C.; et al. Identification of Global DNA Methylation Signatures in Glioblastoma-Derived Cancer Stem Cells. *J. Genet. Genom.* **2015**, *42*, 355–371. [[CrossRef](#)]
10. Weber, A.; Schwarz, S.C.; Tost, J.; Trümbach, D.; Winter, P.; Busato, F.; Tacik, P.; Windhorst, A.C.; Fagny, M.; Arzberger, T.; et al. Epigenome-wide DNA methylation profiling in Progressive Supranuclear Palsy reveals major changes at DLX1. *Nat. Commun.* **2018**, *9*, 2929. [[CrossRef](#)]
11. Aran, D.; Sabato, S.; Hellman, A. DNA methylation of distal regulatory sites characterizes dysregulation of cancer genes. *Genome Biol.* **2013**, *14*, R21. [[CrossRef](#)] [[PubMed](#)]
12. Alexander, N.; Illius, S.; Stalder, T.; Wankerl, M.; Muehlhan, M.; Kirschbaum, C. Serotonin transporter gene methylation predicts long-term cortisol concentrations in hair. *Psychoneuroendocrinology* **2019**, *106*, 179–182. [[CrossRef](#)] [[PubMed](#)]
13. Zhu, H.; Wang, G.; Qian, J. Transcription factors as readers and effectors of DNA methylation. *Nat. Rev. Genet.* **2016**, *17*, 551–565. [[CrossRef](#)] [[PubMed](#)]
14. Li Yim, A.Y.F.; Duijvis, N.W.; Zhao, J.; de Jonge, W.J.; D'Haens, G.R.A.M.; Mannens, M.M.A.M.; Mul, A.N.P.M.; Te Velde, A.A.; Henneman, P. Peripheral blood methylation profiling of female Crohn's disease patients. *Clin. Epigenetics* **2016**, *8*, 65. [[CrossRef](#)] [[PubMed](#)]
15. Tsai, P.C.; Glastonbury, C.A.; Eliot, M.N.; Bollepalli, S.; Yet, I.; Castillo-Fernandez, J.E.; Carnero-Montoro, E.; Hardiman, T.; Martin, T.C.; Vickers, A.; et al. Smoking induces coordinated DNA methylation and gene expression changes in adipose tissue with consequences for metabolic health. *Clin. Epigenetics* **2018**, *10*, 126. [[CrossRef](#)] [[PubMed](#)]
16. Chambers, J.C.; Loh, M.; Lehne, B.; Drong, A.; Kriebel, J.; Motta, V.; Wahl, S.; Elliott, H.R.; Rota, F.; Scott, W.R.; et al. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: A nested case-control study. *Lancet Diabetes Endocrinol.* **2015**, *3*, 526–534. [[CrossRef](#)]
17. Breton, C.V.; Marsit, C.J.; Faustman, E.; Nadeau, K.; Goodrich, J.M.; Dolinoy, D.C.; Herbstman, J.; Holland, N.; LaSalle, J.M.; Schmidt, R.; et al. Small-Magnitude Effect Sizes in Epigenetic End Points are Important in Children's Environmental Health Studies: The Children's Environmental Health and Disease Prevention Research Center's Epigenetics Working Group. *Environ. Health Perspect.* **2017**, *125*, 511–526. [[CrossRef](#)] [[PubMed](#)]

18. Weeding, E.; Sawalha, A.H. Deoxyribonucleic Acid Methylation in Systemic Lupus Erythematosus: Implications for Future Clinical Practice. *Front. Immunol.* **2018**, *9*, 875. [[CrossRef](#)]
19. Ouboussad, L.; Burska, A.N.; Melville, A.; Buch, M.H. Synovial Tissue Heterogeneity in Rheumatoid Arthritis and Changes with Biologic and Targeted Synthetic Therapies to Inform Stratified Therapy. *Front. Med.* **2019**, *6*, 45. [[CrossRef](#)]
20. Padyukov, L.; Seielstad, M.; Ong, R.T.; Ding, B.; Rönnelid, J.; Seddighzadeh, M.; Alfredsson, L.; Klareskog, L.; Epidemiological Investigation of Rheumatoid Arthritis (EIRA) study group. A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. *Ann. Rheum. Dis.* **2011**, *70*, 259–265. [[CrossRef](#)]
21. Van Der Pouw Kraan, T.C.; Van Baarsen, L.G.; Wijbrandts, C.A.; Voskuyl, A.E.; Rustenburg, F.; Baggen, J.M.; Dijkmans, B.A.; Tak, P.P.; Verweij, C.L. Expression of a pathogen-response program in peripheral blood cells defines a subgroup of rheumatoid arthritis patients. *Genes Immun.* **2008**, *9*, 16–22. [[CrossRef](#)] [[PubMed](#)]
22. Qu, Y.; Lennartsson, A.; Gaidzik, V.I.; Deneberg, S.; Bengtzen, S.; Arzenani, M.K.; Bullinger, L.; Döhner, K.; Lehmann, S. Genome-Wide DNA Methylation Analysis Shows Enrichment of Differential Methylation in “Open Seas” and Enhancers and Reveals Hypomethylation in DNMT3A Mutated Cytogenetically Normal AML (CN-AML). *Blood* **2012**, *120*, 653.
23. Chandra, A.; Senapati, S.; Roy, S.; Chatterjee, G.; Chatterjee, R. Epigenome-wide DNA methylation regulates cardinal pathological features of psoriasis. *Clin. Epigenetics* **2018**, *10*, 108. [[CrossRef](#)] [[PubMed](#)]
24. Chen, Z.; Bozec, A.; Ramming, A.; Schett, G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **2019**, *15*, 9–17. [[CrossRef](#)] [[PubMed](#)]
25. Masi, A.T. Articular patterns in the early course of rheumatoid arthritis. *Am. J. Med.* **1983**, *75*, 16–26. [[CrossRef](#)]
26. Lin, Z.; Wu, W.S.; Liang, H.; Woo, Y.; Li, W.H. The spatial distribution of cis regulatory elements in yeast promoters and its implications for transcriptional regulation. *BMC Genom.* **2010**, *11*, 581. [[CrossRef](#)] [[PubMed](#)]
27. Alvizi, L.; Ke, X.; Brito, L.A.; Seselgyte, R.; Moore, G.E.; Stanier, P.; Passos-Bueno, M.R. Differential methylation is associated with non-syndromic cleft lip and palate and contributes to penetrance effects. *Sci. Rep.* **2017**, *7*, 2441. [[CrossRef](#)] [[PubMed](#)]
28. Wang, J.; Zhen, Y.; Zhou, Y.; Yan, S.; Jiang, L.; Zhang, L. Promoter methylation cooperates with SNPs to modulate RAGE transcription and alter UC risk. *Biochem. Biophys. Rep.* **2018**, *17*, 17–22. [[CrossRef](#)]
29. Fan, D.; He, X.; Bian, Y.; Guo, Q.; Zheng, K.; Zhao, Y.; Lu, C.; Liu, B.; Xu, X.; Zhang, G.; et al. Triptolide Modulates TREM-1 Signal Pathway to Inhibit the Inflammatory Response in Rheumatoid Arthritis. *Int. J. Mol. Sci.* **2016**, *17*, 498. [[CrossRef](#)]
30. Liu, Y.; Qu, Y.; Liu, L.; Zhao, H.; Ma, H.; Si, M.; Cheng, L.; Nie, L. PPAR- γ agonist pioglitazone protects against IL-17 induced intervertebral disc inflammation and degeneration via suppression of NF- κ B signaling pathway. *Int. Immunopharmacol.* **2019**, *72*, 138–147. [[CrossRef](#)]
31. Bouhlel, M.A.; Derudas, B.; Rigamonti, E.; Dièvert, R.; Brozek, J.; Haulon, S.; Zawadzki, C.; Jude, B.; Torpier, G.; Marx, N.; et al. PPAR γ activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. *Cell Metab.* **2017**, *6*, 137–143. [[CrossRef](#)] [[PubMed](#)]
32. Shahin, D.; Toraby, E.E.; Abdel-Malek, H.; Boshra, V.; Elsamanoudy, A.Z.; Shaheen, D. Effect of peroxisome proliferator-activated receptor gamma agonist (pioglitazone) and methotrexate on disease activity in rheumatoid arthritis (experimental and clinical study). *Clin. Med. Insights Arthritis Musculoskelet. Disord.* **2011**, *4*, 1–10. [[CrossRef](#)] [[PubMed](#)]
33. Hinks, A.; Cobb, J.; Sudman, M.; Eyre, S.; Martin, P.; Flynn, E.; Packham, J.; Childhood Arthritis Prospective Study (CAPS); UK RA Genetics (UKRAG) Consortium; British Society of Paediatric and Adolescent Rheumatology (BSPAR) Study Group; et al. Investigation of rheumatoid arthritis susceptibility loci in juvenile idiopathic arthritis confirms high degree of overlap. *Ann. Rheum. Dis.* **2012**, *71*, 1117–1121. [[CrossRef](#)] [[PubMed](#)]
34. Hou, Y.C.; Hu, H.Y.; Liu, I.L.; Chang, Y.T.; Wu, C.Y. The risk of autoimmune connective tissue diseases in patients with atopy: A nationwide population-based cohort study. *Allergy Asthma Proc.* **2017**, *38*, 383–389. [[CrossRef](#)] [[PubMed](#)]
35. Zeisbrich, M.; Yanes, R.E.; Zhang, H.; Watanabe, R.; Li, Y.; Brosig, L.; Hong, J.; Wallis, B.B.; Giacomini, J.C.; Assimes, T.L.; et al. Hypermetabolic macrophages in rheumatoid arthritis and coronary artery disease due to glycogen synthase kinase 3 β inactivation. *Ann. Rheum. Dis.* **2018**, *77*, 1053–1062. [[CrossRef](#)] [[PubMed](#)]

36. Baecklund, E.; Sundström, C.; Ekblom, A.; Catrina, A.I.; Biberfeld, P.; Feltelius, N.; Klareskog, L. Lymphoma subtypes in patients with rheumatoid arthritis: Increased proportion of diffuse large B cell lymphoma. *Arthritis Rheum.* **2003**, *48*, 1543–1550. [[CrossRef](#)] [[PubMed](#)]
37. Kudaeva, F.M.; Speechley, M.R.; Pope, J.E. A systematic review of viral exposures as a risk for rheumatoid arthritis. *Semin. Arthritis Rheum.* **2019**, *48*, 587–596. [[CrossRef](#)]
38. Suvas, S.; Singh, V.; Sahdev, S.; Vohra, H.; Agrewala, J.N. Distinct role of CD80 and CD86 in the regulation of the activation of B cell and B cell lymphoma. *J. Biol. Chem.* **2002**, *277*, 7766–7775. [[CrossRef](#)]
39. Catalán, D.; Aravena, O.; Sabugo, F.; Wurmman, P.; Soto, L.; Kalergis, A.M.; Cuchacovich, M.; Aguillón, J.C.; Millennium Nucleus on Immunology and Immunotherapy P-07-088-F. B cells from rheumatoid arthritis patients show important alterations in the expression of CD86 and FcγRIIb, which are modulated by anti-tumor necrosis factor therapy. *Arthritis Res. Ther.* **2010**, *12*, R68.
40. Wang, J.; Shan, Y.; Jiang, Z.; Feng, J.; Li, C.; Ma, L.; Jiang, Y. High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. *Clin. Exp. Immunol.* **2013**, *174*, 212–220. [[CrossRef](#)]
41. Mehta, S.; Cronkite, D.A.; Basavappa, M.; Saunders, T.L.; Adiliaghdam, F.; Amatullah, H.; Morrison, S.A.; Pagan, J.D.; Anthony, R.M.; Tonnerre, P.; et al. Maintenance of macrophage transcriptional programs and intestinal homeostasis by epigenetic reader SP140. *Sci. Immunol.* **2017**, *2*, 3160. [[CrossRef](#)] [[PubMed](#)]
42. Jensen, T.L.; Frasketi, M.; Conway, K.; Villarroel, L.; Hill, H.; Krampis, K.; Goll, J.B. RSEQREP: RNA-Seq Reports, an open-source cloud-enabled framework for reproducible RNA-Seq data processing, analysis, and result reporting. *F1000Research* **2017**, *6*, 2162. [[CrossRef](#)] [[PubMed](#)]
43. David, A.; Arnaud, N.; Fradet, M.; Lascaux, H.; Ouk-Martin, C.; Gachard, N.; Zimmer-Strobl, U.; Feuillard, J.; Faumont, N. c-Myc dysregulation is a co-transforming event for nuclear factor-κB activated B cells. *Haematologica* **2017**, *102*, 883–894. [[CrossRef](#)] [[PubMed](#)]
44. Levin, A.M.; Iannuzzi, M.C.; Montgomery, C.G.; Trudeau, S.; Datta, I.; Adrianto, I.; Chitale, D.A.; McKeigue, P.; Rybicki, B.A. Admixture fine-mapping in African Americans implicates XAF1 as a possible sarcoidosis risk gene. *PLoS ONE* **2014**, *9*, e92646. [[CrossRef](#)] [[PubMed](#)]
45. Lee, J.E.; Nam, H.Y.; Shim, S.M.; Bae, G.R.; Han, B.G.; Jeon, J.P. Expression phenotype changes of EBV-transformed lymphoblastoid cell lines during long-term subculture and its clinical significance. *Cell Prolif.* **2010**, *43*, 378–384. [[CrossRef](#)]
46. Secchiero, P.; di Iasio, M.G.; Melloni, E.; Voltan, R.; Celeghini, C.; Tiribelli, M.; Dal Bo, M.; Gattei, V.; Zauli, G. The expression levels of the pro-apoptotic XAF-1 gene modulate the cytotoxic response to Nutlin-3 in B chronic lymphocytic leukemia. *Leukemia* **2010**, *24*, 480–483. [[CrossRef](#)] [[PubMed](#)]
47. Ellinghaus, E.; Ellinghaus, D.; Krusche, P.; Greiner, A.; Schreiber, C.; Nikolaus, S.; Gieger, C.; Strauch, K.; Lieb, W.; Rosenstiel, P.; et al. Genome-wide association analysis for chronic venous disease identifies EFEMP1 and KCNH8 as susceptibility loci. *Sci. Rep.* **2017**, *7*, 45652. [[CrossRef](#)]
48. Ramos, P.S.; Titus, N.; Sajuthi, S.P.; Divers, J.; Huang, Y.; Nayak, U.; Chen, W.M.; Hunt, K.J.; Kamen, D.L.; Gilkeson, G.S.; et al. Identification of Autoimmune Disease Genes In Regions Under Selection In The Gullah African American Population Of South Carolina. *Arthritis Rheum.* **2013**, *65*, S61.
49. Grimes, C.Z.; Hwang, L.Y.; Wei, P.; Shah, D.P.; Volcik, K.A.; Brown, E.L. Differentially regulated gene expression associated with hepatitis C virus clearance. *J. Gen. Virol.* **2013**, *94*, 534–542. [[CrossRef](#)]
50. Hansen, M.F.; Greibe, E.; Skovbjerg, S.; Rohde, S.; Kristensen, A.C.; Jensen, T.R.; Stentoft, C.; Kjær, K.H.; Kronborg, C.S.; Martensen, P.M. Folic acid mediates activation of the pro-oncogene STAT3 via the Folate Receptor alpha. *Cell Signal.* **2015**, *27*, 1356–1368. [[CrossRef](#)]
51. Bonizzi, G.; Karin, M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* **2004**, *25*, 280–288. [[CrossRef](#)] [[PubMed](#)]
52. Yang, K.; Liang, Y.; Sun, Z.; Xue, D.; Xu, H.; Zhu, M.; Fu, Y.X.; Peng, H. T Cell-Derived Lymphotoxin Is Essential for the Anti-Herpes Simplex Virus 1 Humoral Immune Response. *J. Virol.* **2018**, *92*, e00428-18. [[CrossRef](#)] [[PubMed](#)]
53. Fava, R.A.; Notidis, E.; Hunt, J.; Szanya, V.; Ratcliffe, N.; Ngam-Ek, A.; De Fougerolles, A.R.; Sprague, A.; Browning, J.L. A role for the lymphotoxin/LIGHT axis in the pathogenesis of murine collagen-induced arthritis. *J. Immunol.* **2003**, *171*, 115–126. [[CrossRef](#)] [[PubMed](#)]

54. Hua, C.D.; Bian, E.B.; Chen, E.F.; Yang, Z.H.; Tang, F.; Wang, H.L.; Zhao, B. Repression of Dok7 expression mediated by DNMT1 promotes glioma cells proliferation. *Biomed. Pharm.* **2018**, *106*, 678–685. [[CrossRef](#)] [[PubMed](#)]
55. Zhang, L.; Li, R.; Hu, K.; Dai, Y.; Pang, Y.; Jiao, Y.; Liu, Y.; Cui, L.; Shi, J.; Cheng, Z.; et al. Prognostic role of DOK family adapters in acute myeloid leukemia. *Cancer Gene Ther.* **2018**. [[CrossRef](#)] [[PubMed](#)]
56. Acharya, M.; Edkins, A.L.; Ozanne, B.W.; Cushley, W. SDF-1 and PDGF enhance alphavbeta5-mediated ERK activation and adhesion-independent growth of human pre-B cell lines. *Leukemia* **2009**, *23*, 1807–1817. [[CrossRef](#)]
57. International Multiple Sclerosis Genetics Consortium. Network-based multiple sclerosis pathway analysis with GWAS data from 15,000 cases and 30,000 controls. *Am. J. Hum. Genet.* **2013**, *92*, 854–865. [[CrossRef](#)]
58. Ferreira, M.A.; Vonk, J.M.; Baurecht, H.; Marenholz, I.; Tian, C.; Hoffman, J.D.; Helmer, Q.; Tillander, A.; Ullemar, V.; van Dongen, J.; et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat. Genet.* **2017**, *49*, 1752–1757. [[CrossRef](#)]
59. Kuo, C.F.; Grainge, M.J.; Valdes, A.M.; See, L.C.; Yu, K.H.; Shaw, S.W.S.; Luo, S.F.; Zhang, W.; Doherty, M. Familial aggregation of rheumatoid arthritis and co-aggregation of autoimmune diseases in affected families: A nationwide population-based study. *Rheumatology* **2017**, *56*, 928–933. [[CrossRef](#)]
60. Kozela, E.; Juknat, A.; Gao, F.; Kaushansky, N.; Coppola, G.; Vogel, Z. Pathways and gene networks mediating the regulatory effects of cannabidiol, a nonpsychoactive cannabinoid, in autoimmune T cells. *J. Neuroinflamm.* **2016**, *13*, 136. [[CrossRef](#)]
61. Xie, T.; Liang, J.; Geng, Y.; Liu, N.; Kurciciyan, A.; Kulur, V.; Leng, D.; Deng, N.; Liu, Z.; Song, J.; et al. MicroRNA-29c Prevents Pulmonary Fibrosis by Regulating Epithelial Cell Renewal and Apoptosis. *Am. J. Respir. Cell Mol. Biol.* **2017**, *57*, 721–732. [[CrossRef](#)] [[PubMed](#)]
62. Boucherie, C.; Boutin, C.; Jossin, Y.; Schakman, O.; Goffinet, A.M.; Ris, L.; Gailly, P.; Tissir, F. Neural progenitor fate decision defects, cortical hypoplasia and behavioral impairment in Celsr1-deficient mice. *Mol. Psychiatry* **2018**, *23*, 723–734. [[CrossRef](#)] [[PubMed](#)]
63. Del Giudice, I.; Messina, M.; Chiaretti, S.; Santangelo, S.; Tavolaro, S.; De Propriis, M.S.; Nanni, M.; Pescarmona, E.; Mancini, F.; Pulsoni, A.; et al. Behind the scenes of non-nodal MCL: Downmodulation of genes involved in actin cytoskeleton organization, cell projection, cell adhesion, tumour invasion, TP53 pathway and mutated status of immunoglobulin heavy chain genes. *Br. J. Haematol.* **2012**, *156*, 601–611. [[CrossRef](#)] [[PubMed](#)]
64. Lorenzetti, R.; Janowska, I.; Smulski, C.R.; Frede, N.; Henneberger, N.; Walter, L.; Schleyer, M.T.; Hüppe, J.M.; Staniek, J.; Salzer, U.; et al. Abatacept modulates CD80 and CD86 expression and memory formation in human B-cells. *J. Autoimmun.* **2019**, *101*, 145–152. [[CrossRef](#)] [[PubMed](#)]
65. Imgenberg-Kreuz, J.; Sandling, J.K.; Nordmark, G. Epigenetic alterations in primary Sjögren’s syndrome—An overview. *Clin. Immunol.* **2018**, *196*, 12–20. [[CrossRef](#)] [[PubMed](#)]



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Article

Regulation of Th17 Cytokine-Induced Osteoclastogenesis via SKI306X in Rheumatoid Arthritis

Hae-Rim Kim ^{1,†}, Kyoung-Woon Kim ^{2,†}, Bo-Mi Kim ², Ji-Yeon Won ², Hong-Ki Min ¹,
Kyung-Ann Lee ³, Tae-Young Kim ⁴ and Sang-Heon Lee ^{1,*}

¹ Division of Rheumatology, Department of Internal Medicine, Research Institute of Medical Science, School of Medicine, Konkuk University, Seoul 05030, Korea

² Conversant Research Consortium in Immunologic Disease, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul 06591, Korea

³ Division of Rheumatology, Department of Internal Medicine, Soonchunhyang University Hospital, Seoul 04401, Korea

⁴ Department of Orthopedic Surgery, School of Medicine, Konkuk University, Seoul 05030, Korea

* Correspondence: shlee@kuh.ac.kr; Tel.: +82-2-2030-7541; Fax: +82-2-2030-7748

† These authors contributed equally to this work.

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Abstract: This study aimed to investigate the regulatory effect of SKI306X, a mixed extract of three herbs, in T helper (Th)17 cytokine-induced inflammation and joint destruction in rheumatoid arthritis (RA). Synovial fibroblasts were isolated from RA patients and cultured with Th17 cytokines including interleukin (IL)-17, IL-21, and IL-22 and SKI306X, and tumor necrosis factor (TNF)- α , IL-1 β , and receptor activator of nuclear factor kappa-B ligand (RANKL) expression and production were investigated using real-time PCR and ELISA of culture media. After peripheral blood (PB) cluster of differentiation (CD)14⁺ monocytes were cultured in media supplemented with Th17 cytokines and SKI306X, tartrate-resistant acid phosphatase positive (TRAP⁺) multinucleated giant cells (mature osteoclasts) were enumerated and gene expression associated with osteoclast maturation was assessed via real-time PCR analysis. After PB monocytes were co-cultured with IL-17-stimulated RA synovial fibroblasts in the presence of SKI306X, osteoclast differentiation was assessed. When RA synovial fibroblasts were cultured with IL-17, IL-21, and IL-22, TNF- α , IL-1 β , and RANKL expression and production were increased; however, SKI306X reduced cytokine expression and production. When PB monocytes were cultured in media supplemented with Th17 cytokines, osteoclast differentiation was stimulated; however, SKI306X decreased osteoclast differentiation and osteoclast maker expression. When PB monocytes were co-cultured with IL-17-stimulated RA synovial fibroblasts, osteoclast differentiation was increased; however, SKI306X decreased osteoclast differentiation and osteoclast maker expression. SKI306X reduced Th17 cytokine-induced TNF- α , IL-1 β , and RANKL expression and osteoclast differentiation, providing novel insights into adjuvant therapy for regulating inflammation and joint destruction in RA.

Keywords: rheumatoid arthritis; synovial fibroblasts; cytokine; osteoclast; herbal medicine

1. Introduction

A few decades ago, Korean patients with rheumatoid arthritis (RA) received herbal medication and acupuncture before they visited rheumatologic clinics. They believed the herbal therapies could cure RA without any adverse effects. By the time these patients visited rheumatologists, they already had joint destruction and disability because of delayed treatment with disease-modifying antirheumatic drugs (DMARDs). RA is currently considered an autoimmune inflammatory disease requiring treatment

with DMARDs, which has led to an increase in rheumatologic consultations. However, DMARDs do not always have therapeutic effects in RA, they can display adverse effects and are highly expensive [1]. Hence, some patients prefer complementary and alternative medication, primarily including herbal medication [1,2]. According to the Korean RA registry (KORONA), 10.5% of patients received complementary and alternative medicines de novo and among them, 17% received herbal medication and 55% received acupuncture [3].

SKI306X, a mixed extract of three herbs, is a purified extract prepared from a mixture of three Oriental herbal plants, *Clematis mandshurica*, *Trichosanthes kirilowii*, and *Prunella vulgaris* [4]. According to the Donguibogam, i.e., 'Principles and Practice of Eastern Medicine' written in 1610, *Clematis mandshurica* effectively reduces lower back and knee pain, *Trichosanthes kirilowii* reduces febrile sensations and dry mouth, and *Prunella vulgaris* effectively reduces lymphadenitis, abscess, and ulcers. The anti-inflammatory effects of SKI306X have been reported previously.

SKI306X inhibits tumor necrosis factor (TNF)- α , leukotriene B₄, and nitric oxide (NO) production in macrophages and cyclooxygenase-2 expression [5]. It inhibits TNF- α , prostaglandin E₂, and interleukin (IL)-1 β production by stimulated peripheral blood (PB) mononuclear cells [6] and is commonly used to treat osteoarthritis owing to its chondroprotective effects. SKI306X inhibits the IL-1 β -induced production of proteoglycans, NO, matrix metalloproteinases (MMPs) and degradation of glycosaminoglycan by cartilage [4,6]. A preclinical animal study reported that SKI306X protects against osteoarthritis. Collagenase injection at the knee joint of rabbits led to osteoarthritis-like degeneration of articular cartilage and development of synovial tissues; however, SKI306X reduces osteoarthritis-like histological changes [4]. In chondrocytes cultured in media supplemented with IL-1 α , the cumulative activity of MMP-3 and MMP-13 increased; however, SKI306X significantly reduced their activities and inhibited the activation of the proenzyme MMP-3 to the active MMP-3 [7]. In clinical trials of knee osteoarthritis, SKI306X treatment resulted in greater changes in pain and function than the placebo [8,9]. The change in cartilage volume and thickness of the lateral tibia were reportedly greater after treatment with SKI306X than the placebo [8].

Two studies have reported that SKI306X has similar effects in controlling for pain and disease activity and has greater cardiovascular safety than celecoxib in RA. When SKI306X was compared with celecoxib among RA patients, SKI306X was not inferior to celecoxib with regards to pain score (visual analog scale), American College of Rheumatology (ACR) 20 response rate, frequency of rescue medication, and drug-related adverse effects [10]. Unlike celecoxib, SKI306X does not have a higher risk of cardiovascular events in patients with RA and osteoarthritis [11]. However, the limitation of this clinical study was that celecoxib is not a DMARD and it cannot prevent joint destruction. Thus far, no study has investigated the anti-inflammatory and joint protective effects of SKI306X in RA. Therefore, this study aimed to investigate the regulatory effect of SKI306X in T helper (Th)17 cytokine-induced inflammation and bone destruction in RA.

2. Methods

2.1. Patients

Informed consent was obtained from all patients, and the experimental protocol was approved by the Institutional Review Board for Human Research, Konkuk University Hospital (KUH1010186, approved on January 22nd 2010). Synovial tissues were isolated from eight RA patients (mean age 63.4 \pm 4.6 years, range 38–76 years) undergoing total knee and hip replacement surgery.

2.2. Isolation of Synovial Fibroblasts

Synovial fibroblasts were isolated by enzymatic digestion of synovial tissues, as described previously [12,13]. Synovial fibroblast cell lines were prepared from synovectomized tissue of RA patients undergoing joint replacement surgery. To set up cell lines, synovial tissues were minced into 2–3 mm pieces and treated for 4 h with 4 mg/mL type 1 collagenase (Worthington Biochemicals, Freehold,

NJ, USA) in Dulbecco's modified Eagle's medium (DMEM) at 37 °C in 5% CO₂. Dissociated cells were centrifuged at 500× g and were resuspended in DMEM supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Suspended cells were plated in 75 cm² culture flasks and cultured at 37 °C in 5% CO₂. Medium was replaced every 3 days, and once the primary culture reached confluence, cells were split weekly. Cells at passages 5 to 8 contained a homogeneous population of synovial fibroblasts (<2.5% CD14 positive, <1% CD3 positive, and <1% CD19 positive in flow cytometric analysis).

2.3. Reagents

Recombinant human IL-17 (20 ng/mL), IL-21 (20 ng/mL), IL-22 (20 ng/mL), and macrophage colony-stimulating factor (M-CSF) (25 ng/mL) were purchased from R&D Systems (Minneapolis, MN, USA). SKI306X (at 0 µg/mL, 1 µg/mL, 5 µg/mL and 10 µg/mL) were generously provided by the Life Science R&D Center of SK Chemicals (Seongnam, Korea). Anti-TNF- α , anti-IL-1 β and anti-RANKL antibodies were purchased from R&D Systems.

2.4. Real-Time PCR

Synovial fibroblasts were stimulated with IL-17, IL-21, or IL-22. Synovial fibroblasts were incubated in the presence or absence of SKI306X for 3 h before the addition of IL-17, IL-21, or IL-22. After incubation for 72 h, mRNA was extracted using RNAzol B (Biotex Laboratories, Houston, TX, USA) according to the manufacturer's instructions.

2.5. Enzyme-Linked Immunosorbent Assay (ELISA)

TNF- α , IL-1 β , IL-8 and soluble receptor activator of nuclear factor kappa-B ligand (sRANKL) levels in the culture supernatants from RA synovial fibroblasts were measured using a sandwich ELISA according to R&D System's instructions.

2.6. Osteoclast Formation

PB monocytes were prepared from heparinized blood by Ficoll-Hypaque (GE Healthcare, Pittsburgh, PA, USA) density gradient centrifugation. Monocytes were added to the IL-17-pretreated RA synovial fibroblasts with fresh media. Monocytes were co-cultured for 3 weeks in α -minimal essential medium and 10% fetal bovine serum (FBS) in the presence of 25 ng/mL recombinant human macrophage-colony stimulating factor (rhM-CSF). On day 21, tartrate-resistant acid phosphatase (TRAP)-positive cells were identified using a leukocyte acid phosphatase kit according to Sigma-Aldrich's protocol [14].

2.7. Statistical Analysis

The data are expressed as means \pm standard deviation (SD). Statistical difference was assessed using Mann-Whitney *U* test for analyzing two groups or one-way analysis of variance (ANOVA) with Bonferroni's multiple comparison post-hoc test for analyzing more than three groups. A *p* value < 0.05 was considered statistically significant.

3. Results

3.1. Regulatory Effect of SKI306X on Th17 Cytokine-Induced TNF- α Expression and Production in RA Synovial Fibroblasts

When RA synovial fibroblasts were cultured in media supplemented with IL-17, IL-21, or IL-22, TNF- α was upregulated; however, SKI306X reduced Th17 cytokine-induced TNF- α expression (Figure 1A), TNF- α production was increased upon IL-17 stimulation in culture media, and SKI306X reduced TNF- α production (Figure 1B).

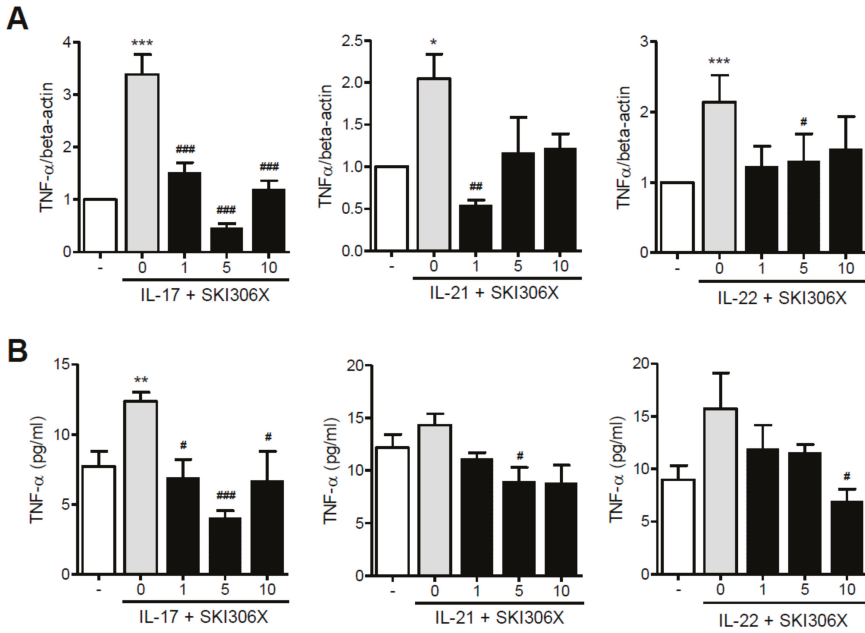


Figure 1. The inhibitory effect of SKI306X, a mixed extract of three herbs, in T helper (Th)17 cytokine-induced tumor necrosis factor (TNF)- α expression and production by synovial fibroblasts in rheumatoid arthritis (RA). (A) RA synovial fibroblasts were cultured in media supplemented with interleukin (IL)-17, IL-21, and IL-22 in the presence of various doses of SKI306X, and TNF- α was examined using real-time PCR, normalized to that of beta-actin and reported in relative expression units. (B) RA synovial fibroblasts were cultured with IL-17, IL-21, and IL-22 in the presence of various concentrations of SKI306X, and TNF- α production in culture media was determined using ELISA. The data represent the mean \pm standard deviation (SD) values from six independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with the nil condition (white bars). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ compared with the Th17 cytokine stimulating condition (gray bars).

3.2. The Regulatory Effect of SKI306X on Th17 Cytokine-Induced IL-1 β Expression and Production in RA Synovial Fibroblasts

When RA synovial fibroblasts were cultured in media supplemented with IL-17 or IL-22, IL-1 β was upregulated; however, SKI306X reduced Th17 cytokine-induced IL-1 β expression. Stimulation with IL-21 resulted in a similar, albeit non-significant, effect (Figure 2A). Furthermore, IL-1 β production was increased after IL-17 or IL-21 supplementation in culture media, and SKI306X reduced IL-1 β production (Figure 2B).

However, IL-21 and IL-22 did not promote IL-8 expression and production. Although IL-17 induced IL-8 expression and production, SKI306X inhibited IL-17-induced IL-8 production in RA synovial fibroblasts but did not affect IL-17-induced IL-8 expression.

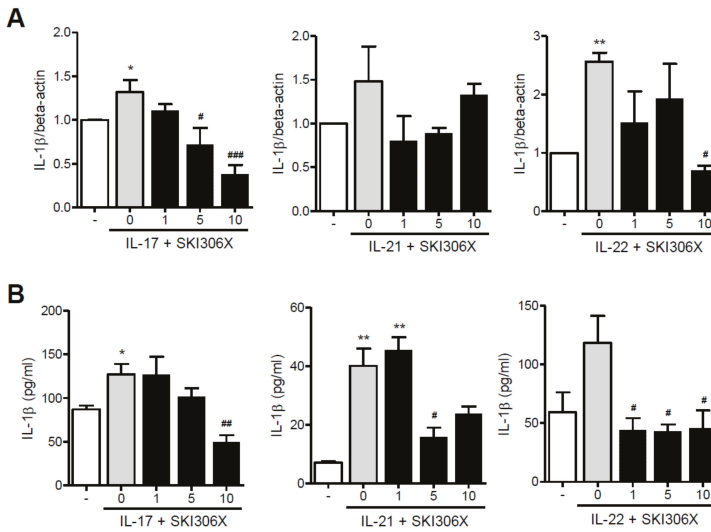


Figure 2. The inhibitory effect of SKI306X on Th17 cytokine-induced IL-1β expression and production in synovial fibroblasts in RA. **(A)** RA synovial fibroblasts were cultured in medium supplemented with IL-17, IL-21, and IL-22 with various concentrations of SKI306X, and IL-1β expression was examined using real-time PCR, normalized to beta-actin and reported in relative expression units. **(B)** RA synovial fibroblasts were cultured in medium supplemented with IL-17, IL-21, and IL-22 in the presence of various doses of SKI306X, and IL-1β production in the culture media was determined via ELISA. The data represent mean ± SD values from six independent experiments. * $p < 0.05$ and ** $p < 0.01$ compared with the nil condition (white bars). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ compared with the Th17 cytokine stimulating condition (gray bars).

3.3. Regulatory Effect of SKI306X on Th17 Cytokine-Induced RANKL Expression and Production in RA Synovial Fibroblasts

On culturing RA synovial fibroblasts in media supplemented with IL-17 or IL-22, RANKL was upregulated; however, SKI306X reduced Th17 cytokine-induced RANKL expression (Figure 3A). Furthermore, RANKL production was increased upon IL-17 supplementation in culture media, and SKI306X reduced IL-17-induced RANKL production (Figure 3B).

3.4. Regulatory Effect of SKI306X on Th17 Cytokine-Induced Osteoclast Differentiation from PB Monocytes

When CD14⁺ monocytes isolated from the PB of healthy donors were cultured in media supplemented with IL-17 and monocyte colony stimulating factor (M-CSF), TRAP-positive multinucleated osteoclasts were differentiated, and SKI306X reduced osteoclast differentiation in a dose-dependent manner. Osteoclast markers including TRAP, cathepsin K, and dendritic cell specific transmembrane protein (DC-STAMP) were upregulated upon IL-17 stimulation, and SKI306X downregulated these factors (Figure 4A). Monocyte stimulation with IL-21 or IL-22 yielded a similar pattern as IL-17 during osteoclast differentiation. On culturing CD14⁺ monocytes in media supplemented with IL-21 or IL-22 and M-CSF, TRAP-positive multinucleated osteoclasts differentiated, and SKI306X reduced osteoclast differentiation in a dose-dependent manner. Osteoclast markers including TRAP, cathepsin K, DC-STAMP, and ATP6v0d2 were also upregulated upon IL-21 and IL-22 stimulation, and SKI306X reduced their expression (Figure 4B,C).

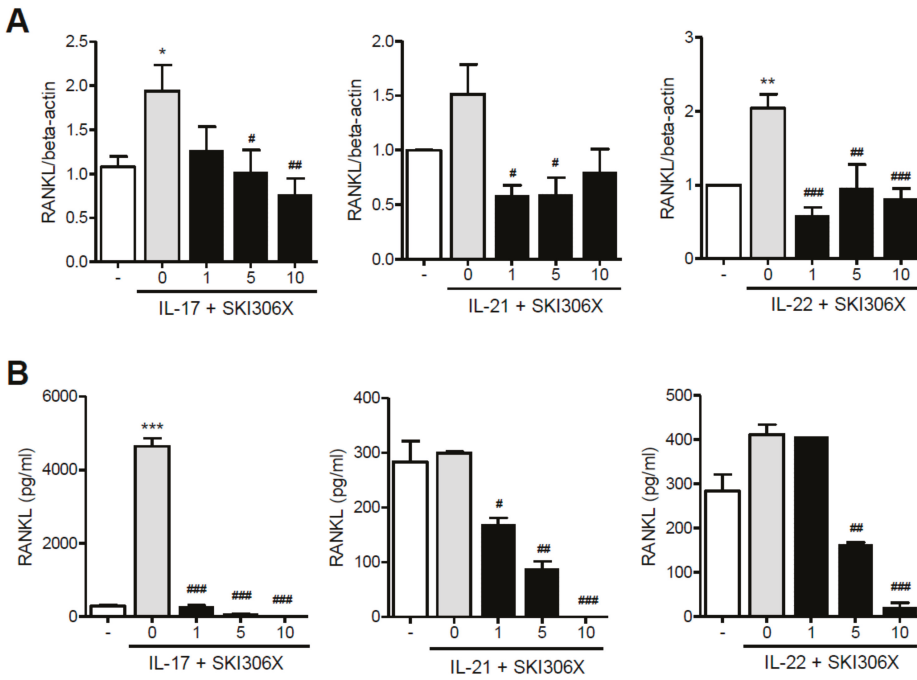


Figure 3. The inhibitory effect of SKI306X in Th17 cytokine-induced receptor activator of nuclear factor kappa-B ligand (RANKL) expression and production in synovial fibroblasts in RA. **(A)** RA synovial fibroblasts were cultured in medium supplemented with IL-17, IL-21, and IL-22 with various concentrations of SKI306X, and RANKL expression was examined using real-time PCR, normalized to beta-actin and reported in relative expression units. **(B)** RA synovial fibroblasts were cultured in medium supplemented with IL-17, IL-21, and IL-22 with various concentrations of SKI306X, and RANKL production in the culture media was examined using ELISA. The data represent mean ± SD values from six independent experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with the nil condition (white bars). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ compared with the Th17 cytokine stimulating condition (gray bars).

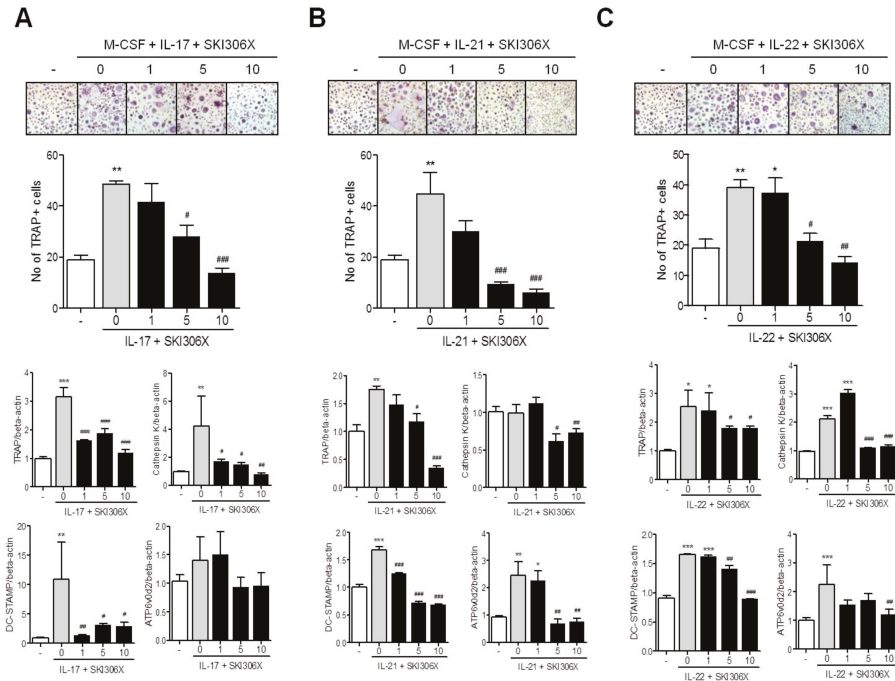


Figure 4. Regulatory effect of SKI306X on Th17 cytokine-induced osteoclast differentiation in peripheral blood (PB) monocytes. PB cluster of differentiation (CD)14⁺ monocytes were cultured in medium supplemented with (A) IL-17, (B) IL-21, or (C) IL-22 and various concentrations of SKI306X in the presence of 25 ng/mL of monocyte colony stimulating factor. After 21 days of culturing, TRAP-positive multinucleated cells were enumerated. The figures represent one of three independent experiments and the bars represent mean ± SD values. The expression of osteoclast markers including tartrate-resistant acid phosphatase (TRAP), cathepsin K, dendritic cell specific transmembrane protein (DC-STAMP), and ATP6v0d2 was quantified using real-time PCR, normalized to beta-actin and reported in relative expression units. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared with the nil condition (white bars). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ compared with the Th17 cytokine stimulating condition (gray bars).

3.5. Regulatory Effect of SKI306X on Osteoclast Differentiation from PB Monocytes Co-Cultured with IL-17-Stimulated RA Synovial Fibroblasts

CD14⁺ monocytes isolated from PB were co-cultured with IL-17-prestimulated RA synovial fibroblasts in media supplemented with M-CSF, and TRAP-positive multinucleated osteoclasts were differentiated and compared with non-stimulated RA synovial fibroblasts. SKI306X reduced osteoclast differentiation (Figure 5A). Furthermore, osteoclast markers including TRAP, cathepsin K, and nuclear factor of activated T-cells, cytoplasmic 1 (NF-ATc1) were upregulated when osteoclast precursors were co-cultured with IL-17-prestimulated RA synovial fibroblasts, and SKI306X reduced their expression (Figure 5B).

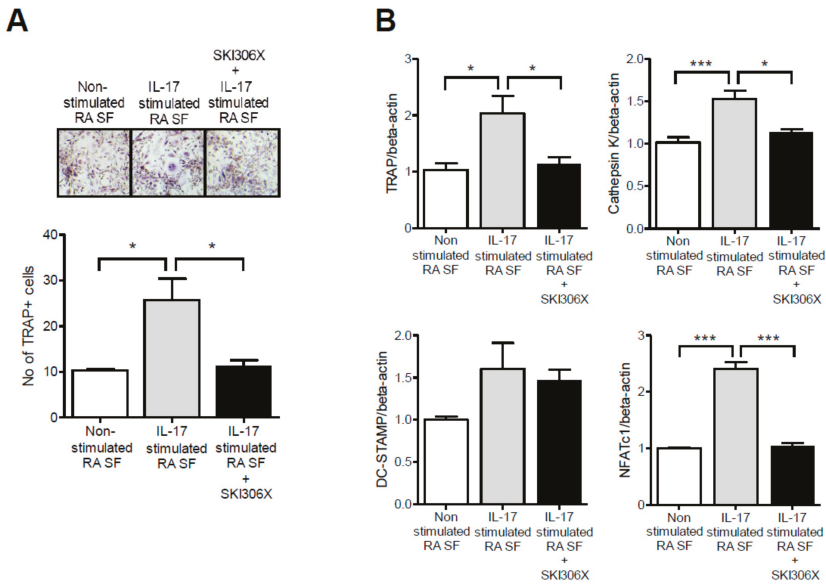


Figure 5. The regulatory effect of SKI306X on osteoclast differentiation in co-cultures of PB monocytes and IL-17-stimulated synovial fibroblasts (SF) in RA. **(A)** After PB monocytes were co-cultured with IL-17 stimulated RA synovial fibroblasts in the presence of monocyte colony stimulating factor and SKI306X for 21 days, the TRAP-positive multinucleated cells were enumerated. The figures represent one of three independent experiments and the bars represent mean ± SD values. **(B)** The expression of osteoclast markers including TRAP, cathepsin K, DC-STAMP, and nuclear factor of activated T-cells, cytoplasmic 1 (NF-ATc1) was quantified using real-time PCR, normalized to beta-actin and reported in relative expression units. * $p < 0.05$ and *** $p < 0.001$.

4. Discussion

In some Asian countries, herbal medicines are commonly used for treating arthritis. Although many patients do not receive prompt diagnosis and treatment for RA, some patients have experienced therapeutic effects using herbal medication [1,3]. SKI306X is widely used to manage osteoarthritis in Korea; however, no study has reported the efficacy of herbal medication in RA. This study aimed to investigate the anti-inflammatory and osteoprotective effects of SKI306X in Th17 cytokine-induced inflammation and osteoclast differentiation in RA.

To determine the anti-inflammatory effect of SKI306X, we examined Th17 cytokine-induced TNF- α and IL-1 β expression in RA synovial fibroblasts. We selected synovial fibroblasts and Th17 cytokines in this study because they are primary targets in RA pathogenesis; however, they are very active effector cells, which can induce and aggravate inflammatory processes [15,16]. We previously reported that IL-21, IL-22, and IL-17 stimulate RA synovial fibroblasts to produce inflammatory cytokines [13,17,18]. Moreover, Th17 overproduction and the differentiation of Th17-positive cells are one of the major events in RA pathogenesis; hence, reduction of the Th17 response regulates inflammation and joint destruction in RA [19,20]. IL-17, IL-21, and IL-22 upregulated TNF- α and IL-1 β in RA synovial fibroblasts and SKI306X effectively reduced their expression. A previous study reported that SKI306X reduces lipopolysaccharide (LPS)-induced TNF- α and IL-1 β production in human peripheral blood mononuclear cells (PBMCs) [20]. Another study reported that SKI306X inhibits TNF- α release from LPS-stimulated human whole blood; however, it does not affect IL-1 β release [5]. LPS is a nonspecific stimulant in the inflammatory processes. In this study, we used RA synovial fibroblasts, which represent target cells, and stimulated them with Th17 cytokines as

specific stimulators and LPS as a non-specific cellular stimulator. Thus, we replicated pathological conditions *in vitro* and investigated the therapeutic effect of SKI306X. Reduction of Th17-induced TNF- α and IL-1 β expression and production by SKI306X indicates that SKI306X plays a potential anti-inflammatory role in Th17-induced inflammation in RA.

Furthermore, we determined the bone protective role of SKI306X in RA. In RA, loss of cartilage and bone erosion cause joint destruction and subsequent joint disability. Bone erosion is caused by bone resorption of synovial osteoclasts, which in turn are activated by RANKL and other inflammatory cytokines. They originate from synovial tissues, subchondral bone, and circulating monocytes in inflammatory conditions [21]. We examined the dual roles of SKI306X in the reduction of RANKL production from synovial fibroblasts and in the inhibition of osteoclast differentiation. Because synovial fibroblasts are major sources of RANKL [13,22,23], SKI306X inhibited Th17 cytokine-induced RANKL expression and production in RA synovial fibroblasts. These findings suggest that SKI306X potentially ameliorates bone destruction because RANKL is a very important molecule in osteoclast activation. In recent clinical trials, denosumab, an anti-RANKL antibody, inhibited the progression of joint destruction in RA patients [24,25]. Although denosumab does not reduce inflammation, combinatorial treatment with conventional or biologic DMARDs can effectively reduce both inflammation and joint destruction.

Finally, we examined the inhibitory effect of SKI306X in osteoclast differentiation. RA treatment is primarily aimed at preventing joint destruction; hence, inhibition of osteoclastogenesis is critical for treatment. Circulating CD14⁺ monocytes are precursors of osteoclasts and they upregulate RANK on the cell surface and interact with RANKL that is primarily produced by RA synovial fibroblasts and Th17 cells [26]. Th17 cytokines independently induce osteoclast differentiation from their precursor [17,18,27]. In this study, when PB monocytes were cultured in media supplemented with Th17 cytokines and SKI306X, SKI306X reduced Th17 cytokine-induced osteoclast differentiation. The underlying mechanism of action of this drug is unclear; hence, further studies are required to determine the cell receptors and signaling pathways involved herein.

Monocytes express various chemokine receptors including C-C chemokine receptor type 2 (CCR2) and (C-X3-C motif) chemokine receptor 1 (CX3CR1) and interact with chemokine ligands expressed by synovial fibroblasts. Their interaction promotes cellular activation, migration, and recruitment into the synovium of RA patients [26]. To investigate their interactions, we co-cultured monocytes with RA synovial fibroblasts under osteoclast-differentiating conditions. RA synovial fibroblasts can potentially augment osteoclastogenesis [28], and TNF- α - or IL-17-stimulated synovial fibroblasts are more effective at osteoclastogenesis [17]. Osteoclast differentiation was augmented when RA synovial fibroblasts were stimulated with IL-17 and then co-cultured with monocytes rather than non-stimulation with IL-17. However, SKI306X restored IL-17-augmented osteoclast differentiation in stimulated synovial fibroblasts. These results suggest that SKI306X reduces osteoclastogenesis through its effects on both osteoclast precursors and cellular interactions with synovial fibroblasts.

There are only two clinical studies of SKI306X in RA; however, the studies have limitations regarding the assessment of the clinical efficacy of SKI306X in RA patients. One study is a six-week, double blinded noninferiority study for assessment of pain relief and tolerability of SKI306X compared with celecoxib. The duration of the study is too short to assess clinical efficacy and protective effect of joint destruction. Because SKI306X is compared with celecoxib rather than DMARDs, the disease modifying effect of SKI306X for RA cannot be assessed [10]. The other study evaluated cardiovascular risk associated with SKI306X use in RA patients which was compared with celecoxib and naproxen. A total of 27,253 patients were studied and the incidence of major cardiovascular events was highest for celecoxib (15.4%), followed by SKI306X (8.6%) and naproxen (8%). SKI306X did not have a higher risk of cardiovascular events than naproxen. This study is a retrospective observational study using data from National Health Insurance Service–National Sample Cohort and it does not assess the therapeutic efficacy of SKI306X. However, this study has a meaningful result because the cardiovascular risk is high in RA patients and it influences their survival [11]. RA is associated with high cardiovascular risk,

affecting patient survival, and inflammation and atherosclerosis are closely linked; the pathological features are similar in both disease states and they share common risk factors [29]. Furthermore, the overall risk of metabolic syndrome is higher in patients with RA than in healthy controls and RA is associated with body weight changes, dyslipidemia, adipokine profile changes and insulin resistance in metabolic syndrome [30]. Although IL-17 has a double-sided effect in atherosclerosis, IL-17 could be involved in the process of atherosclerosis of RA. It induces the release of chemokines and their ligands (chemokine (C-X-C motif) ligand (CXCL)1, CXCL2, CXCL8 and CXCL10), which recruit neutrophils and monocytes to the atherosclerotic lesion. IL-17 stimulates monocytes to produce IL-6, TNF- α and IL-1 β , which enhance plaque instability [31,32]. In early atherosclerosis, increased carotid intima-media thickness is associated with the IL-17-related chemokine eotaxin [33], and in RA patients, IL-17 is the main predictor of microvascular function and arterial compliance, suggesting a significant role for IL-17 in increased cardiovascular risk in RA [34].

This study is the first to report that SKI306X regulates RANKL production and osteoclast differentiation in RA. The mechanism of action of SKI306X is unclear, unlike that of conventional DMARDs. Recently, targeted or biologic DMARDs have been preferred in treating active RA; however, their usage in combination with SKI306X potentially results in additional therapeutic effects. There have been no clinical or experimental studies of the comparison between SKI306X and other DMARDs such as methotrexate or hydroxychloroquine. There are only comparative clinical studies of SKI306X for assessment of pain relief, tolerability and cardiovascular risk. Based upon the results of this study, a comparison study of the therapeutic effects of SKI306X versus DMARDs and the assessment of combination effects of SKI306X with DMARDs in RA patients should be performed in the future.

5. Conclusions

SKI306X reduced both inflammation and osteoclast differentiation in RA, reducing Th17 cytokine-induced TNF α and IL-1 β expression and production in RA synovial fibroblasts during inflammation. SKI306X ameliorated RANKL production in synovial fibroblasts and osteoclast differentiation in circulating monocytes. SKI306X is thus a potential novel therapeutic agent to prevent inflammation and joint destruction in RA.

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Conflicts of Interest: The authors declare no potential conflicts of interest.

Abbreviations

| | |
|---------------|---|
| Th17 | T helper 17 |
| IL-17 | interleukin 17 |
| IL-21 | interleukin 21 |
| IL-22 | interleukin 22 |
| RA | rheumatoid arthritis |
| FLSs | fibroblast-like synoviocytes |
| TRAP | tartrate-resistant acid phosphatase |
| TNF- α | tumor necrosis factor- α |
| IL-1 β | interleukin-1 β |
| M-CSF | macrophage colony-stimulating factor |
| RANKL | receptor activator of nuclear factor kappa-B ligand |

References

1. Rambod, M.; Nazarinia, M.; Raieskarimian, F. The prevalence and predictors of herbal medicines usage among adult rheumatoid arthritis patients: A case-control study. *Complement. Ther. Med.* **2018**, *41*, 220–224. [[CrossRef](#)] [[PubMed](#)]
2. Teekachunhatean, S.; Kunanusorn, P.; Rojanasthien, N.; Sananpanich, K.; Pojchamarnwiputh, S.; Lhieochaiphunt, S.; Pruksakorn, S. Chinese herbal recipe versus diclofenac in symptomatic treatment of osteoarthritis of the knee: A randomized controlled trial [ISRCTN70292892]. *BMC Complement. Altern. Med.* **2004**, *4*, 19. [[CrossRef](#)] [[PubMed](#)]
3. Han, M.; Sung, Y.K.; Cho, S.K.; Kim, D.; Won, S.; Choi, C.B.; Bang, S.Y.; Cha, H.S.; Choe, J.Y.; Chung, W.T.; et al. Factors Associated with the Use of Complementary and Alternative Medicine for Korean Patients with Rheumatoid Arthritis. *J. Rheumatol.* **2015**, *42*, 2075–2081. [[CrossRef](#)] [[PubMed](#)]
4. Choi, J.H.; Kim, D.Y.; Yoon, J.H.; Youn, H.Y.; Yi, J.B.; Rhee, H.I.; Ryu, K.H.; Jung, K.; Han, C.K.; Kwak, W.J.; et al. Effects of SKI 306X, a new herbal agent, on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model. *Osteoarthr. Cartil.* **2002**, *10*, 471–478. [[CrossRef](#)] [[PubMed](#)]
5. Kim, J.H.; Ryu, K.H.; Jung, K.W.; Han, C.K.; Kwak, W.J.; Cho, Y.B. Effects of SKI306X on arachidonate metabolism and other inflammatory mediators. *Biol. Pharm. Bull.* **2005**, *28*, 1615–1620. [[CrossRef](#)] [[PubMed](#)]
6. Hartog, A.; Hougee, S.; Faber, J.; Sanders, A.; Zuurman, C.; Smit, H.F.; van der Kraan, P.M.; Hoijer, M.A.; Garssen, J. The multicomponent phytopharmaceutical SKI306X inhibits in vitro cartilage degradation and the production of inflammatory mediators. *Phytomedicine* **2008**, *15*, 313–320. [[CrossRef](#)] [[PubMed](#)]
7. Kim, J.H.; Ryu, K.H.; Jung, K.W.; Han, C.K.; Kwak, W.J.; Cho, Y.B. SKI306X suppresses cartilage destruction and inhibits the production of matrix metalloproteinase in rabbit joint cartilage explant culture. *J. Pharmacol. Sci.* **2005**, *98*, 298–306. [[CrossRef](#)] [[PubMed](#)]
8. Kim, J.I.; Choi, J.Y.; Kim, K.G.; Lee, M.C. Efficacy of JOINS on Cartilage Protection in Knee Osteoarthritis: Prospective Randomized Controlled Trial. *Knee Surg. Relat. Res.* **2017**, *29*, 217–224. [[CrossRef](#)]
9. Ha, C.W.; Park, Y.B.; Min, B.W.; Han, S.B.; Lee, J.H.; Won, Y.Y.; Park, Y.S. Prospective, randomized, double-blinded, double-dummy and multicenter phase IV clinical study comparing the efficacy and safety of PG201 (Layla) and SKI306X in patients with osteoarthritis. *J. Ethnopharmacol.* **2016**, *181*, 1–7. [[CrossRef](#)]
10. Song, Y.W.; Lee, E.Y.; Koh, E.M.; Cha, H.S.; Yoo, B.; Lee, C.K.; Baek, H.J.; Kim, H.; Suh, Y.; Kang, S.W.; et al. Assessment of comparative pain relief and tolerability of SKI306X compared with celecoxib in patients with rheumatoid arthritis: A 6-week, multicenter, randomized, double-blind, double-dummy, phase III, noninferiority clinical trial. *Clin. Ther.* **2007**, *29*, 862–873. [[CrossRef](#)]
11. Woo, Y.; Hyun, M.K. Evaluation of cardiovascular risk associated with SKI306X use in patients with osteoarthritis and rheumatoid arthritis. *J. Ethnopharmacol.* **2017**, *207*, 42–46. [[CrossRef](#)] [[PubMed](#)]
12. Kim, K.W.; Cho, M.L.; Kim, H.R.; Ju, J.H.; Park, M.K.; Oh, H.J.; Kim, J.S.; Park, S.H.; Lee, S.H.; Kim, H.Y. Up-regulation of stromal cell-derived factor 1 (CXCL12) production in rheumatoid synovial fibroblasts through interactions with T lymphocytes: Role of interleukin-17 and CD40L-CD40 interaction. *Arthritis Rheum.* **2007**, *56*, 1076–1086. [[CrossRef](#)] [[PubMed](#)]
13. Kim, H.R.; Kim, K.W.; Kim, B.M.; Lee, K.A.; Lee, S.H. N-acetyl-l-cysteine controls osteoclastogenesis through regulating Th17 differentiation and RANKL in rheumatoid arthritis. *Korean J. Intern. Med.* **2019**, *34*, 210–219. [[CrossRef](#)] [[PubMed](#)]
14. Kong, Y.Y.; Yoshida, H.; Sarosi, I.; Tan, H.L.; Timms, E.; Capparelli, C.; Morony, S.; Oliveira-dos-Santos, A.J.; Van, G.; Itie, A.; et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* **1999**, *397*, 315–323. [[CrossRef](#)] [[PubMed](#)]
15. Lefevre, S.; Meier, F.M.; Neumann, E.; Muller-Ladner, U. Role of synovial fibroblasts in rheumatoid arthritis. *Curr. Pharm. Des.* **2015**, *21*, 130–141. [[CrossRef](#)] [[PubMed](#)]
16. Neumann, E.; Lefevre, S.; Zimmermann, B.; Gay, S.; Müller-Ladner, U. Rheumatoid arthritis progression mediated by activated synovial fibroblasts. *Trends Mol. Med.* **2010**, *16*, 458–468. [[CrossRef](#)] [[PubMed](#)]
17. Kim, K.W.; Kim, H.R.; Kim, B.M.; Cho, M.L.; Lee, S.H. Th17 cytokines regulate osteoclastogenesis in rheumatoid arthritis. *Am. J. Pathol.* **2015**, *185*, 3011–3024. [[CrossRef](#)] [[PubMed](#)]
18. Kim, K.W.; Kim, H.R.; Park JY Park, J.S.; Oh, H.J.; Woo, Y.J.; Park, M.K.; Cho, M.L.; Lee, S.H. Interleukin-22 promotes osteoclastogenesis in rheumatoid arthritis through induction of RANKL in human synovial fibroblasts. *Arthritis Rheum.* **2012**, *64*, 1015–1023. [[CrossRef](#)]

19. Roelvelde, D.M.; Koenders, M.I. The role of the Th17 cytokines IL-17 and IL-22 in Rheumatoid Arthritis pathogenesis and developments in cytokine immunotherapy. *Cytokine* **2015**, *74*, 101–107. [[CrossRef](#)]
20. Roelvelde, D.M.; van Nieuwenhuijze, A.E.; van den Berg, W.B.; Koenders, M.I. The Th17 pathway as a therapeutic target in rheumatoid arthritis and other autoimmune and inflammatory disorders. *BioDrugs* **2013**, *27*, 439–452. [[CrossRef](#)]
21. Amarasekara, D.S.; Yun, H.; Kim, S.; Lee, N.; Kim, H.; Rho, J. Regulation of Osteoclast Differentiation by Cytokine Networks. *Immune Netw.* **2018**, *18*, e8. [[CrossRef](#)] [[PubMed](#)]
22. Tunyogi-Csapo, M.; Kis-Toth, K.; Radacs, M.; Farkas, B.; Jacobs, J.J.; Finnegan, A.; Mikecz, K.; Glant, T.T. Cytokine-controlled RANKL and osteoprotegerin expression by human and mouse synovial fibroblasts: Fibroblast-mediated pathologic bone resorption. *Arthritis Rheum.* **2008**, *58*, 2397–2408. [[CrossRef](#)] [[PubMed](#)]
23. Kim, H.R.; Kim, B.M.; Won, J.Y.; Lee, K.A.; Ko, H.M.; Kang, Y.S.; Lee, S.H.; Kim, K.W. Quercetin, a Plant Polyphenol, Has Potential for the Prevention of Bone Destruction in Rheumatoid Arthritis. *J. Med. Food* **2019**, *22*, 152–161. [[CrossRef](#)] [[PubMed](#)]
24. Takeuchi, T.; Tanaka, Y.; Soen, S.; Yamanaka, H.; Yoneda, T.; Tanaka, S.; Nitta, T.; Okubo, N.; Genant, H.K.; van der Heijde, D. Effects of the anti-RANKL antibody denosumab on joint structural damage in patients with rheumatoid arthritis treated with conventional synthetic disease-modifying antirheumatic drugs (DESIRABLE study): A randomised, double-blind, placebo-controlled phase 3 trial. *Ann Rheum. Dis.* **2019**, *78*, 899–907. [[PubMed](#)]
25. Ishiguro, N.; Tanaka, Y.; Yamanaka, H.; Yoneda, T.; Ohira, T.; Okubo, N.; Genant, H.K.; van der Heijde, D.; Takeuchi, T. Efficacy of denosumab with regard to bone destruction in prognostic subgroups of Japanese rheumatoid arthritis patients from the phase II DRIVE study. *Rheumatology (Oxford)* **2019**, *58*, 997–1005. [[CrossRef](#)]
26. Rana, A.K.; Li, Y.; Dang, Q.; Yang, F. Monocytes in rheumatoid arthritis: Circulating precursors of macrophages and osteoclasts and, their heterogeneity and plasticity role in RA pathogenesis. *Int. Immunopharmacol.* **2018**, *65*, 348–359. [[CrossRef](#)] [[PubMed](#)]
27. Kim, K.W.; Kim, B.M.; Lee, K.A.; Lee, S.H.; Firestein, G.S.; Kim, H.R. Histamine and Histamine H4 Receptor Promotes Osteoclastogenesis in Rheumatoid Arthritis. *Sci. Rep.* **2017**, *7*, 1197. [[CrossRef](#)] [[PubMed](#)]
28. Takayanagi, H. New developments in osteoimmunology. *Nat. Rev. Rheumatol.* **2012**, *8*, 684–689. [[CrossRef](#)] [[PubMed](#)]
29. Skeoch, S.; Bruce, I.N. Atherosclerosis in rheumatoid arthritis: Is it all about inflammation? *Nat. Rev. Rheumatol.* **2015**, *11*, 390–400. [[CrossRef](#)]
30. Kerekes, G.; Nurmohamed, M.T.; Gonzalez-Gay, M.A.; Seres, I.; Paragh, G.; Kardos, Z.; Baráth, Z.; Tamási, L.; Soltész, P.; Szekanez, Z. Rheumatoid arthritis and metabolic syndrome. *Nat. Rev. Rheumatol.* **2014**, *10*, 691–696. [[CrossRef](#)]
31. Lu, X. The Impact of IL-17 in Atherosclerosis. *Curr. Med. Chem.* **2017**, *24*, 2345–2358. [[CrossRef](#)] [[PubMed](#)]
32. Ryu, H.; Chung, Y. Regulation of IL-17 in atherosclerosis and related autoimmunity. *Cytokine* **2015**, *74*, 219–227. [[CrossRef](#)] [[PubMed](#)]
33. Tarantino, G.; Costantini, S.; Finelli, C.; Capone, F.; Guerriero, E.; La Sala, N.; Gioia, S.; Castello, G. Is serum Interleukin-17 associated with early atherosclerosis in obese patients? *J. Transl. Med.* **2014**, *12*, 214. [[CrossRef](#)] [[PubMed](#)]
34. Marder, W.; Khalatbari, S.; Myles, J.D.; Hench, R.; Yalavarthi, S.; Lustig, S.; Brook, R.; Kaplan, M.J. Interleukin 17 as a novel predictor of vascular function in rheumatoid arthritis. *Ann. Rheum. Dis.* **2011**, *70*, 1550–1555. [[CrossRef](#)] [[PubMed](#)]



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Article

Maintained Clinical Remission in Ankylosing Spondylitis Patients Switched from Reference Infliximab to Its Biosimilar: An 18-Month Comparative Open-Label Study

Evrpidis Kaltsonoudis, Eleftherios Pelechas, Paraskevi V. Voulgari and Alexandros A. Drosos *

Rheumatology Clinic, Department of Internal Medicine, Medical School, University of Ioannina, 45110 Ioannina, Greece

* Correspondence: adrosos@cc.uoi.gr; Tel.: +30-265-100-7503; Fax: +30-265-100-7054

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Abstract: Background: Switching from reference infliximab (RI) to biosimilar infliximab (BI) had no detrimental effects on efficacy and safety. However, long-term follow-up data is missing. Objective: To evaluate patients with Ankylosing Spondylitis (AS) in clinical remission who were switching from RI to BI, in terms of the safety and efficacy of this, in a long-term fashion. Methods: One hundred and nine consecutive unselected AS patients were investigated. All were naïve to other biologics and were followed-up at predefined times receiving RI. Patients in clinical remission were asked to switch from RI to BI. Those who switched to BI were compared with a matched control-group receiving continuous RI. During follow-up, several parameters were recorded for at least 18 months. Disease activity was measured using the Bath Ankylosing Spondylitis disease activity index (BASDAI), and the Ankylosing Spondylitis disease activity score (ASDAS), using the C-reactive protein. Remission was defined as BASDAI < 4 and ASDAS < 1.3. Results: Eighty-eight patients were evaluated (21 excluded for different reasons). From those, 45 switched to BI, while 43 continued receiving RI. No differences between groups regarding demographic, clinical and laboratory parameters were observed. All patients were in clinical remission. During follow-up, five patients from the BI-group and three from the maintenance-group discontinued the study (4 patients nocebo effect, 1 loss of efficacy). After 18 months of treatment, all patients in both groups remained in clinical remission. No significant adverse events were noted between groups. Conclusion: BI is equivalent to RI in maintaining AS in clinical remission for at least 18 months.

Keywords: ankylosing spondylitis; infliximab; biosimilar; switching

1. Introduction

CTP-13 (Inflectra®, Remsima®) the biosimilar infliximab (BI) has been granted all indications, including Ankylosing Spondylitis (AS), of the reference infliximab (RI) in several countries [1]. Clinical evidence for the approval of BI has been obtained from pivotal studies on patients with AS (PLANETAS) [2] and rheumatoid arthritis (RA) (PLANETRA) [3]. Switching from RI to its biosimilar had no detrimental effects on efficacy, safety or immunogenicity compared with continuous RI therapy [4,5]. Therefore, BI is an efficacious alternative to RI in patients with inflammatory arthritis [6]. On the other hand, there are reports emphasizing the role of shared-decision making with patients when it comes to switching to a biosimilar product in order to achieve a better acceptance and higher retention rate, minimizing the nocebo effect [5]. However, long-term follow-up data is missing. The aim of our study was to investigate if BI is equivalent to RI to maintain patients with AS in clinical remission compared with continuing RI in a long-term fashion.

2. Materials and Methods

This is a single-center prospective observational cohort study with a total number of 109 consecutive unselected patients with AS who were treated with RI in a tertiary university center. All patients were followed-up at predefined times receiving RI (5 mg/kg/8 weeks) intravenously and were naïve to previous biologic treatments. Patients who were in clinical remission were asked to switch from RI to BI using the same therapeutic dose after shared-decision making. The allocation of the patients was done randomly using an internet-based allocation program in order to minimize any selection bias (Random.org). Patients switched to BI were compared with a matched control group receiving continuous RI. The switching period was from January 2017 until June 2017 and patients were followed-up until December 2018. During follow-up, the demographic, clinical, and laboratory parameters as well as comorbidities were all recorded for at least 18 months. In addition, all adverse events as well as serious adverse events according to the Food and Drug Administration (FDA.gov) were also recorded. Disease activity was measured using the Bath Ankylosing Spondylitis Activity Index (BASDAI) [7] and the Ankylosing Spondylitis Activity Score (ASDAS) [8,9] using the C-reactive protein (CRP). Clinical remission was defined if patients had BASDAI < 4 and ASDAS < 1.3. Statistical analysis was performed using SPSS statistics version 20.0 (IBM Corporation, Armonk, NY, USA) We used the paired samples t-test for variables with normal distribution and Wilcoxon signed ranks test for variables which were not normally distributed. A *p*-value < 0.05 was considered statistically significant. Written informed consent was obtained from all patients, and the study has been approved by the clinical Research Ethic Committee of the University Hospital of Ioannina, according to the principles in the Declaration of Helsinki (197/2-12-2016).

3. Results

Twenty-one patients were excluded: 9 because they were not in clinical remission and 12 refused to switch from RI to its biosimilar. Thus, the final results comprise 88 patients. From these patients, 45 switched to BI, while 43 continued receiving RI (Figure 1). The demographic and clinical characteristics of our patients are depicted in Table 1. There were no differences between groups regarding the demographic, clinical and laboratory parameters. All patients were in clinical remission with low BASDAI and low ASDAS for approximately 3 years. During the follow-up period, 5 patients from the switched group and 3 from the continuing group discontinued the study (Figure 1). Four patients receiving BI presented nocebo effects after the second infusion while one had recurrent urinary tract infections. The patients with nocebo effects experienced nonspecific, subjective complaints such as headache, somnolence, dizziness, arthralgias, fatigue and pain. The clinical examination of these patients was unremarkable, and the acute phase reactants were within normal limits. These patients were switched to RI. Three responded well, while the fourth did not, and was changed to interleukin-17 (IL-17) inhibitor with good results. On the other hand, from the patients who continued receiving the RI, two patients presented recurrent upper respiratory tract infections while one had a disease flare-up. These patients were treated with an IL-17 inhibitor and responded very well. After 18 months of follow-up, all patients in both groups remained in clinical remission with low BASDAI, low ASDAS as well as low erythrocyte sedimentation rate (ESR) and CRP (Table 2). No significant adverse events, serious adverse events or any comorbidities were noted between the studied groups (Table 3).

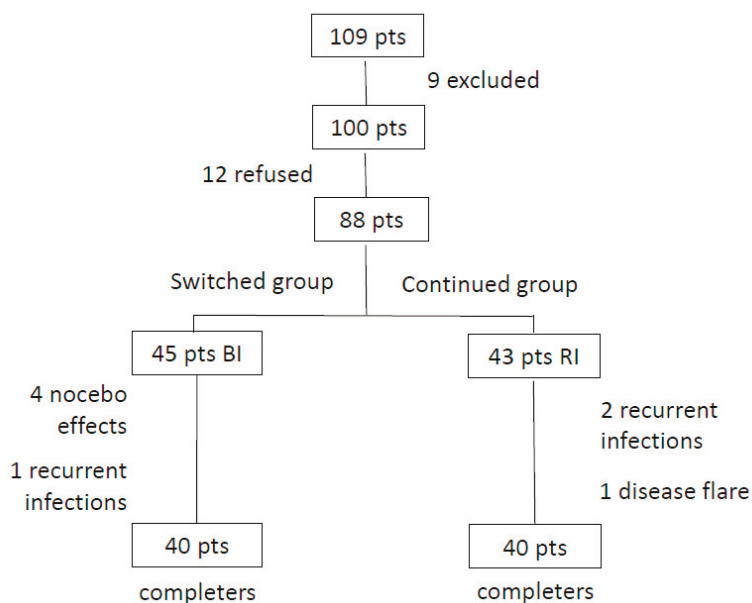


Figure 1. Flow chart of AS patients treated with infliximab.

Table 1. Demographic and clinical characteristic of Ankylosing Spondylitis (AS) patients at switching.

| Parameters | Switched Group (BI) n:45 | Continued Group (RI) n:43 | p-Value |
|--|-----------------------------|------------------------------|---------|
| Mean age (years) (SD) | 36.1 (4.6) | 35.7 (4.3) | NS |
| Male/female | 39/6 | 40 (3) | NS |
| Mean disease duration (years) (SD) | 7.8 (3.0) | 7.6 (2.8) | NS |
| Mean follow-up (years) (SD) | 7.0 (1.1) | 6.9 (0.9) | NS |
| BMI (kg/m ²) >25 | 5 (11.1) | 4 (9.3) | NS |
| Current smokers n (%) | 10 (22.2) | 8 (18.6) | NS |
| Ex-smokers n (%) | 9 (20) | 9 (20.9) | NS |
| Mean treatment with BI/RI | 6.4 (0.9) | 6.5 (0.8) | NS |
| Mean treatment with RI and clinical remission (years) (SD) | | 3.6 (0.8) | NS |
| Axial disease n (%) | 45 (100) | 43 (100) | NS |
| Peripheral disease n (%) | 4 (9) | 3 (7) | NS |
| Methotrexate intake n (%) | 3 (7) | 2 (5) | NS |
| Mean BASDAI (SD) | 3.7 (0.2) | 3.6 (0.4) | NS |
| Mean ASDAS (SD) | 1.0 (0.2) | 1.1 (0.2) | NS |
| Mean ESR mm/h (SD) | 18.5 (2.2) | 19.3 (1.7) | NS |
| Mean CRP mg/L (SD) | 6.0 (0.8) | 5.8 (0.6) | NS |

BI, biosimilar infliximab; RI, reference infliximab; SD, standard deviation; BMI, body mass index; BASDAI, bath ankylosing spondylitis activity index; ASDAS, ankylosing spondylitis disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NS, non-statistical.

Table 2. Response to treatment in AS patients switched to BI versus those continuing RI.

| Parameters | Switched Group (BI) | Continued Group (RI) | p-Value |
|-------------------------|---------------------|----------------------|---------|
| At switching | | | |
| BASDAI (SD) | 3.7 (0.2) | 3.6 (0.4) | NS |
| ASDAS (SD) | 1.0 (0.2) | 1.1 (0.2) | NS |
| ESR mm/h (SD) | 18.5 (2.2) | 19.3 (1.7) | NS |
| CRP mg/l (SD) | 6.0 (0.8) | 5.8 (0.6) | NS |
| End of the study | | | |
| BASDAI (SD) | 3.7 (0.4) | 3.8 (0.2) | N5 |
| ASDAS (SD) | 1.0 (0.2) | 1.1 (0.1) | NS |
| ESR mm/h (SD) | 19.5 (1.5) | 20.0 (1.6) | NS |
| CRP mg/l (SD) | 6.0 (1.0) | 6.1 (1.1) | NS |

BI, biosimilar infliximab; RI, reference infliximab; SD, standard deviation; BASDAI, bath ankylosing spondylitis activity index; ASDAS, ankylosing spondylitis disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NS, non-statistical.

Table 3. Adverse events during follow-up in AS patients switched to BI versus those continuing RI.

| Adverse Events * n (%) | Switched Group (BI) | Continued Group (RI) | p-Value |
|------------------------------------|---------------------|----------------------|---------|
| Upper respiratory tract infections | 3 (6.6) | 2 (4.6) | NS |
| Urinary tract infections | 2 (4.4) | 2 (4.6) | NS |
| Skin infections | 2 (2.2) | 1 (2.3) | NS |
| Increased liver enzymes | 2 (4.4) | 2 (4.6) | NS |
| Diarrhea | 1 (2.2) | 2 (4.6) | NS |
| Viral infections | 2 (4.4) | 1 (2.3) | NS |
| Headache | 1 (2.2) | 0 (0) | NS |
| Hypertension | 1 (2.2) | 1 (2.3) | NS |

*, not requiring discontinuation; BI, Biosimilar Infliximab; RI, Reference Infliximab; n, number of patients; NS, Non-statistical.

4. Discussion

Biosimilars represent an important new generation of drugs in a rheumatologist’s armamentarium [10]. Biosimilars have been approved by the European Medical Association (EMA) for rheumatologic indications and those for which the biological originator is no longer patent-protected. CTP-13, under the commercial name Inflectra/Remsima, was the first biosimilar approved by the EMA in 2013 [1]. Approval of BI was based on findings from two pivotal trials in AS [2] and RA [3]. Data from open-label extension studies of the original trials for AS have been reported [5]. Current data supports the proposal that it was possible to switch from RI to BI without any detrimental effects on safety and efficacy [5]. In addition, all available data regarding switching from RI to its biosimilar are reassuring. Switching is also recommended in the European League Against Rheumatism (EULAR) guidelines [11]. Indeed, a 52-week double-blind trial supports the efficacy and safety of the switch from RI to its biosimilar in patients with stable disease [12]. However, long-term follow-up data are required to confirm the efficacy and safety of the switch. The present study tries to cover this gap.

In our study, 88 AS patients receiving RI who were in clinical remission were asked to switch to BI. Half of them received BI, while the rest continued receiving RI. After 18 months of follow-up, no differences of clinical efficacy and safety were found between groups. Both groups remained in clinical remission. Our findings are in line with the PLANETAS study despite the fact that they used different tools in assessing disease activity [2]. Five patients from the BI group and three from the RI group discontinued the treatment. In the switched group, four patients discontinued the treatment due to nocebo effects. Nocebo effects are complex and individualized clinical phenomena that can induce new worsening pain, nonspecific subjective complaints such as malaise, fatigue, headache, weakness and others which are mainly induced by the patients’ negative expectations [13]. Thus, physicians should be aware of the potential appearance of nocebo effects which may hinder the transition to biosimilars in

some patients [14]. Our patients responded very well to switching from RI to its biosimilar. The reason for this could be the clinical state of the patients that are in clinical remission. Our results are in line with those of other investigators who reported a high retention rate of switching to biosimilars if the patients are stable [5,12]. Another reason could be that the switching was after discussion and decision-making with the patients. Evidence-based recommendations are available for several conditions in order to guide physicians in the switching process with biologics. Data suggests that shared-decision making leads to a better therapeutic response with fewer nocebo effects in contrast to non-medical switching [15,16]. The limitation of our study is that we included a small number of patients. On the other hand, the strength of our study is that it is the longest comparative study regarding switching from the RI to BI in AS.

When biosimilars appeared in the market, they not only had a lower price but also led to the price erosion of the reference products. In our study, there were no differences between the studied groups, and despite the fact that we did not make a cost-effectiveness analysis, we assume that the cost of the BI per patient is lower than that of the RI. Our study offers the promise of substantial savings relative to the RI product, enabling more AS patients to access biological therapy and reducing the cost associated with expensive biological treatment [17].

5. Conclusions

This is the first study in which AS patients in clinical remission receiving RI who were switched to BI remained in clinical remission for at least 18 months. We demonstrated that BI is equivalent to RI in maintaining AS patients in clinical remission.

Author Contributions: E.K., acquisition, analysis and interpretation of the data and manuscript drafting; E.P., acquisition and analysis of the data, editing and manuscript review; P.V.V. acquisition and analysis of the data, editing and manuscript review; and A.A.D. conception and study design and manuscript review. All authors have approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dörner, T.; Kay, J. Biosimilars in rheumatology: Current perspectives and lessons learnt. *Nat. Rev. Rheumatol.* **2015**, *11*, 713–724. [[CrossRef](#)] [[PubMed](#)]
2. Park, W.; Yoo, D.H.; Miranda, P.; Brzosko, M.; Wiland, P.; Gutierrez-Ureña, S.; Mikazane, H.; Lee, Y.A.; Smiyan, S.; Lim, M.J.; et al. Efficacy and safety of switching from reference infliximab to CT-P13 compared with maintenance of CT-P13 in ankylosing spondylitis: 102-week data from the PLANETAS extension study. *Ann. Rheum. Dis.* **2017**, *76*, 346–354. [[CrossRef](#)] [[PubMed](#)]
3. Yoo, D.H.; Prodanovic, N.; Jaworski, J.; Miranda, P.; Ramiterre, E.; Lanzon, A.; Baranauskaite, A.; Wiland, P.; Abud-Mendoza, C.; Oparanov, B.; et al. Efficacy and safety of CT-P13 (biosimilar infliximab) in patients with rheumatoid arthritis: Comparison between switching from reference infliximab to CT-P13 and continuing CT-P13 in the PLANETRA extension study. *Ann. Rheum. Dis.* **2017**, *76*, 355–363. [[CrossRef](#)] [[PubMed](#)]
4. Tanaka, Y.; Yamanaka, H.; Takeuchi, T.; Inoue, M.; Saito, K.; Saeki, Y.; Lee, S.J.; Nambu, Y. Safety and efficacy of CT-P13 in Japanese patients with rheumatoid arthritis in an extension phase or after switching from infliximab. *Mod. Rheumatol.* **2017**, *27*, 237–245. [[CrossRef](#)] [[PubMed](#)]
5. Scherlinger, M.; Germain, V.; Labadie, C.; Barnette, T.; Truchetet, M.E.; Bannwarth, B.; Mehsen-Cetre, N.; Richez, C.; Schaefferbeke, T.; FHU ACRONIM. Switching from originator infliximab to biosimilar CT-P13 in real-life: The weight of patient acceptance. *Joint Bone Spine.* **2018**, *85*, 561–567. [[CrossRef](#)] [[PubMed](#)]
6. Jones, A.; Ciurtin, C.; Ismajli, M.; Leandro, M.; Sengupta, R.; Machado, P.M. Biologics for treating axial spondyloarthritis. *Expert Opin. Biol. Ther.* **2018**, *18*, 641–652. [[CrossRef](#)] [[PubMed](#)]
7. Calin, A.; Garrett, S.; Whitelock, H.; Kennedy, L.G.; O’Hea, J.; Mallorie, P.; Jenkinson, T. A new approach to defining functional ability in ankylosing spondylitis: The development of the Bath Ankylosing Spondylitis Functional Index. *J. Rheumatol.* **1994**, *21*, 2281–2285. [[PubMed](#)]

8. Lukas, C.; Landewé, R.; Sieper, J.; Dougados, M.; Davis, J.; Braun, J.; van der Linden, S.; van der Heijde, D. Assessment of SpondyloArthritis international Society. Development of an ASAS-endorsed disease activity score (ASDAS) in patients with ankylosing spondylitis. *Ann. Rheum. Dis.* **2009**, *68*, 18–24. [[CrossRef](#)] [[PubMed](#)]
9. Machado, P.; Landewé, R.; Lie, E.; Kvien, T.K.; Braun, J.; Baker, D.; van der Heijde, D.; Assessment of SpondyloArthritis international Society. Ankylosing Spondylitis Disease Activity Score (ASDAS): Defining cut-off values for disease activity states and improvement scores. *Ann. Rheum. Dis.* **2011**, *70*, 47–53. [[CrossRef](#)] [[PubMed](#)]
10. Blair, H.A.; Deeks, E.D. Infliximab Biosimilar (CT-P13; Infliximab-dyyb): A Review in Autoimmune Inflammatory Diseases. *Bio Drugs.* **2016**, *30*, 469–480. [[CrossRef](#)] [[PubMed](#)]
11. Kay, J.; Schoels, M.M.; Dörner, T.; Emery, P.; Kvien, T.K.; Smolen, J.S.; Breedveld, F.C. Task Force on the Use of Biosimilars to Treat Rheumatological Diseases. Consensus-based recommendations for the use of biosimilars to treat rheumatological diseases. *Ann. Rheum. Dis.* **2018**, *77*, 165–174. [[CrossRef](#)] [[PubMed](#)]
12. Jørgensen, K.K.; Olsen, I.C.; Goll, G.L.; Lorentzen, M.; Bolstad, N.; Haavardsholm, E.A.; Lundin, K.E.A.; Mørk, C.; Jahnsen, J.; Kvien, T.K.; et al. Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): A 52-week, randomised, double-blind, non-inferiority trial. *Lancet* **2017**, *389*, 2304–2316.
13. Kravvariti, E.; Kitas, G.D.; Mitsikostas, D.D.; Sfakakis, P.P. Nocebos in rheumatology: Emerging concepts and their implications for clinical practice. *Nat. Rev. Rheumatol.* **2018**, *14*, 727–740. [[CrossRef](#)] [[PubMed](#)]
14. Tweehuysen, L.; van den Bemt, B.J.F.; van Ingen, I.L.; de Jong, A.J.L.; van der Laan, W.H.; van den Hoogen, F.H.J.; den Broeder, A.A. Subjective Complaints as the Main Reason for Biosimilar Discontinuation After Open-Label Transition From Reference Infliximab to Biosimilar Infliximab. *Arthritis Rheumatol.* **2018**, *70*, 60–68. [[CrossRef](#)] [[PubMed](#)]
15. Scherlinger, M.; Langlois, E.; Germain, V.; Schaefferbeke, T. Acceptance rate and sociological factors involved in the switch from originator to biosimilar etanercept (SB4). *Semin. Arthritis Rheum.* **2019**, *48*, 927–932. [[CrossRef](#)] [[PubMed](#)]
16. Tweehuysen, L.; Huiskes, V.J.B.; van den Bemt, B.J.F.; Vrieseckolk, J.E.; Teerenstra, S.; van den Hoogen, F.H.J.; van den Ende, C.H.; den Broeder, A.A. Open-Label, Non-Mandatory Transitioning From Originator Etanercept to Biosimilar SB4: Six-Month Results From a Controlled Cohort Study. *Arthritis Rheumatol.* **2018**, *70*, 1408–1418. [[CrossRef](#)] [[PubMed](#)]
17. Lyman, G.H.; Zon, R.; Harvey, R.D.; Schilsky, R.L. Rationale, Opportunities, and Reality of Biosimilar Medications. *N. Engl. J. Med.* **2018**, *378*, 2036–2044. [[CrossRef](#)] [[PubMed](#)]



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Article

Achieving Pain Control in Rheumatoid Arthritis with Baricitinib or Adalimumab Plus Methotrexate: Results from the RA-BEAM Trial

Peter C. Taylor ^{1,*}, Yvonne C. Lee ², Roy Fleischmann ³, Tsutomu Takeuchi ⁴, Elizabeth L. Perkins ⁵, Bruno Fautrel ⁶, Baojin Zhu ⁷, Amanda K. Quebe ⁷, Carol L. Gaich ⁷, Xiang Zhang ⁷, Christina L. Dickson ⁷, Douglas E. Schlichting ⁷, Himanshu Patel ⁷, Frederick Durand ⁷ and Paul Emery ⁸

¹ Botnar Research Centre, University of Oxford, Oxford OX3 7LD, UK

² Division of Rheumatology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA; yvonne.lee@northwestern.edu

³ Metroplex Clinical Research Center, University of Texas Southwestern Medical Center, Dallas, TX 75231, USA; RFleischmann@arthdocs.com

⁴ Division of Rheumatology, Department of Internal Medicine, Keio University, Tokyo 162-5882, Japan; tsutake@z5.keio.jp

⁵ Rheumatology Care Center, Birmingham, AL 35244, USA; eperkins@rheumatologycarecenter.com

⁶ AP-HP, Pitié-Salpêtrière Hospital, Dept of Rheumatology, Sorbonne Université, Pierre Louis Institute for Epidemiology and Public Health, 705013 Paris, France; bruno.fautrel@aphp.fr

⁷ Eli Lilly and Company, Indianapolis, IN 46285, USA; zhu_baojin@lilly.com (B.Z.); amanda.quebe@lilly.com (A.K.Q.); gaich_carol_lynn@lilly.com (C.L.G.); zhang_xiang@lilly.com (X.Z.); dickson_christina_l@lilly.com (C.L.D.); schlichting_douglas_e@lilly.com (D.E.S.); himanshu.patel@lilly.com (H.P.); durand_frederick@lilly.com (F.D.)

⁸ Leeds Institute of Rheumatic and Musculoskeletal Medicine University of Leeds, Leeds LS1 3EX, UK; p.emery@leeds.ac.uk

* Correspondence: peter.taylor@kennedy.ox.ac.uk; Tel.: +44-0186-227323

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Abstract: The purpose of the study was to assess the proportion of patients who achieve pain relief thresholds, the time needed to reach the thresholds, and the relationship between pain and inflammation among patients with rheumatoid arthritis (RA) and an inadequate response to methotrexate in RA-BEAM (NCT0170358). A randomized, double-blind trial was conducted, comparing baricitinib ($N = 487$), adalimumab ($N = 330$), and placebo ($N = 488$) plus methotrexate. Pain was evaluated by patient's assessment on a 0–100 mm visual analog scale (VAS). The following were assessed through a 24-week placebo-controlled period: the proportion of patients who achieved $\geq 30\%$, $\geq 50\%$, and $\geq 70\%$ pain relief, the time to achieve these pain relief thresholds, remaining pain (VAS ≤ 10 mm, ≤ 20 mm, or ≤ 40 mm), and the relationship between inflammation markers and pain relief. Baricitinib-treated patients were more likely ($p < 0.05$) to achieve $\geq 30\%$, $\geq 50\%$, and $\geq 70\%$ pain relief than placebo- and adalimumab-treated patients, as early as Week 1 vs. placebo and at Week 4 vs. adalimumab. A greater proportion of baricitinib-treated patients achieved ≤ 20 mm or ≤ 40 mm remaining pain vs. placebo- and adalimumab-treated patients. Baricitinib-treated patients tended to demonstrate consistent pain relief independent of levels of inflammation control. In RA patients with an inadequate response to methotrexate, baricitinib provided greater and more rapid pain relief than adalimumab and placebo. Analyses suggest the relationship between inflammation and pain may be different for baricitinib and adalimumab treatments.

Keywords: baricitinib; disease-modifying antirheumatic drugs; pain perception; outcomes research; patient perspective; rheumatoid arthritis

1. Introduction

Rapid, sustained pain control is a foremost goal for many patients with rheumatoid arthritis (RA); notably, in a survey approximately two-thirds of patients responded that pain was their treatment priority [1]. Many patients with RA who have achieved control of inflammation associated with good clinical response with RA therapy continue to report pain, including at levels described as moderate to severe [1–4]. This remaining pain may be a result of the multifactorial nature of pain associated with RA, which is not solely a result of inflammation; rather it may also be associated with structural damage, peripheral sensitization, or central amplification [2,5,6]. For other patients, despite a treat-to-target approach, the desired goal of remission cannot be attained. For these patients, ongoing pain is often a predominant symptom.

In RA-BEAM, a Phase 3 clinical trial of baricitinib, an oral, selective inhibitor of Janus kinase (JAK)1 and JAK2, baricitinib plus methotrexate (MTX) was associated with significant clinical improvements compared to patients treated with adalimumab plus MTX or placebo plus MTX. Baricitinib- and adalimumab-treated patients demonstrated similar improvement in swollen joint count (SJC), with both groups demonstrating significantly greater improvement relative to the placebo group beginning at Week 1 that was maintained through the placebo-controlled period (Week 24). For patient-reported pain, however, baricitinib-treated patients reported significantly greater improvements as early as Week 1 compared to placebo-treated patients, and as early as Week 2 when compared with adalimumab-treated patients. These statistical differences in pain relief between the active treatment arms were maintained through the duration of RA-BEAM (Week 52) [7]. This observation prompted us to explore differences in pain relief with baricitinib- and adalimumab-treated patients in RA-BEAM.

Publications of clinical trials in RA traditionally evaluate pain improvement only as central tendencies (i.e., mean change from baseline). To our knowledge, no prior reports have more fully characterized treatment effects or explored the relationship between the control of pain and inflammation with treatment. The objectives of this analysis were two-fold: first, to use the RA-BEAM data to assess the proportion of patients who achieve pain relief thresholds and the time needed to achieve these thresholds, and second, to investigate the relationship between inflammation and patient-reported pain.

2. Patients and Methods

2.1. Trial Design

The design and procedure of RA-BEAM have been described previously [7,8]. Briefly, RA-BEAM was a randomized, double-blind, double-dummy, placebo-controlled and active-controlled, parallel-arm, 52-week study conducted at 281 centers in 26 countries between 2012 and 2015 (ClinicalTrials.gov: NCT01710358). A total of 1305 patients on stable background MTX were randomly allocated (3:2:3) to placebo, adalimumab 40 mg, or baricitinib 4 mg. At Week 16, those patients considered non-responders received open-label rescue treatment with baricitinib 4 mg. After Week 16, patients may have received rescue treatment at investigator discretion. At Week 24, placebo-treated patients were switched to baricitinib. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was approved by each center's institutional review board or ethics committee. All patients provided written informed consent.

2.2. Patients

Patients were ≥ 18 years old with active RA ($\geq 6/68$ tender and $\geq 6/66$ swollen joints; serum high-sensitivity C-reactive protein (CRP) ≥ 6 mg/L). Patients had an inadequate response to MTX and either ≥ 3 joint erosions (based on radiographs), or ≥ 1 joint erosion with seropositivity for rheumatoid factor or anti-citrullinated peptide antibodies [7].

2.3. Pain Measures

Pain was measured with the patient's assessment of pain visual analog scale (VAS), consisting of one question, "How much pain are you currently having because of your rheumatoid arthritis?" Responses range from 0 mm (no pain) to 100 mm (worst possible pain). The pain VAS was administered at every study visit.

Pain Thresholds

Our initial observation of differential pain response between baricitinib and adalimumab was based on mean change from baseline. We wanted to understand if these differences persisted when pain relief was evaluated against various thresholds of success, as is typical for other patient-reported outcomes in RA. Because there are no established, standard pain thresholds in RA, we reviewed the literature and selected two approaches. First, we applied percent change from baseline threshold recommendations from the general chronic pain literature, specifically those from the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT), a multidisciplinary organization with the mission to develop consensus reviews and recommendations and to improve clinical trials of treatments for pain [9,10]. A 30% improvement threshold is described as "much improved, meaningful differences" and 50% represents "very much improved, substantial improvement" in chronic pain conditions. A 70% improvement threshold, although not defined in IMMPACT, was also evaluated because it is analogous to American College of Rheumatology response endpoints and was observed with patients in RA-BEAM. Second, while relative improvement is important, so is absolute pain; thus we evaluated thresholds of remaining pain (i.e., the absolute value of patient-reported pain) of ≤ 10 mm, ≤ 20 mm, or ≤ 40 mm, at Week 24. The ≤ 10 mm threshold reflects limited pain to no pain and is extrapolated from data by Wells et al. [11]. The ≤ 20 mm threshold represents a threshold when satisfaction with health is not negatively affected by pain [11,12]. The ≤ 40 mm threshold was derived from observed cut-off points between the pain VAS and the Patient Acceptable Symptom State (PASS) [13].

2.4. Outcomes

The proportion of patients achieving $\geq 30\%$, $\geq 50\%$, or $\geq 70\%$ improvement from baseline by Week 24 was assessed, as was the median time when 50% of patients achieved these thresholds of pain relief. The proportion of patients achieving remaining pain VAS values of ≤ 10 mm, ≤ 20 mm, or ≤ 40 mm was assessed at Week 24. To evaluate if the differences in pain response were associated with inflammation, we assessed the relationship between levels of inflammation and pain relief at Week 24.

2.5. Statistical Analyses

All analyses were conducted with an intention-to-treat approach in which data from patients who received ≥ 1 dose of study drug were assessed, regardless of whether they completed the trial. Missing values were imputed with modified last observation carried forward for all analyses where applicable. Analyses were not adjusted for multiplicity.

Comparisons were made on the percent change in pain VAS from baseline to Week 24 using analysis of covariance (ANCOVA) and on the proportion of patients achieving pain relief at Week 24 between treatment arms using logistic models, adjusted for randomization factors (region, baseline joint erosion status (1–2 erosions plus seropositivity vs. ≥ 3 erosions)) and baseline pain VAS score. The median time needed for patients to achieve these pain relief thresholds were assessed through Week 24 using the cumulative incidence estimate with 'competing risks' which included rescue or discontinuation due to lack of efficacy before reaching the pain relief threshold. The Cox proportional hazards model with 'competing risks' (proportional sub-distribution hazards model) [14,15] was used to obtain the hazard ratio.

Remaining pain was analyzed across treatment groups with logistic regression models. Pain relief at Week 24 by CRP was evaluated using ANCOVA.

A mediation analysis with multiple mediators was conducted to evaluate the relationship between levels of inflammation and pain relief. The effects of change in inflammatory factors (CRP, erythrocyte sedimentation rate (ESR), SJC) as multiple mediators on change in pain outcome for each treatment over placebo during the 24-week period were evaluated in this analysis [16]. The total treatment effect on pain relief over placebo that can be accounted for by changes in CRP, ESR, and SJC in the mediation analysis is the 'indirect' or mediation effect, while the total treatment effect that cannot be accounted for by the 'indirect' effect is called the 'direct' effect. Observed data were used for the mediation analysis.

Statistical analyses were performed in SAS (SAS Institute; Cary, NC, USA, version 9.4). A two-sided p value < 0.05 was considered statistically significant.

3. Results

3.1. Pain Relief

As noted by Taylor et al. [7], patients had established and active RA. The mean baseline pain scores were well matched across treatment groups in this study and ranged from 60 to 62 mm with the median baseline pain of 62 mm [7]. Other baseline characteristics were well-balanced between the treatment arms [7]. A detailed description of the safety of baricitinib and adalimumab is available in the RA-BEAM publication [7]. In brief, adverse events were more frequent with baricitinib (71%) and adalimumab (68%) than with placebo (60%) through Week 24. Rates of serious adverse events through Week 24 were 5% with placebo, 5% with baricitinib, and 2% with adalimumab.

As early as Week 1, significantly greater improvement in pain relief was observed between baricitinib and placebo (25% for baricitinib vs. 4% for placebo, $p < 0.0001$). At Week 24, the mean percentage reduction in pain from baseline for baricitinib, adalimumab, and placebo, respectively, were 51%, 39%, 17% ($p = 0.001$ for baricitinib and adalimumab vs. placebo and $p = 0.030$ for baricitinib vs. adalimumab).

A greater proportion of patients treated with baricitinib or adalimumab achieved the $\geq 30\%$, $\geq 50\%$, or $\geq 70\%$ pain relief thresholds compared with placebo-treated patients at Week 1 (Figure 1). Compared with adalimumab-treated patients, a greater proportion ($p < 0.05$) of baricitinib-treated patients achieved $\geq 30\%$ and $\geq 50\%$ pain relief as early as Week 4 and $\geq 70\%$ pain relief at Week 8. Differences between baricitinib and adalimumab for $\geq 50\%$ and $\geq 70\%$ pain relief were maintained through Week 24 (Figure 1).

At Week 24, for the placebo-, adalimumab-, and baricitinib-treated patients, respectively, the proportion of patients who achieved $\geq 30\%$ pain relief were 49%, 69%, and 74%; for $\geq 50\%$ pain relief, the values were 32%, 52%, and 61%; and for $\geq 70\%$ pain relief, the values were 16%, 32%, and 41%.

The median time to achieve the $\geq 30\%$ pain relief threshold was 2 weeks for baricitinib- and adalimumab-treated patients and 5 weeks for those on placebo (Figure 2). For $\geq 50\%$ pain relief, the median time was 4 weeks for baricitinib, 8 weeks for adalimumab, and 14 weeks for placebo (Figure 2). For $\geq 70\%$ pain relief, the median time was 12 weeks for baricitinib, 20 weeks for adalimumab, and >24 weeks for placebo (Figure 2). Compared with placebo, baricitinib-treated patients were more likely to achieve $\geq 30\%$, $\geq 50\%$, or $\geq 70\%$ pain relief with Hazard Ratio (HR) values of 1.7, 1.9, and 2.5, respectively ($p \leq 0.001$). Compared with adalimumab, baricitinib-treated patients were more likely to achieve $\geq 50\%$ or $\geq 70\%$ pain relief; the HR values for the $\geq 30\%$, $\geq 50\%$, or $\geq 70\%$ pain relief thresholds, respectively, were 1.1 ($p = 0.145$), 1.2 ($p = 0.032$), and 1.3 ($p = 0.003$).

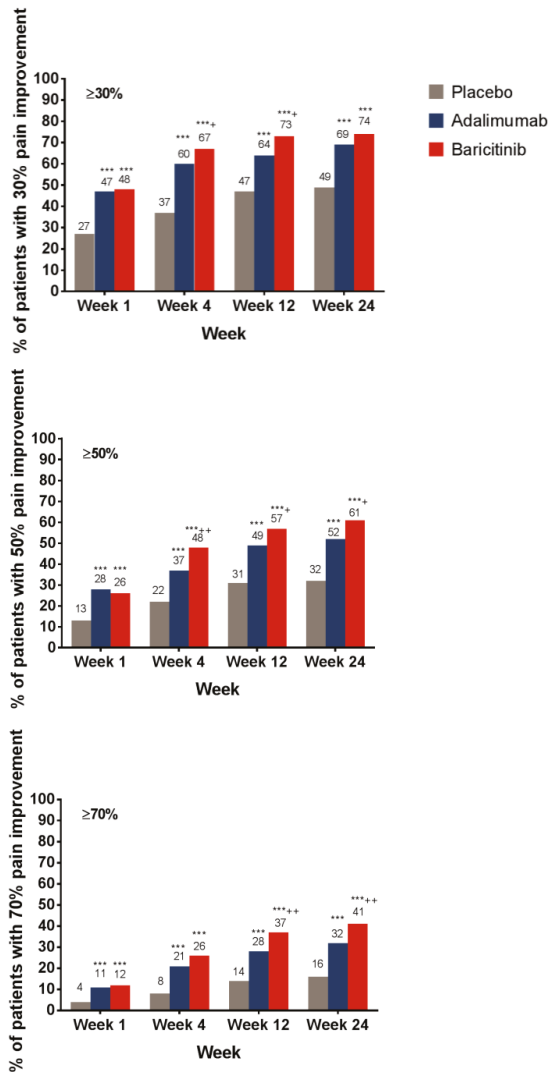
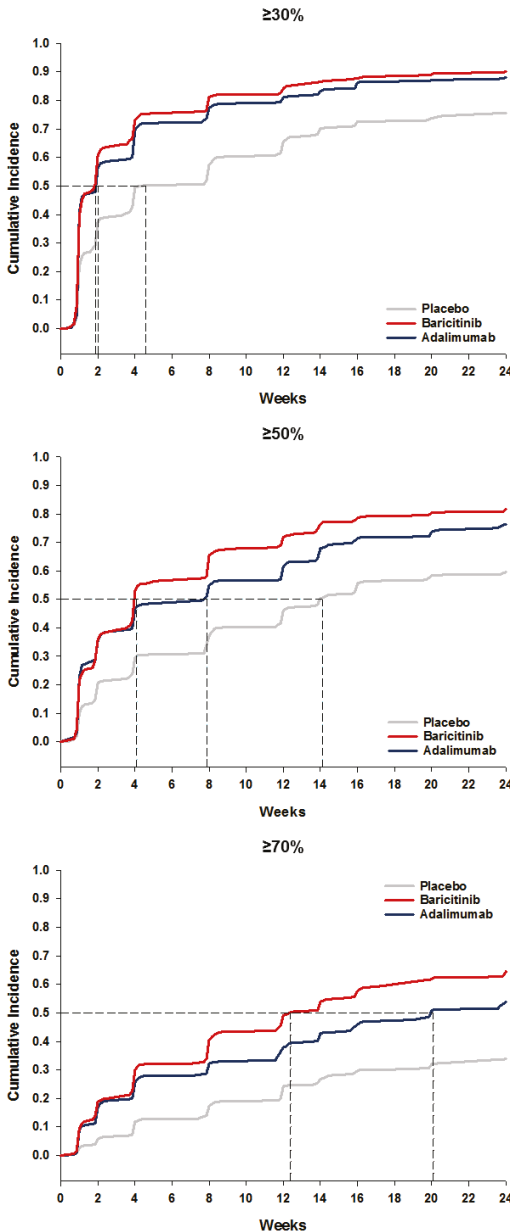


Figure 1. Percentage of patients who achieved pain relief thresholds from baseline, as measured by the pain VAS. *** $p \leq 0.001$ vs. placebo; † $p \leq 0.05$; †† $p \leq 0.01$, ††† $p \leq 0.001$ vs. adalimumab. Abbreviations: VAS = visual analog scale. Number of respondents who answered the pain question by week: placebo, $n = 481$ at Week 1 and $n = 483$ at all other weeks; adalimumab, $n = 325$ at Week 1 and $n = 327$ at all other weeks; baricitinib, $n = 482$ at all weeks.



The median time (weeks) for patients to attain pain VAS improvement $\geq 30\%$ was 1.9 weeks for baricitinib, 2.0 weeks for adalimumab, and 4.6 weeks for placebo. Compared with placebo, baricitinib-treated patients were more likely to achieve $\geq 30\%$ pain relief with Hazard Ratio (HR) of 1.7 ($p \leq 0.001$).

The median time (weeks) for patients to attain pain VAS improvement $\geq 50\%$ was 4.0 weeks for baricitinib, 7.9 weeks for adalimumab, and 14.0 weeks for placebo. Compared with placebo, baricitinib-treated patients were more likely to achieve $\geq 50\%$ pain relief with an HR of 1.9 ($p \leq 0.001$). Compared with adalimumab, baricitinib-treated patients were more likely to achieve $\geq 50\%$ pain relief with an HR of 1.2 ($p = 0.032$).

The median time (weeks) for patients to attain pain VAS improvement $\geq 70\%$ was 12.4 weeks for baricitinib, 20.0 weeks for adalimumab, and >24.0 weeks for placebo. Compared with placebo, baricitinib-treated patients were more likely to achieve $\geq 70\%$ pain relief with an HR of 2.5 ($p \leq 0.001$). Compared with adalimumab, baricitinib-treated patients were more likely to achieve $\geq 70\%$ pain relief; the HR value was 1.3 ($p = 0.003$).

Figure 2. Time course for patients attaining pain relief thresholds.

3.2. Remaining Pain

The differences in the proportion of patients who achieved the ≤ 40 mm, ≤ 20 mm, and ≤ 10 mm remaining pain thresholds were significantly greater for baricitinib and adalimumab compared with placebo as early as Week 1 (Table 1). Compared with adalimumab-treated patients, a greater proportion of baricitinib-treated patients achieved the ≤ 40 mm threshold ($p \leq 0.001$) at Week 4, and a difference

was observed between the active treatment groups at the ≤ 20 mm threshold by Week 12 ($p \leq 0.001$; Table 1). Differences were maintained through Week 24. The percentage of patients who achieved the ≤ 10 mm remaining pain threshold was greater for baricitinib compared to adalimumab, but the difference reached statistical significance only at Week 12.

Table 1. Percentage of patients who met the thresholds of remaining pain (VAS) over time by treatment groups. *** $p \leq 0.001$ vs. placebo; † $p \leq 0.05$; ††† $p \leq 0.001$ vs. adalimumab.

| Threshold of Remaining Pain at Each Time Point (Week) | Placebo n (%) | Adalimumab n (%) | Baricitinib n (%) |
|---|---------------|------------------|-------------------|
| ≤ 40 mm | | | |
| 1 | 150 (31) | 132 (41) *** | 208 (43) *** |
| 4 | 193 (40) | 169 (52) *** | 298 (62) ***,††† |
| 12 | 225 (46) | 202 (62) *** | 335 (69) ***,† |
| 24 | 236 (49) | 218 (66) *** | 351 (73) ***,† |
| ≤ 20 mm | | | |
| 1 | 51 (11) | 64 (20) *** | 90 (19) *** |
| 4 | 80 (17) | 93 (28) *** | 158 (33) *** |
| 12 | 103 (21) | 120 (37) *** | 209 (43) ***,† |
| 24 | 105 (22) | 121 (37) *** | 239 (49) ***,††† |
| ≤ 10 mm | | | |
| 1 | 20 (4) | 32 (10) *** | 40 (8) *** |
| 4 | 29 (6) | 49 (15) *** | 88 (18) *** |
| 12 | 52 (11) | 63 (19) *** | 124 (26) ***,† |
| 24 | 56 (12) | 86 (26) *** | 144 (30) *** |

3.3. Relationship between Inflammation and Pain Relief

At Week 24, among patients with varying levels of inflammation, as measured by CRP as an objective marker of inflammation, baricitinib-treated patients tended to demonstrate consistent pain relief regardless of the CRP levels. In contrast, patients treated with adalimumab and placebo demonstrated less pain relief at higher CRP levels (Figure 3).

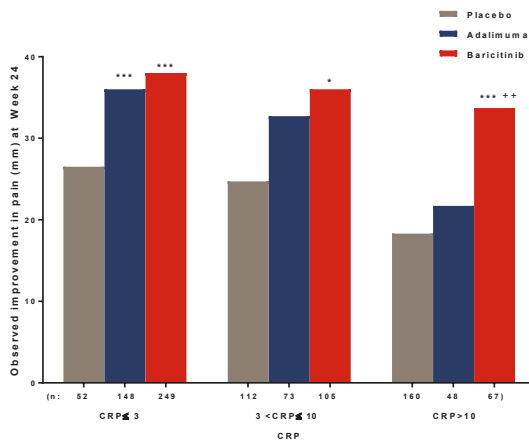


Figure 3. Pain improvement by remaining inflammation (CRP, mg/L) at Week 24. * $p \leq 0.05$, *** $p \leq 0.001$ vs. placebo; †† $p \leq 0.01$ vs. adalimumab. Abbreviations: CRP = C-reactive protein.

While the total effect of baricitinib on pain relief over placebo at Week 24 was greater than that for adalimumab, changes in inflammation accounted for approximately 40% of the pain improvement with baricitinib and 50% of pain improvement with adalimumab (Figure 4; Table S1). In this analysis, the direct effects (i.e., those not associated with these markers of inflammation) of drug on pain relief were higher for baricitinib than for adalimumab after accounting for indirect inflammatory effects (Figure 4).

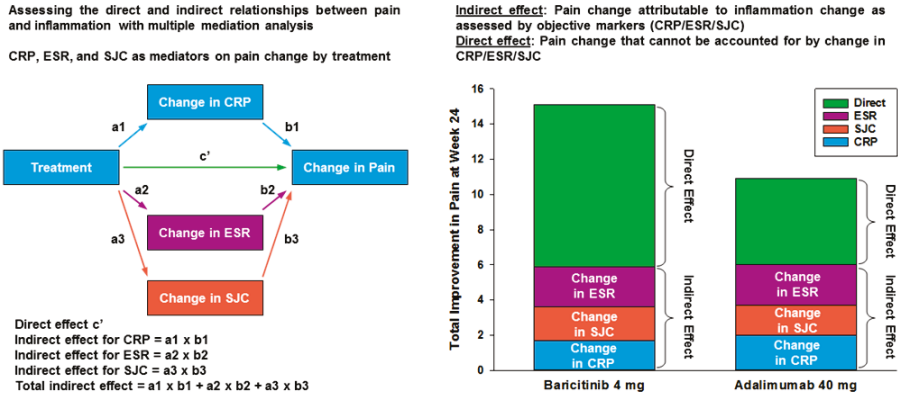


Figure 4. Relative contribution of inflammatory control to pain control.

4. Discussion

In the RA-BEAM trial, patient-reported improvements in disease activity, physical function, and pain were greater for baricitinib plus MTX than for adalimumab plus MTX within 4 weeks of starting treatment and were maintained throughout the 52-week observation period [7]. In this analysis, we further explored pain relief experienced by patients. Baricitinib demonstrated greater and more rapid achievement of clinically significant levels of pain relief than adalimumab or placebo through Week 24. Furthermore, this differential effect became more marked as the pain relief thresholds increased, with approximately 40% of the patients receiving baricitinib achieving $\geq 70\%$ pain relief from baseline by Week 24.

Another striking feature of this analysis was the rapid onset of effective mean pain relief at a cohort level with baricitinib plus MTX. Here, we show that for those patients achieving $\geq 50\%$ or $\geq 70\%$ pain relief, baricitinib had a shorter median time to achieving these pain relief thresholds than placebo or adalimumab. Specifically, for $\geq 50\%$ pain relief, the 4 weeks needed for baricitinib was approximately half that of adalimumab treated patients (8 weeks). For patients achieving $\geq 30\%$ pain relief, baricitinib and adalimumab had similar median time to onset (approximately 2 weeks).

Remaining pain is commonly reported by patients with RA despite achieving satisfactory disease control by adopting the treat-to-target approach in disease management. A threshold of ≤ 20 mm remaining pain is considered to represent a point where health satisfaction is not adversely affected [11,12]. In our study, we found that patients treated with either baricitinib plus MTX or adalimumab plus MTX were significantly more likely to achieve ≤ 10 mm, ≤ 20 mm, and ≤ 40 mm thresholds for remaining pain compared to placebo plus MTX. Baricitinib separated from adalimumab by Week 4 for the ≤ 40 mm and by Week 8 for the ≤ 20 mm threshold.

To explore the relative contribution of anti-inflammatory and other mechanisms of pain relief obtained with either baricitinib or adalimumab, we explored relationships between changes in patient-reported pain and an objective marker of inflammation, namely CRP. This analysis suggests that the difference in pain relief between baricitinib and adalimumab cannot be solely accounted for by differential effects on inflammation.

While it is clear from multiple clinical studies that baricitinib has a profound anti-inflammatory effect, as would be expected of a multi-cytokine inhibitor, these observations imply that JAK1 and JAK2 inhibition also has anti-nociceptive effects that are independent of at least certain aspects of the inflammatory process [17]. A rodent model indicated that treatment with baricitinib attenuates complete Freund's adjuvant-induced joint deficits, a surrogate measure of joint pain [18]. At present, the mechanisms by which baricitinib modulates the pain experience independently of at least some generic features of inflammation is unknown. One possible mechanism could involve granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF is a cytokine that signals through JAK2 homodimers. In a rodent collagenase-induced instability model of osteoarthritis, pain was shown to be GM-CSF dependent, and therapeutic neutralization of GM-CSF rapidly and completely abolished arthritis pain [19]. Another possible pathway is through phosphorylation of signal transducer and activator of transcription 3 (STAT3). In rodent models of neuropathic pain following spinal nerve ligation, STAT3 phosphorylation was induced centrally in the dorsal spinal cord with upregulation of interleukin-6 (IL-6) mRNA in the dorsal root ganglia and elevated IL-6 concentrations in the dorsal spinal cord. Intrathecal administration of a JAK2 inhibitor blocked this STAT3 phosphorylation pathway with accompanying attenuation of both mechanical allodynia and thermal hyperalgesia [20]. It is known that the JAK-STAT3 system is activated through IL-6 signaling in spinal microglia and that this transduction pathway participates in development of pain associated with nerve alteration. However, it is not known whether such mechanisms have relevance to pain in established RA.

In rodent arthritis models, autoantibodies to citrullinated proteins (ACPA) are reported to induce joint pain independent of inflammation via a chemokine-dependent mechanism [21]. However, this is unlikely to account for the differences in pain relief between TNF blockade and JAK inhibition in our study as we did not observe statistically significant differences in ACPA change from baseline between baricitinib and adalimumab at Week 24.

This analysis has limitations. Specifically, the present findings represent post hoc analyses in which patients were not randomized according to their baseline pain. Pain studies may be complicated by the subjective experience of pain and the inherent limitations of a VAS as an instrument to measure pain experience. Additionally, the pain relief thresholds and remaining pain values are not yet firmly established for RA [12,13]. Further, some concomitant medication use (e.g., glucocorticoids) are controlled within a clinical trial, precluding assessment of any potential relationship between pain improvement and medication changes.

Pain relief with treatment may have clinical and holistic implications. Patients who experience pain relief are likely to report clinically significant improvements in other patient-reported outcomes, such as the Patient's Global Assessment of Disease Activity and functional disability [22,23]. More broadly, reductions in pain have been associated with improvements in daily activity and work productivity [24]. In this analysis, we have presented evidence that baricitinib rapidly provides pain relief in patients with active RA on concomitant MTX to a magnitude greater than that observed with the TNF inhibitor, adalimumab. We have expanded prior research through a new and detailed analysis of the range of magnitude and kinetics of pain relief in a head-to-head study of baricitinib versus adalimumab and by exploring the relationship between the control of pain and inflammation with treatment. We observed that the inhibition of JAK1/JAK2 or TNF similarly ameliorate inflammatory markers, but the overall pain and the non-inflammatory component are faster and more markedly improved by baricitinib. Our findings merit further investigation into the biological mechanisms underlying pain relief.

The observations from this analysis may be of importance in managing the unmet needs of adequate pain relief in RA, whether in patients attaining the treatment targets of remission or low disease activity or in those who are unable to achieve these targets with biologic anti-TNF treatment.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/8/6/831/s1>, Table S1: Multiple mediator analysis coefficients for adalimumab vs. placebo and baricitinib vs. placebo at Week 24.

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References

1. Taylor, P.C.; Manger, B.; Alvaro-Gracia, J.; Johnstone, R.; Gomez-Reino, J.; Eberhardt, E.; Wolfe, F.; Schwartzman, S.; Furfaro, N.; Kavanaugh, A. Patient perceptions concerning pain management in the treatment of rheumatoid arthritis. *J. Int. Med. Res.* **2010**, *38*, 1213–1224. [[CrossRef](#)] [[PubMed](#)]
2. Boyden, S.D.; Hossain, I.N.; Wohlfahrt, A.; Lee, Y.C. Non-inflammatory causes of pain in patients with rheumatoid arthritis. *Curr. Rheumatol. Rep.* **2016**, *18*, 30. [[CrossRef](#)] [[PubMed](#)]
3. Altawil, R.; Saevarsdottir, S.; Wedren, S.; Alfredsson, L.; Klareskog, L.; Lampa, J. Remaining Pain in Early Rheumatoid Arthritis Patients Treated with Methotrexate. *Arthritis Care Res.* **2016**, *68*, 1061–1068. [[CrossRef](#)] [[PubMed](#)]
4. Ishida, M.; Kuroiwa, Y.; Yoshida, E.; Sato, M.; Krupa, D.; Henry, N.; Ikeda, K.; Kaneko, Y. Residual symptoms and disease burden among patients with rheumatoid arthritis in remission or low disease activity: A systematic literature review. *Mod. Rheumatol.* **2018**, *28*, 789–799. [[CrossRef](#)] [[PubMed](#)]
5. Meeus, M.; Vervisch, S.; De Clerck, S.; Moorkens, G.; Hans, G.; Nijs, J. Central sensitization in patients with rheumatoid arthritis: A systematic literature review. *Semin. Arthritis Rheum.* **2012**, *41*, 556–567. [[CrossRef](#)]
6. McWilliams, D.F.; Walsh, D.A. Pain mechanisms in rheumatoid arthritis. *Clin. Exp. Rheumatol.* **2017**, *35* (Suppl. 107), 94–101.
7. Taylor, P.C.; Keystone, E.C.; van der Heijde, D.; Weinblatt, M.E.; del Carmen Morales, L.; Reyes Gonzaga, J.; Yakushin, S.; Ishii, T.; Emoto, K.; Beattie, S.; et al. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N. Engl. J. Med.* **2017**, *376*, 652–662. [[CrossRef](#)]
8. Keystone, E.C.; Taylor, P.C.; Tanaka, Y.; Gaich, C.; DeLozier, A.M.; Dudek, A.; Zamora, J.V.; Cobos, J.A.C.; Rooney, T.; de Bono, S.; et al. Patient-reported outcomes from a phase 3 study of baricitinib versus placebo or adalimumab in rheumatoid arthritis: Secondary analyses from the RA-BEAM study. *Ann. Rheum. Dis.* **2017**, *76*, 1853–1861. [[CrossRef](#)]
9. Dworkin, R.H.; Turk, D.C.; Wyrwich, K.W.; Beaton, D.; Cleeland, C.S.; Farrar, J.T.; Haythornthwaite, J.A.; Jensen, M.P.; Kerns, R.D.; Ader, D.N.; et al. Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: IMMPACT recommendations. *J. Pain* **2008**, *9*, 105–121. [[CrossRef](#)]
10. IMMPACT: Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials. Available online: <http://www.immpact.org/> (accessed on 24 April 2019).

11. Wells, G.A.; Boers, M.; Shea, B.; Brooks, P.M.; Simon, L.S.; Strand, C.V.; Aletaha, D.; Anderson, J.J.; Bombardier, C.; Dougados, M.; et al. Minimal disease activity for rheumatoid arthritis: A preliminary definition. *J. Rheumatol.* **2005**, *32*, 2016–2024.
12. Wolfe, F.; Michaud, K. Assessment of pain in rheumatoid arthritis: Minimal clinically significant difference, predictors, and the effect of anti-tumor necrosis factor therapy. *J. Rheumatol.* **2007**, *34*, 1674–1683. [[PubMed](#)]
13. Tubach, F.; Ravaud, P.; Martin-Mola, E.; Awada, H.; Bellamy, N.; Bombardier, C.; Felson, D.T.; Hajjaj-Hassouni, N.; Hochberg, M.; Logeart, I.; et al. Minimum clinically important improvement and patient acceptable symptom state in pain and function in rheumatoid arthritis, ankylosing spondylitis, chronic back pain, hand osteoarthritis, and hip and knee osteoarthritis: Results from a prospective multinational study. *Arthritis Care Res.* **2012**, *64*, 1699–1707.
14. Gooley, T.A.; Leisenring, W.; Crowley, J.; Storer, B.E. Estimation of failure probabilities in the presence of competing risks: New representations of old estimators. *Stat. Med.* **1999**, *18*, 695–706. [[CrossRef](#)]
15. Fine, J.P.; Gray, R.J. A proportional hazards model for the subdistribution of a competing risk. *J. Am. Stat. Assoc.* **1999**, *94*, 496–509. [[CrossRef](#)]
16. Preacher, K.J.; Hayes, A.F. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behav. Res. Methods* **2008**, *40*, 879–891. [[CrossRef](#)] [[PubMed](#)]
17. Busch-Dienstfertig, M.; González-Rodríguez, S. IL-4, JAK-STAT signaling, and pain. *JAK-STAT* **2013**, *2*, e27638. [[CrossRef](#)] [[PubMed](#)]
18. Knopp, K.L.; Kato, A.; Wall, T.M.; McDermott, J.S.; Nisenbaum, E.S.; Adams, B.L.; Johnson, M. SAT0051 Baricitinib improves joint mobility after injury in a rodent forced-ambulation model. *Ann. Rheum. Dis.* **2019**, *78*, A1089.
19. Cook, A.D.; Pobjoy, J.; Steidl, S.; Durr, M.; Braine, E.L.; Turner, A.L.; Lacey, D.C.; Hamilton, J.A. Granulocyte-macrophage colony-stimulating factor is a key mediator in experimental osteoarthritis pain and disease development. *Arthritis Res. Ther.* **2012**, *14*, R199. [[CrossRef](#)] [[PubMed](#)]
20. Dominguez, E.; Rivat, C.; Pommier, B.; Mauborgne, A.; Pohl, M. JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat. *J. Neurochem.* **2008**, *107*, 50–60. [[CrossRef](#)] [[PubMed](#)]
21. Wigerblad, G.; Bas, D.B.; Fernandes-Cerqueira, C.; Krishnamurthy, A.; Nandakumar, K.S.; Rogoz, K.; Kato, J.; Sandor, K.; Su, J.; Jimenez-Andrade, J.M.; et al. Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via chemokine-dependent mechanism. *Ann. Rheum. Dis.* **2016**, *74*, 730–738. [[CrossRef](#)]
22. Augustsson, J.; Neovius, M.; Cullinane-Carli, C.; Eksborg, S.; van Vollenhoven, R.F. Patients with rheumatoid arthritis treated with tumour necrosis factor antagonists increase their participation in the workforce: Potential for significant long-term indirect cost gains (data from a population-based registry). *Ann. Rheum. Dis.* **2010**, *69*, 126–131. [[CrossRef](#)] [[PubMed](#)]
23. Taylor, P.C.; Moore, A.; Vasilescu, R.; Alvir, J.; Tarallo, M. A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: A current perspective. *Rheumatol. Int.* **2016**, *36*, 685–695. [[CrossRef](#)] [[PubMed](#)]
24. Michaud, K.; Pope, J.; Emery, P.; Zhu, B.; Gaich, C.; DeLozier, A.M.; Zhang, X.; Dickson, C.; Smolen, J.S. Relative impact of pain and fatigue on work productivity in patients with rheumatoid arthritis from the RA-BEAM baricitinib trial. *Rheumatol. Ther.* **2019**, in press.



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Article

Infliximab Induced a Dissociated Response of Severe Periodontal Biomarkers in Rheumatoid Arthritis Patients

Mélanie Rinaudo-Gaujous ¹, Vincent Blasco-Baque ², Pierre Miossec ³, Philippe Gaudin ⁴, Pierre Farge ⁵, Xavier Roblin ⁶, Thierry Thomas ^{7,8}, Stephane Paul ^{1,†} and Hubert Marotte ^{7,8,†,*}

¹ GIMAP EA3064, Laboratory of Immunology and Immunomonitoring, CIC CIE3 Inserm Vaccinology, Hôpital Nord, CHU Saint-Etienne, 42270 Saint-Priest-en-Jarez, France; melanie.rinaudogaujous@chpg.mc (M.R.-G.); stephane.paul@chu-st-etienne.fr (S.P.)

² Institute of Cardiovascular and Metabolic Diseases, CHU Rangueil, 31400 Toulouse, France; vincent.blasco@inserm.fr

³ Clinical Immunology Unit, Departments of Immunology and Rheumatology, Hôpital Edouard Herriot, CHU Lyon, 69003 Lyon, France; pierre.miossec@univ-lyon1.fr

⁴ Department of Rheumatology, CHU Grenoble, 38130 Échirolles, France; pgaudin@chu-grenoble.fr

⁵ Faculty of Odontology, University Lyon I., 69622 Villeurbanne, France; pierre.farge@univ-lyon1.fr

⁶ Department of Gastroenterology, Hôpital Nord, CHU Saint-Etienne, 42270 Saint-Priest-en-Jarez, France; xavier.roblin@chu-st-etienne.fr

⁷ Department of Rheumatology, Hôpital Nord, CHU Saint-Etienne, 42055 Saint-Etienne, France; thierry.thomas@chu-st-etienne.fr

⁸ INSERM U1059, Université de Lyon–Université Jean Monnet, 42023 Saint-Etienne, France

* Correspondence: hubert.marotte@chu-st-etienne.fr; Tel.: +33-477-12-76-43; Fax: +33-477-12-75-77

† These authors contribute equally to this work.

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Abstract: Objective: Rheumatoid arthritis and periodontal disease are associated together, but the effect of therapy provided for one disease to the second one remained under-investigated. This study investigated effect of infliximab therapy used to treat rheumatoid arthritis (RA) on various biomarkers of periodontal disease (PD) severity including serologies of *Porphyromonas gingivalis* and *Prevotella intermedia* and matrix metalloproteinase 3. Methods: Seventy nine RA patients were enrolled at the time to start infliximab therapy and the 28 joint disease activity score (DAS28), anti-cyclic citrullinated peptides 2nd generation (anti-CCP2), anti-*P. gingivalis* antibody, and Matrix metalloproteinase 3 (MMP-3) were monitored before and at 6 months of infliximabtherapy. Joint damage and severe periodontal disease were assessed at baseline. Anti-CCP2, anti-*P. gingivalis* antibody, and MMP-3 weredetermined by enzyme-linked immunosorbent assay (ELISA). Results: At baseline, anti-CCP2 titers were associated with anti-*P. gingivalis* lipopolysaccharide (LPS)-specific antibodies titers ($p < 0.05$). Anti-*P. gingivalis* antibodies were not significantly correlated with clinical, biological, or destruction parameters of RA disease. At 6 months of infliximab therapy, MMP-3 level decreased (from 119 ± 103 ng/mL to 62.44 ± 52 ng/mL; $p < 0.0001$), whereas *P. gingivalis* antibody levels remained at the same level. DAS28 and inflammation markers C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR) also decreased significantly during infliximab therapy ($p < 0.05$) as anti-CCP2 levels ($p < 0.001$). Only high MMP-3 level at baseline was associated with infliximab efficacy ($p < 0.01$). Conclusion: MMP-3 level can be a useful marker of the efficacy of infliximab in RA patients. The treatment did not affect anti-*P. gingivalis* antibodies.

Keywords: Rheumatoid arthritis; *Porphyromonas gingivalis*; periodontal disease; matrix metalloproteinase 3; infliximab

1. Introduction

Rheumatoid arthritis (RA) is the most frequently chronic inflammatory joint disease characterized by synovial hypertrophy and inflammation with joint and subchondral bone destruction, which correlates with disability and loss of function [1]. Epidemiological studies suggest an association between RA and periodontal disease (PD) and confirmed by a recent meta-analysis [2]. Both diseases (RA and PD) share important similarities in their pathogenesis involving similar genetic background [3] or production of large amount of proinflammatory cytokines such as tumor necrosis factor alpha (TNF) [4]. In, P.D., inflammation is initiated and perpetuated by a subset of bacteria, including *Porphyromonas gingivalis* (*P. gingivalis*) and *Prevotella intermedia* (*P. intermedia*), two specific gram-negative anaerobic bacteria, which colonize the gingival sulcus and proliferate in the gingival plaque. The resulting chronic inflammatory response by the host induces destruction of the supporting structures of the teeth defining severe PD. *P. gingivalis* presence seems to be specific of severe PD. This was reinforced by recent data from an experimental rat model confirming the specific involvement of *P. gingivalis* in arthritis onset [5]. Furthermore, bacterial colonization was also described in the gut of RA patients [6].

Anti-citrullinated protein antibodies (ACPA) are the highest specific biomarker for RA diagnosis or prognosis and are now included in the new RA criteria [1]. Endogenous or exogenous peptidyl-arginine deiminases (PADs) induced citrullinated proteins by conversion of peptidyl-arginine to peptidyl-citrulline. This is part of many physiological processes [7]. However, smoking or *P. gingivalis* infection could induce excess of citrullination in some conditions [8]. Since ACPA occurred some years before RA clinical onset [9], *P. gingivalis* infection could precede RA onset and be a key player for initiation and maintenance of the autoimmune inflammatory responses in RA [10]. *P. gingivalis* is the unique known pathogen to have a specific enzyme PAD (PPAD) [11], which induce citrullination of proteins [12] and could provide a rupture of tolerance with ACPA induction.

Indirect presence of *P. gingivalis* by serology demonstrated that high concentrations of anti-*P. gingivalis* antibody in established [12] or early RA patients [13]. This indirect biomarker of *P. gingivalis* correlated with the gingival bacteria load assessed by polymerase chain reaction [13,14]. PD is related to many other anaerobic periodontal pathogens including *P. intermedia*, which was also detected in both the serum and synovial fluids of RA patients [15].

Matrix metalloproteinase 3 (MMP-3) is one of the major MMPs expressed in rheumatoid synovial tissue [16]. MMP-3 is mainly involved in bone and cartilage degradation in RA or bone destruction in PD [17]. Thus, MMP-3 is already considered as a biomarker for RA and PD destruction [18]. In, P.D., a MMP-3 polymorphism was described as associated to PD [19]. Furthermore, strategy based on MMP-3 monitoring improved clinical response and reduced joint destruction in RA [20]. In both PD and, R.A., production of proinflammatory cytokines, as TNF, is increased and specific blocking of TNF improves two-third of RA patients [21]. Only few studies have reported predictive factors of response to infliximab in RA [21], but no relevant clinical or biological markers can be used in the daily practice. In only one previous study, PD was related to be a predictive factor for a non-response to TNF blocker therapy in RA patients [22]. Persistence of *P. gingivalis* in gingival tissue could participate to maintain local and systemic inflammation in relation with treatment resistance [23]. Only few studies explored therapeutic effect for PD on RA [24] and *vice versa* [25].

Since both diseases are associated at the susceptibility and severity level [24], therapy from one disease should be efficient to the second one. This concept was recently reinforce by the first demonstration of PD severity on RA activity [26]. We already reviewed previously [24] impact of some biologic disease modifying anti-rheumatic drugs (bDMARDs) on PD. Infliximab treatment worsened the gingival inflammation, but decreased the gingival destruction of bone [25]. At the opposite, rituximab [27] or tocilizumab [28] decreased gingival inflammation or gingival bone destruction related to the PD. Accordingly, in case of severe PD B-cell blocker or IL-6 receptor blockers could be considered preferentially compared to TNF blocker. At the opposite, some non-surgical PD therapy reported decreased of anti-*P. gingivalis* antibodies without effect on ACPA level [29].

Thus, our aim in this study was to correlate marker of PD severity (MMP-3, anti-*P. gingivalis* and anti-*P. intermedia* antibodies) and to assess effect of infliximab therapy on PD severe biomarkers in RA patients. In addition, the usefulness of these biomarkers was assessed for prediction of clinical response to infliximab therapy.

2. Patients and methods

2.1. Patients and Controls

Seventy nine RA patients treated with methotrexate with active disease and starting infliximab therapy were included consecutively. Following clinical parameters were recorded: Age, sex, disease duration, patient's global assessment of disease activity, 28 tender and swollen joint counts, and the 28 joint disease activity score (DAS28). Joint damage and severe PD were defined by a right Larsen wrist score ≥ 2 and Hugoson and Jordan criteria, respectively as previously used [3]. Wrist X-rays were examined by the same reader (HM) as panoramic X-rays (PF). Clinical response to infliximab was defined by a decrease of DAS28 > 1.2 . Blood samples were collected before and at 6 months of infliximab therapy to assess anti-cyclic citrullinated peptide second generation (CCP2), rheumatoid factor (RF), MMP-3, and antibodies against *P. gingivalis* and *P. intermedia*. Sera from two control groups were used in this study. We enrolled 27 healthy blood donors as control healthy volunteers and 28 patients with inflammatory bowel disease (IBD) and 35 patients with systemic lupus erythematosus (SLE), as inflammatory disorders controls. Local clinical ethics committee approved the protocol and all patients gave their written informed consent.

2.2. Methods

Determination of anti-*P. gingivalis* and anti-*Escherichia coli* LPS-specific antibodies by enzyme-linked immunosorbent assay (ELISA). To optimise our evaluation anti-*P. gingivalis* antibody assessed by ELISA, we performed two standardised ways for coating: Whole extract or lipopolysaccharide (LPS) components. LPS from *P. gingivalis* (InvivoGen, Toulouse, France) was coated on 96-well plates (Nunc, Dominique Dutscher, Brumath, France) at 10 $\mu\text{g}/\text{mL}$ (diluted in carbonate buffer, pH 9.6) and incubated overnight at 4 °C. We used LPS from *Escherichia coli* (*E. coli*, InvivoGen, Toulouse, France) as control with the same dilution. Wells were washed three times with phosphate buffered saline (PBS)-Tween (0.005%). Plasma were diluted to 1:600 in PBS containing 1% of bovine serum albumin (BSA) and incubated (in duplicate) for 2 h at room temperature. Plates were washed as described above and incubated with peroxidase-conjugated goat anti-human IgG H + L (Jackson ImmunoResearch, West Grove, PA, USA) (diluted 1:50 000 in PBS) for 2 h at room temperature. After a final wash, detection was made by tetramethylbenzidine substrate (R&D Systems, Minneapolis, MN., USA). The reaction was stopped by the addition of H_2SO_4 (1M) solution and absorbances were measured at 450 nm.

2.2.1. Determination of Anti-*P. gingivalis* and Anti-*P. intermedia* Whole Extract Antibodies by ELISA

P. gingivalis strain ATCC 33277 and *P. intermedia* CIP 103607 were grown on sterile non-selective agar containing defibrinated sheep blood, supplemented with 0.0002% menadione sodium bisulfite and 0.4% hemin chloride. Cultures were then placed in an anaerobic chamber for 7 days at 37 °C. Then, colonies were recovered in a sterile water solution and centrifuged for 20 min at 5000 \times g. Pellets were diluted in sterile PBS at 50 mg/mL and were frozen at -20 °C until use. *P. gingivalis* or *P. intermedia* solution was then washed twice with carbonate buffer (pH 9.6) and diluted in the same buffer to obtain a solution of 1 McFarland (DensiCHEK plus, Biomérieux, Craponne, France). The solution was heated to 60 °C for 45 min, filtered (0.22 μm), diluted 1:10, coated on a 96-well plate and then incubated overnight at 4 °C. Plasma were diluted to 1:900 in PBS containing 1% BSA. Following steps are identical to those described for the LPS-specific ELISA. Cut-off values for seropositivity to *P. gingivalis* (LPS and whole extract) and *P. intermedia* were determined by concentrations higher than the 95th percentile in 27 healthy blood donors.

A calibration curve was systematically done for each plate with dilutions of a pool of positive plasma diluted six times from 1:100 to 1:16200 to correct for plate-to-plate variation. Two plasma controls (high and low positives) were included on all plates. All intra assay coefficients of variation (CV) were below 6.5%. Inter assay CV for the high positive control were 13%, 8%, and 28% for *P. gingivalis* whole extract, LPS assay and *P. intermedia* assay, respectively. Results are expressed in Arbitrary Units (AU) defined by the pool dilution (10 AU = 1:16200 to 2430 AU = 1:100).

Furthermore, determination of citrullinated proteins in *P. gingivalis* whole extract or LPS was assessed by using the specific anti-CCP2 detection antibody (Phadia, Thermo Fisher Scientific, Uppsala, Sweden).

2.2.2. Assessments of ACPA, R.F., and MMP-3

MMP-3 blood concentrations were determined by a commercial ELISA method (AESKU.diagnostics, Wendelsheim, Germany). ACPA was assessed by anti-CCP2, and RF (IgA and IgM) were measured by ELIA method on ImmunoCap 250 (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). Anti-CCP2 was considered to be positive at a cut off value of 10 U/mL, RF IgA at 14 IU/mL and RF IgM at 3.5 IU/mL, as recommended by the manufacturer.

2.3. Statistical Analysis

Since data were not normally distributed, they were expressed as median and interquartile range 25–75% (IQR 25–75) or number (%). Correlations were performed by Spearman tests. Comparisons between controls and RA patients were performed by Mann Whitney test. Comparisons between baseline and 6 months of infliximab treatment were performed by Wilcoxon test. Statistics were done with the software GraphPad Prism (version 5.0). *p* values less than 0.05 were considered as statistically significant.

3. Results

3.1. RA Population

Our RA population had the main characteristics of RA patients treated with TNF inhibitors as reported in the Table 1. In this cohort, severe PD was present in 51 RA patients and severe PD was associated with joint damage (χ^2 test = 4.4; *p* = 0.0276).

Table 1. Characteristics of rheumatoid arthritis (RA) patients at baseline and after infliximab therapy.

| | Baseline | 6 Months | <i>p</i> Values |
|---|------------------|---------------|-----------------|
| Sex (female/male) | 63/16 | | |
| Age, years | 52.8 (43.3–59.4) | | |
| Disease duration (years) | 9 (5–13) | | |
| No destruction, <i>n</i> (%) | 12 (15.2%) | | |
| Wrist destruction, <i>n</i> (%) | 56 (70.9%) | | |
| Periodontal disease, <i>n</i> (%) | 51 (64.6%) | | |
| DAS28 | 5.1 (4.1–5.7) | 3.5 (2.7–4.3) | <0.0001 |
| ESR (mm/h) | 34 (20–49) | 18 (10–32) | <0.0001 |
| CRP (mg/L) | 18 (7–33) | 7 (2–17) | <0.05 |
| Anti-CCP2 (U/mL) | 97 (9–275) | 43 (5–189) | <0.001 |
| RF IgM (IU/mL) | 38 (11–96) | 22 (6–66) | <0.0001 |
| RF IgA (IU/mL) | 25 (10–61) | 17 (8–47) | 0.0001 |
| MMP-3 (ng/mL) | 90 (40–177) | 45 (24–91) | <0.0001 |
| Anti- <i>P. gingivalis</i> whole extract (AU) | 238 (148–377) | 274 (173–557) | <0.01 |
| Anti- <i>P. gingivalis</i> LPS (AU) | 86 (67–146) | 97 (77–152) | NS |
| Anti- <i>P. intermedia</i> whole extract (AU) | 390 (131–1558) | 436 (246–853) | <0.05 |

Values are indicated as number of patients (%) or median (1st and 3rd quartiles). DAS28: Disease Activity Score 28; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Anti-CCP2: anti-cyclic citrullinated peptides 2nd generation; RF: rheumatoid factor; MMP-3: Matrix metalloproteinase-3; LPS: lipopolysaccharide; U: unit; IU: international units; AU: arbitrary units, NS: non-significant.

3.2. Immunity Against Oral Pathogens is Related to Established RA

Anti-*P. gingivalis* whole extract antibodies were more frequently positive in established RA patients (97.5%) than in healthy blood donors (5%) with higher concentrations of anti-*P. gingivalis* antibody in established RA patients (238 (148–377) AU) than in healthy blood donors (43 (24–79) AU; Figure 1A; $p < 0.001$). Similar results were observed for anti-*P. gingivalis* LPS specific antibody (data not shown). Analogous pattern was also observed for anti-*P. intermedia* whole extract antibodies with more positive in established RA patients (84.8%) than in healthy blood donors (17.8%; Figure 1A; $p < 0.001$). Anti-*P. intermedia* antibody concentrations were also higher in established RA patients (390 (131–1558) AU) than in healthy blood donors (96 (67–179) AU; Figure 1A; $p < 0.001$). Thus immunity against oral pathogens was higher in established RA patients than in healthy blood donors reinforcing association between RA and PD. Furthermore, the two ways for anti-*P. gingivalis* antibody determination strongly correlated together (Figure 1B; $p < 0.0001$) and also correlated with anti-*P. intermedia* antibody concentrations (Figure 1C; $p < 0.0001$). Since we observed a correlation between immunity against these two bacteria from the oral cavity, we investigated the specificity of our assay. For this purpose, we tested the same plasma for anti-LPS fraction of *E. coli*, another commensal bacterium of the gut. Only one patient has serum with anti-*E. coli* antibody without anti-*P. gingivalis* antibody. At the opposite, many patients had anti-*P. gingivalis* antibody without anti-*E. coli* antibody, demonstrating the absence of crossreaction between these two antibodies (Figure 1D). As *P. gingivalis* whole extract may contain citrullinated proteins, we then investigated a putative crossreactivity between anti-CCP2 and anti-*P. gingivalis* antibodies. Presence of citrullinated proteins was not observed in the whole *P. gingivalis* extract by using the monoclonal antibody to detect anti-CCP2 (data not shown).

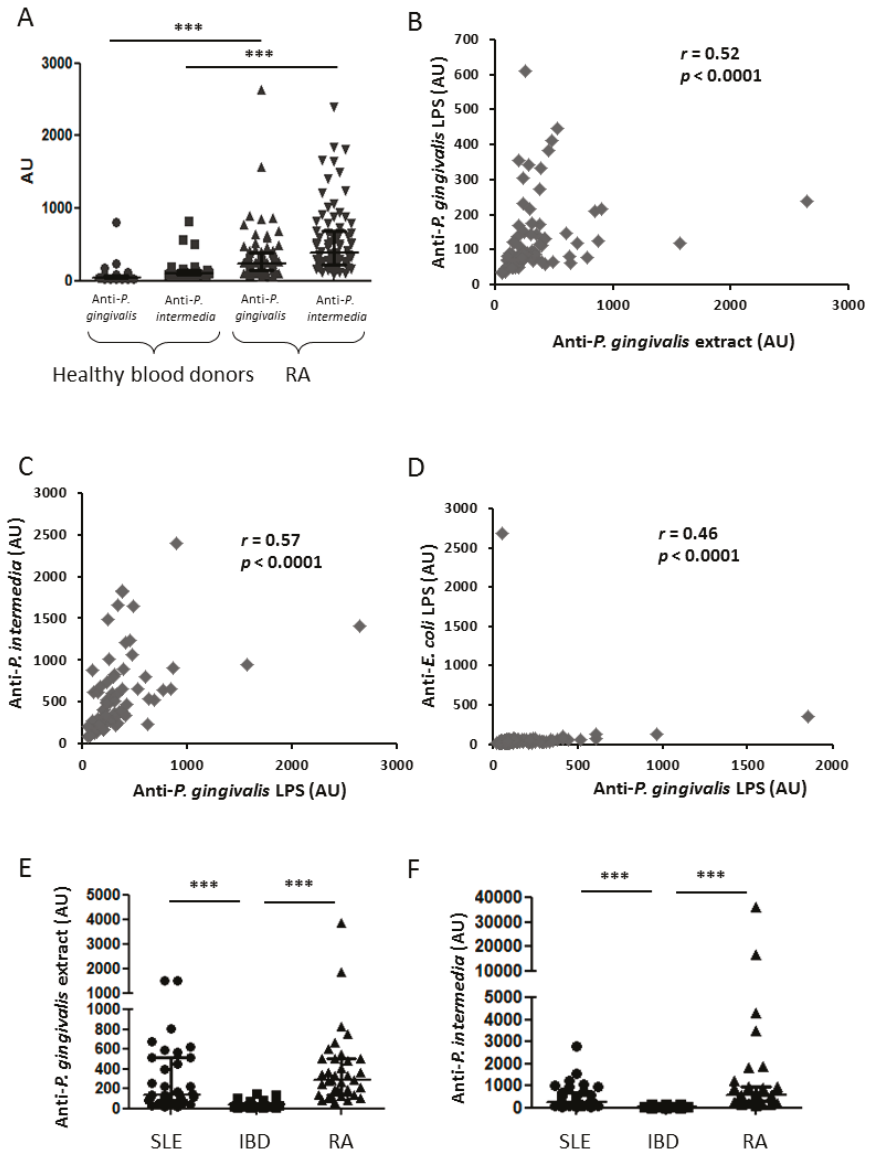


Figure 1. Evaluation of the measure of anti-*P. gingivalis* whole extract and LPS specific antibodies by ELISA. Anti-*P. gingivalis* and anti-*P. intermedia* (A) whole extract antibodies were measured in healthy blood donors and RA patients. Correlations between both anti-*P. gingivalis* LPS and whole extract (two ways to assess the same germ; (B); anti-*P. intermedia* and anti-*P. gingivalis* whole extract (assessment of two oral germs; (C) in RA patients. No correlation between anti-*P. gingivalis* and anti-*E. coli* LPS antibodies in RA patients (assessment of one oral germ and on commensal gut germ; (D)). Anti-*P. gingivalis* (E) and anti-*P. intermedia* (F) whole extract antibodies were measured in SLE., IBD., and RA patients. From the bottom up, the bars indicate the interquartile range and the median. AU: arbitrary units; r : Spearman correlation coefficient; LPS: lipopolysaccharide; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; IBD: inflammatory bowel disease; ***, $p < 0.001$.

3.3. Anti-*P. gingivalis* and anti-*P. Intermedia* Antibodies and RA Specificity

To assess the specificity of these antibodies to, R.A., we then assessed them in IBD and SLE., two other inflammatory auto-immune diseases. Anti-*P. gingivalis* and anti-*P. intermedia* antibody concentrations were lower in IBD and SLE patients and established RA patients (Figure 1E,F; $p < 0.001$ for *P. gingivalis* and *P. intermedia* in RA and SLE vs. IBD). Thus high antibody concentrations against oral pathogens were specific for RA or SLE diseases involving joint.

3.4. Differential Implication of *P. Gingivalis* and *P. Intermedia* Antibodies in Immune Response in RA Patients

Since high antibody concentrations against *P. gingivalis* and *P. intermedia* were observed in RA patients, association of these antibodies with clinical and biological RA parameters was investigated. RF IgA concentrations were correlated with DAS28 at baseline (Figure 2A; $p < 0.01$). Furthermore, anti-CCP2 concentrations correlated with both anti-*P. gingivalis* (whole extract and LPS) antibody concentrations (Figure 2B,C; $p < 0.001$ and $p < 0.01$; respectively), without correlation with IgM RF concentrations. Interestingly, anti-*P. intermedia* antibody concentrations correlated with IgM RF concentrations (Figure 2D; $p < 0.05$), but not with anti-CCP2 concentrations. Since only anti-*P. gingivalis* concentrations correlated with anti-CCP2 concentrations, these data reinforced the association between *P. gingivalis* and citrullination in RA.

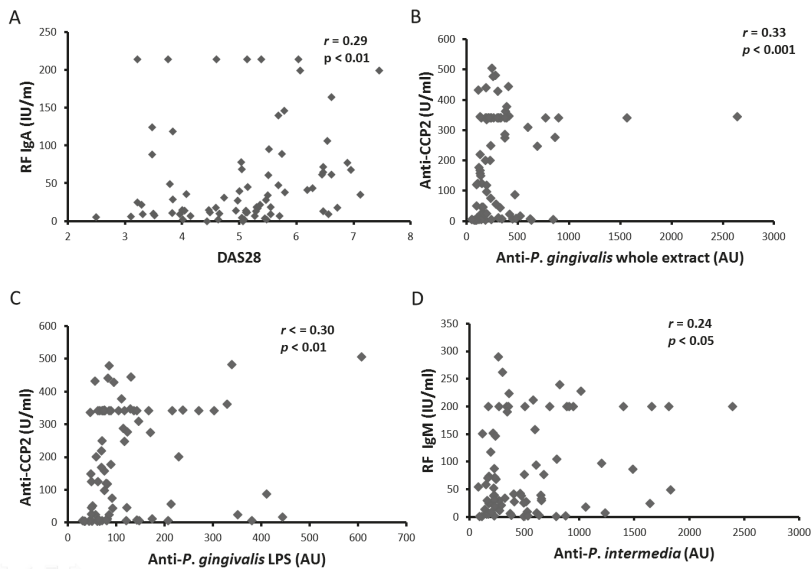


Figure 2. Correlation between clinical and biological parameters at baseline. Correlations between RF IgA and DAS28 (A); anti-CCP2 and anti-*P. gingivalis* whole extract antibodies (B); anti-CCP2 and anti-*P. gingivalis* LPS specific antibodies (C); and RF IgG and anti-*P. intermedia* antibodies (D). RF: rheumatoid factors; IU: international units; DAS28: disease activity score 28; AU: arbitrary units; anti-CCP2: anti-cyclic citrullinated peptides 2nd generation; r : Spearman correlation coefficient; LPS: lipopolysaccharide; U: Unit; IU: International unit; NS: non-significant.

3.5. Clinical Response to Infliximab Therapy and Joint or Periodontal Destructions

As expected, DAS28 and inflammation markers (CRP and ESR) strongly decreased at 6 months of infliximab treatment (Table 1). DAS28 improvement was similar in RA patients with or without severe PD (1.9 (0.7–3.0) vs. 1.7 (0.5–2.5); not significant) and according to the joint damage status. Interestingly, anti-CCP2 concentrations decreased significantly during infliximab therapy (Figure 3A;

$p < 0.001$) as IgM RF concentrations (Figure 3B; $p < 0.001$). As expected, all RA parameters decreased during infliximab therapy.

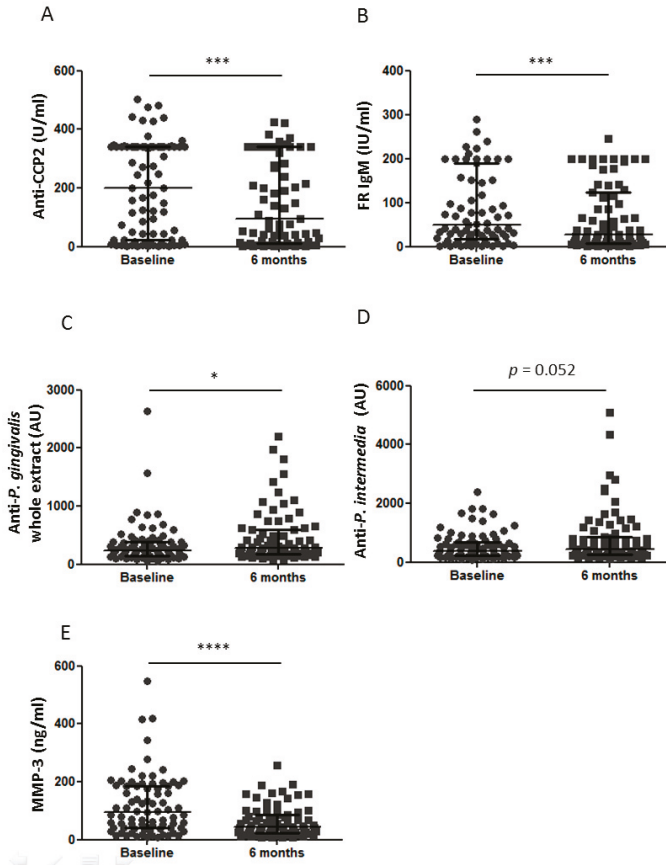


Figure 3. Effect of infliximab treatment on biological markers. Anti-CCP2 (A), RF IgM (B), anti-*P. gingivalis* antibodies (C), anti-*P. intermedia* antibodies (D), and MMP3 (E) were evaluated at baseline and after 6 months of infliximab therapy. Dots represent results for each patient, with values at baseline in round and at 6 months of infliximab therapy in square. From the bottom up, the bars indicate the interquartile range and the median. Anti-CCP2: anti-cyclic citrullinated peptides 2nd generation; RF: rheumatoid factor; MMP-3: metalloproteinase 3; AU: arbitrary units; IU: International units; *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$.

3.6. Dissociated Effect of Infliximab Therapy on PD Biological Markers

Anti-*P. gingivalis* whole extract antibody concentrations slightly increased at 6 months of infliximab therapy (Figure 3C; $p < 0.05$) with the same trend for anti-*P. intermedia* antibody concentrations (Figure 3D; $p = 0.052$). During infliximab therapy, MMP-3 concentrations strongly decreased from 90 (14–259) ng/mL to 45 (10–160) ng/mL (Figure 3E; $p < 0.0001$). Thus, infliximab therapy induced a strong reduction of MMP-3, but slightly increased concentrations of antibodies against *P. gingivalis* and *P. intermedia*.

3.7. Biological Markers and Prediction of Clinical Response

Among biomarkers assessed in this study, baseline MMP-3 concentrations were higher in patients with a good response to infliximab (111 (49–187) ng/mL) compared to non-responders (57 (29–110) ng/mL; $p < 0.05$; Figure 4A). However, neither baseline anti-CCP2 concentrations (Figure 4B; NS) nor anti-*P. gingivalis* and anti-*P. intermedia* antibody concentrations (data not shown) were not associated with clinical response to infliximab. Furthermore, reduction of MMP-3 during infliximab therapy was higher in responders than in non-responders patients (Figure 4C; $p < 0.05$). Only a trend was observed for higher reduction of anti-CCP2 in responders compared to non-responders to infliximab (Figure 4D; $p = 0.09$).

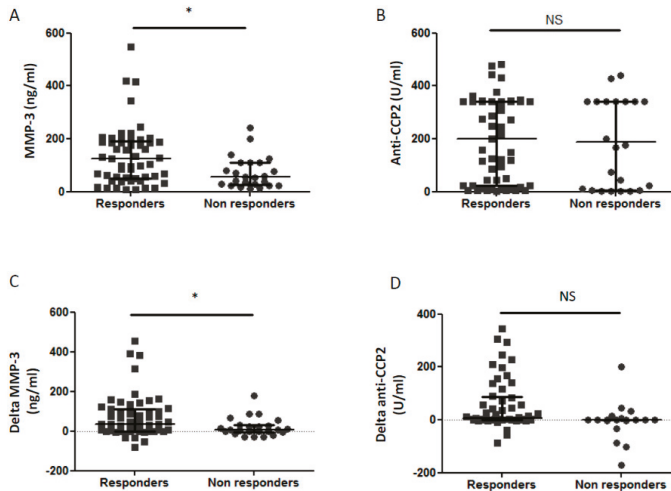


Figure 4. Predictive factors for clinical response to infliximab therapy. Baseline values of MMP-3 (A) and anti-CCP2 (B) were represented. Delta MMP-3 (C) and delta anti-CCP2 (D) represent difference of MMP-3 and ACPA (baseline value minus 6 month value) and are shown according to clinical response to infliximab. Dots represent results for each patient, with responders in square (■) and non-responders in round (●). From the bottom up, the bars indicate the interquartile range and the median. Response to infliximab treatment was defined by an improvement > 1.2 of DAS28 at 6 months. MMP-3: matrix metalloproteinase-3; anti-CCP2: anti-cyclic citrullinated peptides 2nd generation; *: $p < 0.05$; NS: non significant.

4. Discussion

We confirmed a higher prevalence of PD in established RA patients (64.5%) than in the general population (30–40%) [30], as previously reported in early RA [31]. PD is related to many anaerobic periodontal pathogens including *P. intermedia*, *P. melaninogenica*, or *T. forsythia*. Antibodies against these periodontal pathogens were also more frequently detected in the serum from RA patients than controls [32]. We first confirmed higher concentrations of antibodies against *P. gingivalis* and *P. intermedia* in established RA than in healthy blood donors [32] or in two other inflammatory diseases (SLE and IBD). This is in link with a recent review considering high *P. gingivalis* antibody as a biomarker of RA [33]. Serology determination is the easiest way to assess presence of *P. gingivalis* and indirectly presence of severe PD. Gingival presence of *P. gingivalis* detected by polymerase chain reaction is strongly correlated with concentrations of anti-*P. gingivalis* antibody [13,14]. Previous studies have explored antibodies against *P. gingivalis* in RA patients by using either LPS or whole extract [12]. Here, we observed a correlation between these two assessments (LPS and bacterial extract) of antibodies against *P. gingivalis*, suggesting that LPS should be the easiest way to assess

anti-*P. gingivalis*. Furthermore, antibody concentrations against *P. gingivalis* and *P. intermedia* correlated together, which was not surprising since both are involved in PD pathogenesis [34]. Despite the quite high r value for the correlation between antibodies against *P. gingivalis* LPS and against *E. coli*, we still believe that each antibody is specific due to absence of high level of antibodies for both pathogens. Our assay to detect antibodies against *P. gingivalis* LPS or *E. coli* LPS without crossreaction. This was not surprising since LPS from *P. gingivalis* has a specific structure compared to other bacteria with the ability to activate TLR2 and TLR4 [35], whereas LPS from *E. coli* activates mainly TLR4 [35].

Anti-*P. gingivalis* antibody concentrations correlated with anti-CCP2 concentrations without correlation with RF concentrations [15]. We did not expect crossreactivity between *P. gingivalis* and anti-CCP2 due to LPS structure containing only sugars and lipids and absence of detection of citrullinated proteins in *P. gingivalis* extracts by using the monoclonal detection antibody from anti-CCP2 assay. Contrary to anti-*P. gingivalis* antibodies, anti-*P. intermedia* antibodies were not correlated with anti-CCP2, but with RF IgM concentrations, as previously described in a human [36] or in a rat model [5]. This suggested different mechanisms of action between these two bacteria in immune response induction. Assessment of several oral bacteria is required to have a discriminate role for each pathogen. For instance, recent data suggested high anti-*P. intermedia* antibodies in clinical remission RA patients with persistent PD US activity [23]. On the other hand, *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), a periodontal pathogen associated with PD severity, has been suspected to be the culprit of the association of RA and PD [37] without confirmation since. Taken all together, our data support the specific implication of *P. gingivalis* compared to *P. intermedia* in RA pathogenesis with induction of anti-CCP2 in response to gingival citrullinated proteins, as suggested by a recent rodent model [5].

MMP-3 is a biomarker of both destruction in RA [38] and in PD [39]. So, we confirmed the reduction of inflammatory markers (ESR, CRP) and MMP-3 over infliximab treatment, as previously described with infliximab [40] or other TNF blocker [41].

Despite the growing interest for *P. gingivalis* in RA, this was the first observation that anti-*P. gingivalis* and *P. intermedia* antibodies were increasing over time during infliximab therapy. Only a recent study observed a stability of anti-*P. gingivalis* antibody concentrations in 50 early RA patients mainly treated with methotrexate [42]. As previously reported with methotrexate therapy [42], no patient developed seroconversion for *P. gingivalis* or *P. intermedia* with combination of infliximab and methotrexate. Despite the growing interest for *P. gingivalis* pre-existing immunity in RA patients, this was the first observation that serum anti-*P. gingivalis* antibodies were increasing over time during infliximab therapy or bDMARDs. It is not surprising that bDMARDs promotes infections including gingival infection. Our results are in line with previous report with infliximab showing increasing of gingival inflammation during infliximab therapy [25]. However, this increasing of gingival inflammation was not observed with rituximab or tocilizumab, two other bDMARDs targeting B cell and IL-6, respectively [27,28]. On the contrary, some studies investigated the effect of periodontal therapy on RA activity. A recent metaanalysis suggested a small effect of periodontal therapy (full-mouth scaling and root planning) on RA disease activity [43]. Reduction of ACPA during infliximab therapy was also already described [44]. Despite increased of anti-*P. gingivalis* antibody concentrations during infliximab therapy, a trend for a reduction of anti-CCP2 during infliximab therapy was observed in our study. This kind of dissociation response was also observed after non-surgical therapy for periodontal disease with a decreased of anti-*P. gingivalis* levels without effect on anti-CCP2 levels [29]. This dissociated response to infliximab of anti-*P. gingivalis* and anti-CCP2 concentrations reinforced role of *P. gingivalis* only in anti-CCP2 induction.

Prediction of TNF response remains a huge challenge and to date no biomarkers can be used in daily practice [21]. Here, we observed that high baseline MMP-3 concentrations were associated with a good clinical response. This emphasizes the role of MMP-3 for RA management strategy [20]. However, baseline MMP-3 concentrations were similar in 47 good responders vs. 29 non-responders

according to response assessed earlier than in our study at 14 weeks [45]. Further studies are needed before to consider MMP-3 as a validate biomarker to predict clinical response in the daily practice [21].

Our study also has some weakness. PD assessment was only performed at baseline on panoramic X-rays without clinical assessment. Clinical PD assessment was not planned in this study. However, clinical severe PD was already extremely reported [2] with a dissociated effect of infliximab therapy. In fact, infliximab treatment worsens the gingival inflammation, but decreases the gingival destruction of bone [25]. Panoramic X-rays was not repeated due to low variation of bone loss.

5. Conclusions

Concentrations of anti-*P. gingivalis* antibody, a biomarker of PD severity correlated with anti-CCP2 concentrations in one hand and anti-*P. intermedia* antibody concentrations in another hand. Besides, anti-*P. intermedia* antibody concentrations correlated only with RF concentrations suggesting different immunologic response induced by both oral bacteria. Whereas MMP-3 is strongly decreased by infliximab therapy, serology against oral pathogen slightly increased. Furthermore, MMP-3, another biomarker of PD and RA severity, is a predictive biomarker of response to infliximab therapy. Our data reinforce interest of PD in RA pathogenesis and RA therapeutic management. They also confirm the possible involvement of *P. gingivalis* in RA physiopathology due to the correlation with ACPA.

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References

1. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O.; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **2010**, *69*, 1580–1588. [[CrossRef](#)] [[PubMed](#)]
2. Fuggle, N.R.; Smith, T.O.; Kaul, A.; Sofat, N. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis. *Front. Immunol.* **2016**, *7*, 80. [[CrossRef](#)]
3. Marotte, H.; Farge, P.; Gaudin, P.; Alexandre, C.; Mouglin, B.; Miossec, P. The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. *Ann. Rheum. Dis.* **2006**, *65*, 905–909. [[CrossRef](#)] [[PubMed](#)]
4. Rosenstein, E.D.; Greenwald, R.A.; Kushner, L.J.; Weissmann, G. Hypothesis: The humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* **2004**, *28*, 311–318. [[CrossRef](#)] [[PubMed](#)]
5. Courbon, G.; Rinaudo-Gaujous, M.; Blasco-Baque, V.; Auger, I.; Caire, R.; Mijola, L.; Vico, L.; Paul, S.; Marotte, H. Porphyromonas gingivalis experimentally induces periodontitis and an anti-CCP2-associated arthritis in the rat. *Ann. Rheum. Dis.* **2019**, *78*, 594–599. [[CrossRef](#)]
6. Zhang, X.; Zhang, D.; Jia, H.; Feng, Q.; Wang, D.; Liang, D.; Wu, X.; Li, J.; Tang, L.; Li, Y.; et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat. Med.* **2015**, *21*, 895–905. [[CrossRef](#)] [[PubMed](#)]
7. György, B.; Tóth, E.; Tarcsa, E.; Falus, A.; Buzás, E.I. Citrullination: A posttranslational modification in health and disease. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 1662–1677. [[CrossRef](#)]
8. Klareskog, L.; Rönnelid, J.; Lundberg, K.; Padyukov, L.; Alfredsson, L. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu. Rev. Immunol.* **2008**, *26*, 651–675. [[CrossRef](#)] [[PubMed](#)]

9. Nielen, M.M.J.; Van Schaardenburg, D.; Reesink, H.W.; Van De Stadt, R.J.; Van Der Horst-Bruinsma, I.E.; De Koning, M.H.M.T.; Habibuw, M.R.; Vandenbroucke, J.P.; Dijkmans, B.A.C.; Van Der Horst-Bruinsma, I.E. Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors. *Arthritis Rheum.* **2004**, *50*, 380–386. [[CrossRef](#)] [[PubMed](#)]
10. Lundberg, K.; Wegner, N.; Yucel-Lindberg, T.; Venables, P.J. Periodontitis in RA—the citrullinated enolase connection. *Nat. Rev. Rheumatol.* **2010**, *6*, 727–730. [[CrossRef](#)]
11. Mangat, P.; Wegner, N.; Venables, P.J.; Potempa, J. Bacterial and human peptidylarginine deiminases: Targets for inhibiting the autoimmune response in rheumatoid arthritis? *Arthritis Res. Ther.* **2010**, *12*, 209. [[CrossRef](#)] [[PubMed](#)]
12. Wegner, N.; Wait, R.; Sroka, A.; Eick, S.; Nguyen, K.-A.; Lundberg, K.; Kinloch, A.; Culshaw, S.; Potempa, J.; Venables, P.J.; et al. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α -enolase: Implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum.* **2010**, *62*, 2662–2672. [[CrossRef](#)] [[PubMed](#)]
13. Seror, R.; Le Gall-David, S.; Bonnefroid, M.; Schaeferbeke, T.; Cantagrel, A.; Minet, J.; Gottenberg, J.-E.; Chanson, P.; Ravaud, P.; Mariette, X. Association of Anti-*Porphyromonas gingivalis* Antibody Titers with Nonsmoking Status in Early Rheumatoid Arthritis: Results from the Prospective French Cohort of Patients with Early Rheumatoid Arthritis: Anti-*Porphyromonas Gingivalis* Antibody and Early RA. *Arthritis Rheumatol.* **2015**, *67*, 1729–1737. [[CrossRef](#)] [[PubMed](#)]
14. Mikuls, T.R.; Payne, J.B.; Yu, F.; Thiele, G.M.; Reynolds, R.J.; Cannon, G.W.; Markt, J.; McGowan, D.; Kerr, G.S.; Redman, R.S.; et al. Periodontitis and *Porphyromonas gingivalis* in Patients with Rheumatoid Arthritis. *Arthritis Rheumatol.* **2014**, *66*, 1090–1100. [[CrossRef](#)] [[PubMed](#)]
15. Mikuls, T.R.; Payne, J.B.; Reinhardt, R.A.; Thiele, G.M.; Maziarz, E.; Cannell, A.C.; Holers, V.M.; Kuhnc, K.A.; O'Dell, J.R. Antibody responses to *Porphyromonas gingivalis* (*P. gingivalis*) in subjects with rheumatoid arthritis and periodontitis. *Int. Immunopharmacol.* **2009**, *9*, 38–42. [[CrossRef](#)]
16. Okada, Y.; Nagase, H.; Harris, E.D. A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. Purification and characterization. *J. Biol. Chem.* **1986**, *261*, 14245–14255.
17. Toyman, U.; Tüter, G.; Kurtiş, B.; Kıvrak, E.; Bozkurt, Ş.; Yücel, A.A.; Serdar, M. Evaluation of gingival crevicular fluid levels of tissue plasminogen activator, plasminogen activator inhibitor 2, matrix metalloproteinase-3 and interleukin 1- β in patients with different periodontal diseases. *J. Periodontol.* **2015**, *50*, 44–51. [[CrossRef](#)]
18. Zhao, Y.; Jin, Y.; Ren, Y.; Song, L.M.; Li, J.; Lin, X.P. Expression of matrix metalloproteinase-3 in patients with rheumatoid arthritis and its correlation with chronic periodontitis and rheumatoid arthritis. *Zhonghua Kou Qiang Yi Xue Za Zhi* **2019**, *54*, 164–169.
19. da Silva, M.K.; de Carvalho, A.C.G.; Alves, E.H.P.; da Silva, F.R.P.; Pessoa, L.D.S.; Vasconcelos, D.F.P. Genetic Factors and the Risk of Periodontitis Development: Findings from a Systematic Review Composed of 13 Studies of Meta-Analysis with 71,531 Participants. *Int. J. Dent.* **2017**, *2017*, 1914073. [[CrossRef](#)]
20. Urata, Y.; Uesato, R.; Tanaka, D.; Nakamura, Y.; Motomura, S. Treating to target matrix metalloproteinase 3 normalisation together with disease activity score below 2.6 yields better effects than each alone in rheumatoid arthritis patients: T-4 Study. *Ann. Rheum. Dis.* **2012**, *71*, 534–540. [[CrossRef](#)]
21. Marotte, H.; Miossec, P. Biomarkers for prediction of TNF α blockers response in rheumatoid arthritis. *Jt. Bone Spine* **2010**, *77*, 297–305. [[CrossRef](#)] [[PubMed](#)]
22. Savioli, C.; Ribeiro, A.C.M.; Fabri, G.M.C.; Calich, A.L.; Carvalho, J.; Silva, C.A.; Viana, V.S.; Bonfá, E.; Siqueira, J.T.T. Persistent periodontal disease hampers anti-tumor necrosis factor treatment response in rheumatoid arthritis. *J. Clin. Rheumatol.* **2012**, *18*, 180–184. [[CrossRef](#)] [[PubMed](#)]
23. Kimura, Y.; Yoshida, S.; Takeuchi, T.; Yoshikawa, A.; Hiramatsu, Y.; Ishida, T.; Makino, S.; Takasugi, Y.; Hanafusa, T. Periodontal pathogens participate in synovitis in patients with rheumatoid arthritis in clinical remission: A retrospective case–control study. *Rheumatology* **2015**. [[CrossRef](#)] [[PubMed](#)]
24. Marotte, H. Tooth-brushing: An impact on rheumatoid arthritis. *Jt. Bone Spine* **2016**, *83*, 619–621. [[CrossRef](#)]
25. Pers, J.-O.; Saraux, A.; Pierre, R.; Youinou, P. Anti-TNF- α immunotherapy is associated with increased gingival inflammation without clinical attachment loss in subjects with rheumatoid arthritis. *J. Periodontol.* **2008**, *79*, 1645–1651. [[CrossRef](#)] [[PubMed](#)]

26. Rodríguez-Lozano, B.; González-Febles, J.; Garnier-Rodríguez, J.L.; Dadlani, S.; Bustabad-Reyes, S.; Sanz, M.; Sánchez-Alonso, F.; Sánchez-Piedra, C.; González-Dávila, E.; Díaz-González, F. Association between severity of periodontitis and clinical activity in rheumatoid arthritis patients: A case-control study. *Arthritis Res. Ther.* **2019**, *21*, 27. [[CrossRef](#)]
27. Coat, J.; Demoersman, J.; Beuzit, S.; Cornec, D.; Devauchelle-Pensec, V.; Saraux, A.; Pers, J.-O.; Devauchelle-Pensec, V. Anti-B lymphocyte immunotherapy is associated with improvement of periodontal status in subjects with rheumatoid arthritis. *J. Clin. Periodontol.* **2015**, *42*, 817–823. [[CrossRef](#)] [[PubMed](#)]
28. Kobayashi, T.; Okada, M.; Ito, S.; Kobayashi, D.; Ishida, K.; Kojima, A.; Narita, I.; Murasawa, A.; Yoshie, H. Assessment of interleukin-6 receptor inhibition therapy on periodontal condition in patients with rheumatoid arthritis and chronic periodontitis. *J. Periodontol.* **2014**, *85*, 57–67. [[CrossRef](#)] [[PubMed](#)]
29. Okada, M.; Kobayashi, T.; Ito, S.; Yokoyama, T.; Abe, A.; Murasawa, A.; Yoshie, H. Periodontal Treatment Decreases Levels of Antibodies to Porphyromonas gingivalis and Citrulline in Patients with Rheumatoid Arthritis and Periodontitis. *J. Periodontol.* **2013**, *84*, e74–e84. [[CrossRef](#)] [[PubMed](#)]
30. Burt, B. Research, Science and Therapy Committee of the American Academy of Periodontology. Position paper: Epidemiology of periodontal diseases. *J. Periodontol.* **2005**, *76*, 1406–1419. [[CrossRef](#)] [[PubMed](#)]
31. Scher, J.U.; Ubeda, C.; Equinda, M.; Khanin, R.; Buischi, Y.; Viale, A.; Lipuma, L.; Attur, M.; Pillinger, M.H.; Weissmann, G.; et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. *Arthritis Rheum.* **2012**, *64*, 3083–3094. [[CrossRef](#)]
32. Ogrendik, M.; Kokino, S.; Ozdemir, F.; Bird, P.S.; Hamlet, S. Serum antibodies to oral anaerobic bacteria in patients with rheumatoid arthritis. *MedGenMed* **2005**, *7*, 2. [[PubMed](#)]
33. Bender, P.; Bürgin, W.B.; Sculean, A.; Eick, S. Serum antibody levels against Porphyromonas gingivalis in patients with and without rheumatoid arthritis—A systematic review and meta-analysis. *Clin. Oral Invest.* **2017**, *21*, 33–42. [[CrossRef](#)]
34. Cheng, Z.; Meade, J.; Mankia, K.; Emery, P.; Devine, D.A. Periodontal disease and periodontal bacteria as triggers for rheumatoid arthritis. *Best Pr. Res. Clin. Rheumatol.* **2017**, *31*, 19–30. [[CrossRef](#)] [[PubMed](#)]
35. Netea, M.G.; van Deuren, M.; Kullberg, B.J.; Cavallion, J.M.; Van der Meer, J.W. Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? *Trends Immunol.* **2002**, *23*, 135–139. [[CrossRef](#)]
36. Goh, C.E.; Kopp, J.; Papapanou, P.N.; Molitor, J.A.; Demmer, R.T. Association Between Serum Antibodies to Periodontal Bacteria and Rheumatoid Factor in the Third National Health and Nutrition Examination Survey: Periodontal Bacteria Antibodies and Rheumatoid Factor. *Arthritis Rheumatol.* **2016**, *68*, 2384–2393. [[CrossRef](#)]
37. König, M.F.; Abusleme, L.; Reinholdt, J.; Palmer, R.J.; Teles, R.P.; Sampson, K.; Rosen, A.; Nigrovic, P.A.; Sokolove, J.; Giles, J.T.; et al. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci. Transl. Med.* **2016**, *8*, 369ra176. [[CrossRef](#)]
38. Omura, K.; Takahashi, M.; Omura, T.; Miyamoto, S.; Kushida, K.; Sano, Y.; Nagano, A. Changes in the concentration of plasma matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases-1 (TIMP-1) after total joint replacement in patients with arthritis. *Clin. Rheumatol.* **2002**, *21*, 488–492. [[CrossRef](#)] [[PubMed](#)]
39. Reddy, N.R.; Roopa, D.; Babu, D.S.M.; Kumar, P.M.; Raju, C.M.; Kumar, N.S.; Reddy, N.; Raju, C.; Kumar, P.; Reddy, N.R.; et al. Estimation of matrix metalloproteinase-3 levels in gingival crevicular fluid in periodontal disease, health and after scaling and root planing. *J. Indian Soc. Periodontol.* **2012**, *16*, 549–552. [[CrossRef](#)]
40. Ban, A.; Inaba, M.; Furumitsu, Y.; Okamoto, K.; Yukioka, K.; Goto, H.; Nishizawa, Y. Time-course of health status in patients with rheumatoid arthritis during the first year of treatment with infliximab. *Biomed. Pharmacother.* **2010**, *64*, 107–112. [[CrossRef](#)]
41. Kawashiri, S.-Y.; Kawakami, A.; Ueki, Y.; Imazato, T.; Iwamoto, N.; Fujikawa, K.; Aramaki, T.; Tamai, M.; Nakamura, H.; Origuchi, T.; et al. Decrement of serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis (RA) patients achieving remission after 6 months of etanercept treatment: Comparison with CRP, IgM-RF, MMP-3 and anti-CCP Ab. *Jt. Bone Spine* **2010**, *77*, 418–420. [[CrossRef](#)] [[PubMed](#)]
42. Arvikar, S.L.; Collier, D.S.; Fisher, M.C.; Unizony, S.; Cohen, G.L.; McHugh, G.; Kawai, T.; Strle, K.; Steere, A.C. Clinical correlations with Porphyromonas gingivalis antibody responses in patients with early rheumatoid arthritis. *Arthritis Res. Ther.* **2013**, *15*, R109. [[CrossRef](#)]

43. Kaur, S.; Bright, R.; Proudman, S.M.; Bartold, P.M. Does periodontal treatment influence clinical and biochemical measures for rheumatoid arthritis? A systematic review and meta-analysis. *Semin. Arthritis Rheum.* **2014**, *44*, 113–122. [[CrossRef](#)] [[PubMed](#)]
44. Bobbio-Pallavicini, F.; Alpini, C.; Caporali, R.; Avalle, S.; Bugatti, S.; Montecucco, C. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. *Arthritis Res. Ther.* **2004**, *6*, R264–R272. [[CrossRef](#)] [[PubMed](#)]
45. Lequerré, T.; Jouen, F.; Brazier, M.; Clayssens, S.; Klemmer, N.; Ménard, J.-F.; Mejjad, O.; Daragon, A.; Tron, F.; Le Loët, X.; et al. Autoantibodies, metalloproteinases and bone markers in rheumatoid arthritis patients are unable to predict their responses to infliximab. *Rheumatology* **2007**, *46*, 446–453. [[CrossRef](#)] [[PubMed](#)]



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Article

Periodontal Health and Oral Microbiota in Patients with Rheumatoid Arthritis

Kaja Eriksson ^{1,*}, Guozhong Fei ², Anna Lundmark ¹, Daniel Benchimol ³, Linkiat Lee ¹, Yue O. O. Hu ^{4,5}, Anna Kats ¹, Saedis Saevarsdottir ⁶, Anca Irinel Catrina ⁶, Björn Klinge ^{1,7}, Anders F. Andersson ⁴, Lars Klareskog ⁶, Karin Lundberg ⁶, Leif Jansson ^{1,8} and Tülay Yucel-Lindberg ^{1,*}

¹ Department of Dental Medicine, Division of Periodontology, Karolinska Institutet, 14104 Huddinge, Sweden; anna.m.l.lundmark@gmail.com (A.L.); linkiatlee@gmail.com (L.L.); anna.kats@hotmail.com (A.K.); bjorn.klinge@ki.se (B.K.); leif.jansson@sll.se (L.J.)

² Center for Rheumatology, Academic Specialist Center, Stockholm Health Services, 10235 Stockholm, Sweden; guozhong.fei@sll.se

³ Department of Dental Medicine, Division of Orofacial Diagnostics and Surgery—Image and Functional Odontology, Karolinska Institutet, Huddinge 14104, Sweden; daniel.benchimol@ki.se

⁴ Science for Life Laboratory School of Biotechnology, KTH Royal Institute of Technology, 17121 Stockholm, Sweden; yue.hu@ki.se (Y.O.O.H.); anders.andersson@scilifelab.se (A.F.A.)

⁵ Department of Microbiology, Tumor and Cell Biology, Centre for Translational Microbiome Research (CTMR), Karolinska Institutet, 17164 Stockholm, Sweden

⁶ Department of Medicine, Rheumatology Unit, Karolinska University Hospital, Solna, 17176 Stockholm, Sweden; Saedis.Saevarsdottir@ki.se (S.S.); anca.catrina@ki.se (A.I.C.); Lars.Klareskog@ki.se (L.K.); Karin.Lundberg@ki.se (K.L.)

⁷ Department of Periodontology, Faculty of Odontology, Malmö University, 20506 Malmö, Sweden

⁸ Department of Periodontology at Eastmaninstitutet, Stockholm County Council, 11382 Stockholm, Sweden

* Correspondence: Kaja.Eriksson@ki.se (K.E.); Tulya.Lindberg@ki.se (T.Y.-L.); Tel.: +46-73-522 4998 (K.E.); +46-70-508 8126 (T.Y.-L.)

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Abstract: This study aimed to investigate the periodontal health of patients with established rheumatoid arthritis (RA) in relation to oral microbiota, systemic and oral inflammatory mediators, and RA disease activity. Forty patients underwent full-mouth dental/periodontal and rheumatological examination, including collection of blood, saliva, gingival crevicular fluid (GCF) and subgingival plaque. Composition of plaque and saliva microbiota were analysed using 16S rRNA sequencing and levels of inflammatory mediators by multiplex-immunoassay. The majority of the patients (75%) had moderate or severe periodontitis and the rest had no/mild periodontitis. Anti-citrullinated protein antibody (ACPA) positivity was significantly more frequent in the moderate/severe periodontitis (86%) compared to the no/mild group (50%). No significance between groups was observed for RA disease duration or activity, or type of medication. Levels of sCD30/TNFRSF8, IFN- α 2, IL-19, IL-26, MMP-1, gp130/sIL-6R β , and sTNF-R1 were significantly higher in serum or GCF, and April/TNFSF13 was significantly higher in serum and saliva samples in moderate/severe periodontitis. The microbial composition in plaque also differed significantly between the two groups. In conclusion, the majority of RA patients had moderate/severe periodontitis and that this severe form of the disease was significantly associated with ACPA positivity, an altered subgingival microbial profile, and increased levels of systemic and oral inflammatory mediators.

Keywords: rheumatoid arthritis; periodontitis; periodontal disease; anti-citrullinated protein autoantibodies; rheumatoid factor; smoking; medication; *Porphyromonas gingivalis*

1. Introduction

An increased risk of periodontitis has been reported in patients with rheumatoid arthritis (RA) as compared to healthy controls [1] and conversely, an association between chronic periodontal infection and risk of developing RA has been suggested [2,3]. The relationships between periodontitis and RA include similar pathological mechanisms of chronic inflammation and bone destruction [4–7], increased production of cytokines, prostaglandins and matrix-degrading enzymes, as well as shared risk factors where cigarette smoking is the most highlighted [2,4–6,8–10]. In periodontitis, the chronic inflammation, which results in the destruction of tooth-supporting structures, is initiated by periodontal pathogens such as *Porphyromonas gingivalis* and a dysbiotic microbial community surrounding the periodontium [11].

The underlying etiological processes in RA are not fully understood, although ever since the identification of antibodies to citrullinated protein antigens (ACPAs) as specific markers predictive for the development of RA, associated also with disease severity and joint destruction [12], increased attention has been given to the etiological mechanisms of ACPA production. These antibodies are directed against post-translationally modified proteins containing the amino acid citrulline generated by the enzyme peptidyl arginine deiminase (PAD) during the process of citrullination [12,13]. Some recent studies suggest that immunity against citrullinated proteins may be initiated at a mucosal site (e.g., the lung or gingiva), and others point to a cross-reactivity scenario between microbial amino acid sequences and citrullinated self-proteins resulting in ACPA production [11,14,15]. The periodontal pathogens *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* have been suggested to be involved in the generation of citrullinated antigens and the subsequent production of ACPA. Interestingly, *A. actinomycetemcomitans* was reported to induce hypercitrullination in host neutrophils, with hypercitrullination patterns similar to those observed in synovial fluid of RA patients [16]. *P. gingivalis*, on the other hand, has for some time been implicated in RA autoimmunity because of its unique ability to express a microbial PAD enzyme (*P. gingivalis* PAD, PPAD), which has the ability to citrullinate proteins, similar to the human PAD enzymes [2,17]. By citrullinating proteins in the periodontium, PPAD could trigger the production of ACPAs, which through epitope spreading may cross-react with citrullinated proteins in the joints resulting in a chronic inflammation and eventually joint destruction [2].

A relationship between periodontitis and RA has recently been supported by a systematic review and meta-analysis [1]. One of the largest studies that report an association (OR = 1.16; 95% CI: 1.12–1.20) between these two diseases was based on a register study including 13,779 Taiwanese patients with newly diagnosed RA and 137,790 controls [18]. A significant association between RA and periodontitis (OR = 1.17; 95% CI: 1.15–1.19, $p < 0.001$) was also reported in a Korean population based registry study comprising 57,024 patients with RA out of which 26,320 had periodontitis [19]. However, none of these studies were able to account for smoking, which is an important risk factor for periodontitis, as well as RA, and the studies lacked information about autoantibody status. Thus, the strength of the relationship between periodontitis and RA is still an area of interest for researchers and clinicians, as several studies have also failed to report an association between these two diseases. For example, in the largest prospective study conducted to date where 81,132 female nurses (292 RA and 80,840 controls) were followed for more than 12 years, no association was found between RA and periodontal surgery and/or tooth loss [20]. Likewise, in our recently published study of 6682 Swedish patients with established RA and matched healthy controls included in the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) registry, we reported no increased prevalence of periodontitis diagnosis in patients with RA as compared to controls, and no differences in periodontitis prevalence based on ACPA or rheumatoid factor (RF) status [21]. Importantly, however, in that study, we were not able to assess the severity of the periodontal diagnosis in patients with RA using clinical parameters of periodontal disease. The aim of this study was, therefore, to investigate the severity of periodontitis (defined as severe, moderate or no/mild) [22] in Swedish patients fulfilling the 2010 American College of Rheumatology (ACR) criteria for RA in relation to autoantibody status (ACPA and RF), inflammatory mediators, RA disease activity and medication as well as the microbiota in saliva and subgingival plaque.

2. Experimental Section

2.1. Study Population

A total of forty-five volunteers (age 29 to 80) with chronic arthritis (mean disease duration 11 years) were recruited from two Rheumatology clinics at Karolinska University Hospital in Solna and Huddinge (Stockholm, Sweden) between January 2016 and January 2017. Five participants were excluded from the study due to not fulfilling the inclusion criteria for RA (the 2010 ACR Criteria for RA) [23], or having other types of chronic arthritis. The recruited participants underwent a full mouth dental and periodontal examination (third molars not included), performed by a single dentist (KE) calibrated by a periodontist (LJ). The examiner (KE) did not have information about the patients periodontal or rheumatological measurements (clinical or laboratory) beforehand. Based on the results from the dental examinations, the patients were divided according to their periodontal status, following the standardised clinical definition of periodontitis (intended for use in population-based studies) established by the Centers for Disease Control and Prevention and the American Academy of Periodontology [22]. All patients completed a health screen questionnaire including information about comorbidities, medication, smoking and alcohol habits, body mass index (BMI) as well as questions regarding education and place of birth. The participants had not received any periodontal treatment for at least 3 months prior to the dental examination. Exclusion criteria included pregnancy, lactation, other forms of arthritis as well as the use of antibiotics the last 3 months prior to examination. This study was approved by the Regional Ethical Review Board in Stockholm (Dnr 2009/792-31/4 and 2015/766-32) and a written informed consent was obtained from all participants.

2.2. Clinical Assessments

The RA disease activity was assessed by using the DAS28 CRP (Disease Activity Score in 28-joints) [24] measuring total tender- and swollen- joint count (ranging from 0–28 joints), C-reactive protein (CRP, mg/dL), as well as the patient's global assessment of health on a 10 cm visual analogue scale. A DAS28 score of >5.1 was considered high disease activity, whereas <3.2 equaled low disease activity and ≤ 2.6 reflected remission [25]. Patients' self-reported functional status was measured using the Health Assessment Questionnaire (HAQ) [26], where functional ability was assessed by addressing eight general component categories (reach, grip, hygiene, dressing and grooming, eating, arising, walking and common daily activities). In the HAQ disability index, each question was scored from 0 to 3, corresponding to "without any difficulty" (score 0), "with some difficulty" (score 1), "with much difficulty" (score 2) and "unable to do" (score 3).

The periodontal condition was assessed by probing pocket depth (PPD), clinical attachment level (CAL) and bleeding index (BI) determined at 6 sites per tooth, and the presence of supragingival plaque (PI) at 4 sites per tooth. Stimulated salivary flow rate, number of missing and mobile teeth as well as the number of multirrooted teeth with furcation involvement were also recorded. Periodontitis, defined by following international consensus criteria, was based on the interproximal measurements of CAL and PPD, excluding the third molars [22]. The severity of the disease was defined as severe (corresponding to ≥ 2 interproximal sites with CAL ≥ 6 mm, not on the same tooth, and ≥ 1 interproximal sites with PPD ≥ 5 mm), moderate (corresponding to ≥ 2 interproximal sites with CAL ≥ 4 mm, not on the same tooth, or ≥ 2 interproximal sites with PPD ≥ 5 mm) or no/mild (corresponding to neither severe nor moderate criteria) [22]. In addition to the periodontal status, the examination also included assessment of soft tissue pathologies and the number of decayed, missing and filled permanent teeth and tooth surfaces (DMFT/DMFS) describing the amount of dental caries, where the DMFT can range from 0 to 28 and the DMFS from 0 to 128 [27].

2.3. Collection and Preparation of Gingival Crevicular Fluid, Plaque, Saliva and Blood Samples

Gingival crevicular fluid (GCF) was collected by inserting two paper strips (Periopaper, Proflow Inc., Amityville, NY, USA) until slight resistance, at both the mesiobuccal and the distobuccal

sites of the teeth with the deepest pockets. The paper strips were left within the gingival crevice for 30 s, pooled together and frozen at -80°C until processing [28].

Subgingival plaque samples were collected from the deepest pockets by inserting four sterile paper points (Nordenta, Enköping, Sweden), two at the distolingual and two at the mesiallingual sites of the tooth, and left for 20 s [29]. Before the paper points were inserted supragingival plaque was removed by using cotton pellets and the tooth surface was dried with air. The samples from the same tooth were pooled together and frozen at -80°C awaiting analysis.

Stimulated saliva samples were collected by using paraffin wax (1g, Ivoclar Vivadent, Liechtenstein), which the participants were instructed to chew on for a duration of 2 min. The volume of collected stimulated saliva was determined, the salivary flow rate recorded and the samples kept on ice until processing. Saliva samples were then centrifuged at $500\times g$ for 10 min at 5°C and supernatants collected and stored at -80°C until analysis [28].

Blood samples were collected in BD Vacutainer SST tubes (MediCarrier AB, Stockholm, Sweden). Tubes were left standing at room temperature for at least 30 min before storing, in order to remove cells and clotting factors by allowing a clot to form. The samples were then centrifuged at $200\times g$ for 10 min at 20°C and the serum stored at -80°C until analysis. The preparation of the samples is described in the Supplementary Methods (Preparation of samples).

2.4. Immunoassay Analysis

Serological markers of RA (ACPA and RF antibody status) were analysed using a multiplex immunoassay (Bio-Plex[®] 2200 system, Bio-Rad, Hercules, CA, USA) for ACPA and nephelometry for RF (Karolinska University Hospital Laboratory, Sweden). Results were interpreted as ACPA-positive/RF-positive following the cut-off values for seropositivity (3 E/mL for ACPA and 20 E/mL for RF).

Levels of CRP in serum (measured between intervals 0.2–380 mg/L) were analysed at Karolinska University Hospital Laboratory using a near-infrared particle immunoassay (NIPIA) method and Beckman reagents on Synchron LX20 automated equipment (Beckman Coulter, Fullerton, CA, USA) [30]. Concentrations of CRP in saliva and GCF samples were analysed via commercially available ELISA kit (USCN Life Science, Wuhan, China), according to the manufacturer's instructions. Briefly, PBS-diluted saliva (diluted 1:3) and GCF samples (diluted 1:2) were incubated with biotinylated CRP antibodies, followed by incubation with horseradish peroxidase conjugate. CRP-antibody levels were detected with tetramethylbenzidine reagent and the concentrations expressed as pg/ml. The detection limit of the CRP ELISA was 19.2 pg/ml with <12% inter-assay and <10% intra-assay variation.

Immunoassay kits (37-Plex inflammation panel, Bio-Rad, Hercules, CA, USA) were used to investigate the cytokine profile in serum (diluted 1:4), saliva and GCF (undiluted), according to manufacturer's protocol. The analysed cytokines (sensitivities in pg/ml, in brackets) were APRIL/TNFSF13 (190), BAFF/TNFSF13B (34.7), sCD30/TNFRSF8 (1.0), sCD163 (16.8), Chitinase 3-like 1 (10.3), gp130/sIL-6R β (16.9), IFN- α 2 (0.7), sIL-6R α (1.5), IL-8 (2.7), IL-10 (0.6), IL-11 (0.05), IL-12 (p70) (0.1), IL-19 (0.2), IL-20 (3.6), IL-22 (1.1), IL-26 (1.2), IL-27 (p28) (0.1), IL-29/IFN- λ 1 (1.6), IL-32 (12.3), IL-34 (51.9), IL-35 (3.7), LIGHT/TNFSF14 (10.2), MMP-1 (33.7), MMP-2 (39.7), MMP-3 (28.5), Osteocalcin (23.4), Osteopontin (91.3), Pentraxin-3 (0.8), sTNF-R1 (0.2), sTNF-R2 (3.2), TSLP (0.8) and TWEAK/TNFSF12 (0.5). The mean concentrations were expressed as pg/ml and the inflammatory mediators that were below the detection limit were excluded.

2.5. 16S rRNA Sequencing and Quantitative Polymerase Chain Reaction (qPCR)

The amount of bacterial DNA from saliva and subgingival plaque samples of the 40 included patients was determined by using the 7500 Fast Real-Time qPCR System (Applied Biosystems, Foster City, CA, USA). Ten samples with less than in total 0.5 ng/ μL DNA were excluded from qPCR analysis, whereas 3 samples with less than 0.5 ng/ μL DNA were excluded from 16S rRNA gene sequencing. For the preparation of the 16S rRNA amplicon libraries, 2 ng genomic DNA (gDNA) from each saliva and plaque sample were used as template to amplify the V3-V4 regions of the 16S

rRNA gene, with final concentrations for PCR reactions being 1X KAPA HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), 1.0 μM 341'F primer (CCTAHGGGRBGCAGCAG), 1.0 μM 805R primer (GACTACHVGGGTATCTAATCC) [31,32] and 0.1 ng/ μL gDNA. The PCR during amplification was programmed as follows, initial incubation at 98 °C for 2 min, 26 cycles of 98 °C for 20 s, 54 °C for 20 s, 72 °C for 15 s, followed by an elongation step of 72 °C for 2 min. The samples were then purified with Polyethylene Glycol 6000 (Merck Millipore, Darmstadt, Germany) and carboxylic acid beads (Dynabeads® MyOne™, Thermo Fisher Scientific, Waltham, MA, USA) [33]. For indexing the sample amplicons, 12 μL of the purified product, 0.4 μM forward and reverse indexing primers and KAPA HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA) were mixed for PCR amplification. The indexing step followed the PCR programme: 98 °C for 2 min, 10 cycles of 98 °C for 20 s, 62 °C for 30 s, 72 °C for 30 s and a final elongation step of 72 °C for 2 min. Equimolar amounts of indexed samples were mixed and sequenced on the Illumina MiSeq platform (Illumina Inc, San Diego, CA, USA) at NGI/SciLifeLab Stockholm. Three samples were excluded due to their low number of reads (cutoff 10,000 reads). The median depth of sequencing, after exclusion of low-depth libraries, was 188,600 reads per sample (IQR: 166,669–209,905 reads).

For the qPCR analysis of subgingival plaque samples, two sets of primers were used for bacteria detection, one species-specific pair for *P. gingivalis* and the other pair targeting the bacterial 16S rRNA gene [34,35]. The qPCR analyses were performed as described in the Supplementary Methods (Quantitative Polymerase Chain Reaction, qPCR).

2.6. Sequence Count Data Processing and Analysis

To process the sequencing data the DADA2 pipeline [36] was used. Amplicon reads with low quality and primers were trimmed and filtered. The remaining reads were then dereplicated, the sequence variants were inferred, and the paired-end reads were merged by requiring 30 bp overlap with no mismatches. Results of two sequencing runs were then merged and a sequence table was constructed, followed by removal of chimeras. The reads were taxonomically assigned using RDP training set 14 [37]. A phylogenetic tree was constructed using the scripts `align_seqs.py`, `filter_alignment.py`, and `make_phylogeny.py`, as provided by QIIME [38]. The sequence table, taxonomic assignment, and phylogenetic tree were used to create a phyloseq object [39], which was used in all subsequent analyses.

In total 6538 taxa were identified from the merged sequencing table. The final dataset included 1090 taxa, after removing the sequence variants that only present in 5% or less of the samples. The analysis of differential abundance of microbes, reported as genus species (*sp.*), between saliva samples and plaque samples as well as between samples from RA patients with no/mild and moderate/severe periodontitis was performed using DESeq2 [40]. All statistical analyses were conducted in R, version 3.3.3 (R Core Team 2015. R Foundation for Statistical Computing, Vienna, Austria).

2.7. Statistical Analysis

The characteristics of patients with moderate/severe periodontitis were compared to the subjects with no/mild periodontal disease. Mann–Whitney U test or independent two-sample t-test was performed for comparing ordinal and continuous variables. For dichotomous variables, Chi-square test or Fisher's exact test was used. The association between ACPA status, periodontitis severity and periodontal bacteria, estimated as odds ratio (OR) with 95% Confidence Interval (CI), was analysed using logistic regression analysis and adjusted for gender, age and smoking habits. All analyses were performed using the IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA) package. Statistical significance was determined at p value < 0.05.

3. Results

3.1. Characteristics of the Study Population

The characteristics of the RA study population are summarised in Table 1. The group consisted of 40 patients fulfilling the ACR criteria for RA. The mean age of the total study population was 60 ± 11 years and the majority of the participants were women (88%). With regard to the periodontal health, the majority, 75%, of the included subjects had moderate/severe periodontitis ($n = 30$), with the rest ($n = 10$) having no/mild periodontal disease. Based on periodontal diagnosis, there were no significant differences between the two groups with regard to gender, BMI, RA disease duration, place of birth, alcohol consumption or the level of education. The groups were also comparable with respect to comorbidities and medications. Subjects with moderate/severe periodontitis were significantly ($p = 0.010$) older (mean age 64 ± 7.8 years) compared to those with no/mild periodontal disease (mean age 50 ± 14 years). With regard to smoking, there were significantly ($p = 0.014$) more never smokers in the group with no/mild disease as compared to the group with moderate/severe periodontitis (60% and 17%, respectively).

Table 1. Characteristics of subjects with rheumatoid arthritis (RA) in relation to periodontal diagnosis.

| Characteristics | No/Mild Periodontitis ($n = 10$) | Moderate/Severe Periodontitis ($n = 30$) | <i>p</i> Value |
|--|------------------------------------|--|----------------|
| Gender, no (%) | | | |
| Female | 10 (100) | 25 (83) | |
| Male | 0 (0) | 5 (17) | 0.306 |
| Age, mean (\pm SD) | 50 (14) | 64 (7.8) | 0.004 |
| BMI, mean (\pm SD) | 26 (6.8) | 24 (5.6) | 0.325 |
| RA duration in years, mean (\pm SD) | 12 (11) | 10 (10) | 0.406 |
| Comorbidities, no (%) | | | |
| Diabetes | 0 (0) | 2 (6.7) | 1.000 |
| Cardiovascular disease | 3 (30) | 8 (27) | 1.000 |
| High blood pressure | 2 (20) | 5 (17) | 1.000 |
| Gastrointestinal disorders | 1 (10) | 6 (20) | 0.739 |
| Osteoporosis | 0 (0) | 3 (11) | 0.552 |
| Asthma | 0 (0) | 5 (17) | 0.306 |
| Sjögren's syndrome | 0 (0) | 2 (7.4) | 1.000 |
| TMJ | 4 (40) | 8 (27) | 0.451 |
| Medication, no (%) | | | |
| Analgesics | 6 (60) | 13 (43) | 0.473 |
| NSAIDs | 1 (10) | 10 (33) | 0.233 |
| DMARDs | 6 (60) | 22 (73) | 0.451 |
| Biological DMARDs | 5 (50) | 11 (37) | 0.482 |
| Glucocorticoids | 5 (50) | 18 (60) | 0.717 |
| Bisphosphonates | 1 (10) | 1 (3.0) | 0.442 |
| Smoking habits, no (%) | | | |
| Current smokers | 0 (0) | 5 (17) | 0.306 |
| Ex-smokers | 4 (40) | 19 (73) | 0.119 |
| Never smokers | 6 (60) | 5 (17) | 0.014 |
| Alcohol consumption, no (%) | | | |
| Monthly | 7 (70) | 20 (67) | 1.000 |
| Weekly | 5 (50) | 14 (47) | 1.000 |
| Daily | 0 (0) | 3 (10) | 0.560 |
| Never | 2 (20) | 3 (10) | 0.584 |
| Education, no (%) | | | |
| University degree | 4 (40) | 13 (46) | |
| No university degree | 6 (60) | 5 (54) | 1.000 |
| Place of birth, no (%) | | | |
| Sweden | 10 (100) | 24 (83) | |
| Other | 0 (0) | 5 (17) | 0.302 |

RA, rheumatoid arthritis; BMI, body mass index; TMJ, disorders involving the temporomandibular joint; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs; SD, standard deviation. Differences between the groups were analysed by Chi-square test or Fisher's exact test for categorical variables, and Mann-Whitney U-test continuous demographics. p value < 0.05 was considered statistically significant.

3.2. Periodontal and Dental Characteristics in Relation to Periodontitis Severity

During the periodontal and dental examinations, variables such as PI, BI, PPD, CAL, number of missing teeth, mobile and furcation involved teeth, DMFT/DMFS as well as stimulated salivary flow rate were recorded. The subjects with moderate/severe periodontitis had significantly higher (*p* values demonstrated in Supplementary Table S1) frequencies of most of the investigated variables, including PPD ≥ 4 mm, interproximal sites with PPD ≥ 5 mm, sites with CAL 1–2 mm, 3–4 mm or ≥ 5 mm, interproximal sites with CAL ≥ 4 mm or ≥ 6 mm, as well as significantly higher numbers of missing, mobile and furcation involved teeth, as compared to patients with no/mild periodontal disease (Supplementary Table S1). The group with no/mild periodontitis had no interproximal sites with PPD ≥ 5 mm or CAL ≥ 6 mm. Moreover, subjects with no/mild periodontitis had significantly less DMFT and DMFS as compared to those with moderate/severe disease. There were, however, no significant differences in PI or BI between the two groups, and also no differences in salivary flow rate (Supplementary Table S1).

3.3. Rheumatological Characteristics in Relation to Periodontal Status

The periodontal status in relation to rheumatological characteristics was also investigated. The majority (75%) of the study participants were ACPA-positive. When dividing the patients with regard to periodontitis severity, ACPA positivity was significantly (*p* = 0.032) more frequent in patients with moderate/severe periodontitis (86%), compared to the group with no/mild disease (50%) (Table 2). In addition, RF positivity showed a similar trend, although did not reach statistical significance. There were no significant differences between no/mild and moderate/severe periodontitis with regard to self-reported HAQ score, the DAS28 score reported by the patients rheumatologists or the CRP-levels in serum, saliva or GCF. With regard to RA disease activity, most of the participants had moderate disease activity (5 in the no/mild and 9 in moderate/severe group) or were in remission (4 and 10, respectively), irrespective of periodontal status (Table 2).

Table 2. Rheumatological and serological characteristics in relation to periodontal diagnosis.

| Characteristics | No/Mild Periodontitis (n = 10) | Moderate/Severe Periodontitis (n = 30) | <i>p</i> Value |
|--|--------------------------------|--|----------------|
| ACPA status, no (%) | | | |
| ACPA-positive | 5 (50) | 25 (86) | 0.032 |
| ACPA-negative | 5 (50) | 4 (14) | |
| RF status, no (%) | | | |
| RF-positive | 5 (50) | 22 (73) | 0.246 |
| RF-negative | 5 (50) | 8 (27) | |
| HAQ score (range 0–3), mean (\pmSD) | 0.7 (0.5) | 0.9 (0.6) | 0.380 |
| DAS28 score (range 1–10), mean (\pmSD) | 2.7 (1.5) | 3.3 (1.4) | 0.411 |
| Disease activity (DAS28 score), no (%) | | | |
| Remission (score < 2.6) | 4 (44) | 10 (37) | 1.000 |
| Low activity (score 2.6–3.2) | 0 (0) | 4 (15) | 0.553 |
| Moderate activity (score 3.2–5.1) | 5 (56) | 9 (33) | 0.432 |
| High activity (score >5.1) | 0 (0) | 4 (15) | 0.553 |
| Serum CRP (ng/mL), mean (\pmSD) | 5100 (7300) | 4300 (5200) | 0.353 |
| Salivary CRP (ng/mL), mean (\pmSD) | 1.4 (1.9) | 2.1 (5.4) | 0.749 |
| GCF CRP (ng/mL), mean (\pmSD) | 2.2 (3.6) | 2.3 (5.4) | 0.617 |

ACPA, anti-citrullinated protein antibodies; RF, rheumatoid factor; HAQ, health assessment questionnaire; DAS28, 28-joint disease activity score; CRP, C-reactive protein; GCF, gingival crevicular fluid; SD, standard deviation. Differences between the groups were analysed by chi-square test or Fisher’s exact test for categorical variables, and Mann–Whitney U-test for ordinal or continuous demographics. *p* value < 0.05 was considered statistically significant.

In the next series of studies, the participants were also analysed with regard to ACPA status. The ACPA-positive patients were taking significantly (*p* = 0.040) more non-steroidal anti-inflammatory drugs (NSAIDs) compared to the ACPA-negative subjects with RA (37% versus 0%, respectively) (Supplementary Table S2). There were, however, no differences in other medications (analgesics,

DMARDs, biological DMARDs, glucocorticoids or bisphosphonates) based on ACPA status. Moreover, in the ACPA-positive group there were significantly ($p = 0.038$) more ex-smokers, as compared to the ACPA-negative group. In contrast, the ACPA-negative participants were mostly never smokers ($p = 0.032$). There were no significant differences in gender, age, BMI, RA disease duration, comorbidities, alcohol consumption, education or place of birth based on ACPA status (Supplementary Table S2). Moreover, based on ACPA status, there were no significant differences between the groups regarding plaque index (ACPA-positive $51\% \pm 19\%$; ACPA-negative $40\% \pm 18\%$) or bleeding index (ACPA-positive $32\% \pm 22\%$; ACPA-negative $41\% \pm 14\%$).

3.4. Levels of Inflammatory Mediators in Serum, Saliva and GCF Samples

The levels of inflammatory mediators were analysed in serum, saliva and GCF samples from RA patients with no/mild and moderate/severe periodontitis (Figure 1 and Table 3). In serum, RA subjects with moderate/severe periodontitis had significantly higher levels of APRIL (TNFSF13) ($p = 0.013$), sCD30 (TNFRSF8) ($p = 0.048$) and gp130 (sIL-6R β) ($p = 0.01$) compared to patients with no/mild form of periodontitis (Figure 1A–C). Similarly, patients with moderate/severe periodontitis had significantly higher salivary levels of APRIL ($p = 0.048$) compared to those no/mild periodontal disease (Figure 1D). In contrast, the salivary levels of Chitinase 3-like 1 were significantly ($p = 0.041$) lower in patients with moderate/severe periodontitis compared to no/mild periodontitis group (Figure 1E). In GCF samples, significantly ($p < 0.05$) higher levels of IFN- α 2, IL-19, IL-26, MMP-1 and sTNF-R1 were observed in RA patients with moderate/severe periodontitis compared to corresponding RA subjects with no/mild periodontitis (Table 3). However, there were no significant differences in total protein concentrations determined in saliva or GCF samples (Figure 1F and Table 3, respectively) between the two groups.

Table 3. Levels of inflammatory mediators (mean \pm SD) and total protein (mg/mL) in gingival crevicular fluid (GCF) of subjects with RA in relation to periodontal diagnosis.

| Variables | No/Mild Periodontitis (n = 9) | Moderate/Severe Periodontitis (n = 29) | p Value |
|-----------------------------|----------------------------------|---|---------|
| APRIL (TNFSF13) | 5289 (2451) | 4584 (935) | 0.652 |
| BAFF (TNFSF13B) | 2229 (736) | 2982 (1767) | 0.440 |
| sCD30 (TNFRSF8) | 9.0 (11) | 21 (18) | 0.077 |
| sCD163 | 2980 (2727) | 3678 (2835) | 0.400 |
| Chitinase 3-like 1 | 7284 (3881) | 12,885 (9084) | 0.118 |
| gp130 (sIL-6R β) | 585 (424) | 713 (828) | 0.823 |
| IFN- α 2 | 20 (27) | 43 (31) | 0.036 |
| sIL-6R α | 456 (339) | 786 (594) | 0.175 |
| IL-8 | 761 (372) | 1274 (995) | 0.345 |
| IL-10 | 2.0 (1.6) | 4.3 (5.5) | 0.104 |
| IL-11 | 1.9 (2.1) | 2.6 (4.4) | 0.692 |
| IL-12 (p70) | 2.9 (3.2) | 3.8 (4.4) | 0.635 |
| IL-19 | 14 (13) | 32 (37) | 0.035 |
| IL-20 | 68 (51) | 76 (26) | 0.718 |
| IL-22 | 6.6 (4.5) | 10 (9.1) | 0.353 |
| IL-26 | 15 (11) | 36 (62) | 0.046 |
| IL-27 (p28) | 21 (11) | 21 (10) | 0.810 |
| IL-29 (IFN- γ 1) | 52 (61) | 103 (186) | 0.198 |
| IL-32 | 103 (55) | 140 (92) | 0.319 |
| IL-34 | 205 (127) | 184 (101) | 0.436 |
| LIGHT (TNFSF14) | 269 (93) | 314 (117) | 0.328 |
| MMP-1 | 897 (636) | 1294 (534) | 0.049 |
| MMP-3 | 394 (298) | 489 (371) | 0.579 |
| Pentraxin-3 | 375 (261) | 429 (272) | 0.718 |
| sTNF-R1 | 259 (130) | 523 (361) | 0.015 |
| sTNF-R2 | 2075 (5013) | 1976 (5322) | 0.311 |
| TSLP | 24 (20) | 25 (15) | 0.796 |
| TWEAK (TNFSF12) | 27 (17) | 38 (16) | 0.074 |
| Total protein concentration | 0.2 (0.2) | 0.3 (0.3) | 0.503 |

GCF, gingival crevicular fluid; RA, rheumatoid arthritis; SD, standard deviation. Differences between the groups were analysed by Mann–Whitney U-test. p value < 0.05 was considered statistically significant.

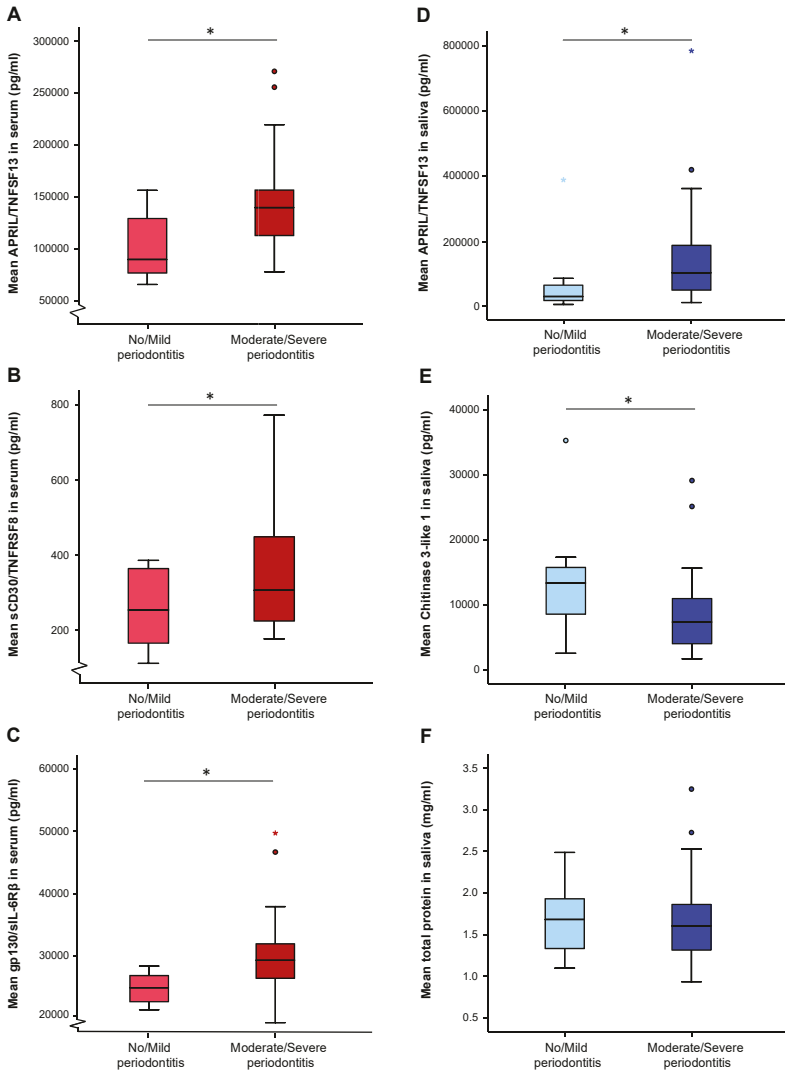


Figure 1. Levels of inflammatory mediators in serum and saliva samples. Mean (\pm SD) serum concentrations of APRIL/TNFSF13 (A), sCD30/TNFRSF8 (B) and gp130/sIL-6R β (C); mean (\pm SD) saliva concentrations of APRIL/TNFSF13 (D), Chitinase 3-like 1 (E) and levels of total protein in saliva (F) from RA patients with no/mild and moderate/severe periodontitis. The differences between the groups were analysed by Mann–Whitney U-test. * p value < 0.05 was considered statistically significant. Circle indicates outlier and star indicates far outlier.

3.5. Microbial Profile of Subgingival Plaque and Saliva Samples

The oral microbial profiles of the RA patients were analysed by 16S rRNA gene sequencing plaque and saliva samples. The most highly differentially abundant microbes in plaque samples compared to saliva samples, irrespective of periodontal status, are demonstrated in Figure 2. The ten most significantly ($p > 0.05$) enriched microbes detected in plaque compared to saliva were *Actinomyces meyeri*, *Prevotella nigrescens*, *Treponema socranskii*, *Treponema* sp., *Eubacterium infirmum*, *Prevotella oris*,

Actinomyces massiliensis, *Catonella* sp. and two non-identified species (NA spp.). In contrast, in saliva samples the most enriched microbes included *Butyrivibrio* sp., *Atopobium parvulum*, *Prevotella pallens*, *Solobacterium moorei*, *Centipeda* sp., two *Veillonella* spp., as well as three *Prevotella* spp. (Figure 2). The oral microbial profiles of patients with RA were also investigated in relation to periodontal status (Figure 3). The results showed that patients with moderate/severe periodontitis had significantly ($p < 0.05$) higher abundance of *Desulfobulbus* sp., *Prevotella* sp., *Bulleidia* sp., *Capnocytophaga* sp., *Tannerella forsythia* and a NA sp. in plaque, when compared to RA with no/mild periodontitis. In contrast, *Prevotella oris* and a *Porphyromonas* sp. were more abundant in patients with no or mild periodontitis (Figure 3). In saliva, there were no significant differences in the detected bacterial species based on periodontal diagnosis (Supplementary Table S3).

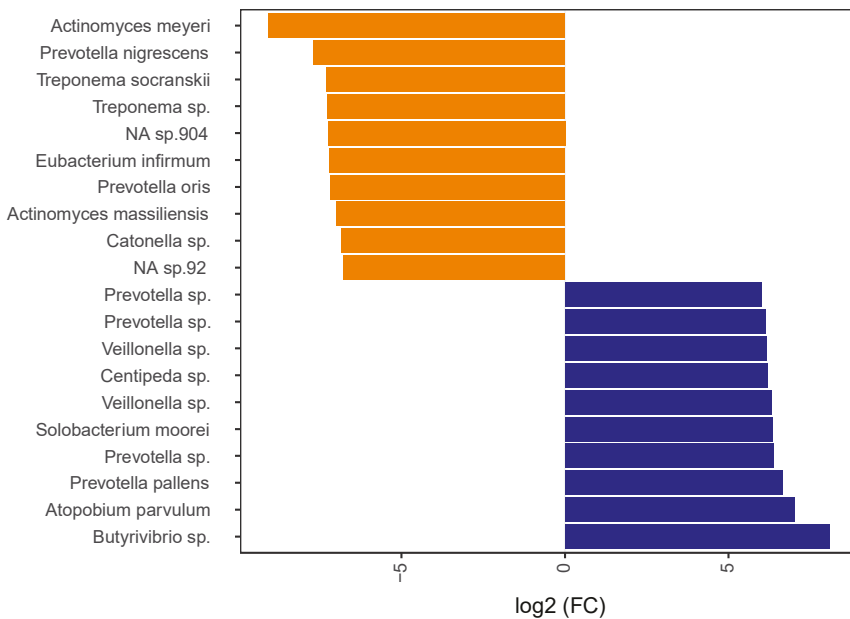


Figure 2. Most highly differentially abundant bacterial species in plaque compared to saliva samples. The graph demonstrates log2 Fold Change (FC) for the ten most enriched as well as the ten most depleted bacteria in plaque compared to saliva from patients with RA irrespective of periodontitis. Negative log2 FC indicates higher abundance in plaque samples (orange bars) and positive values indicate higher abundances in saliva samples (blue bars). NA, genome reference not available. The differences between the groups were analysed using DESeq2 [40]. *Adjusted p value < 0.05 was considered statistically significant.

Subgingival plaque samples of patients were also analysed for the presence and quantity of *P. gingivalis* using qPCR. The results showed that *P. gingivalis* was present in higher degree (62%) in the moderate/severe periodontitis group compared to no/mild periodontitis group (50%), although the difference was not significant (Supplementary Table S4).

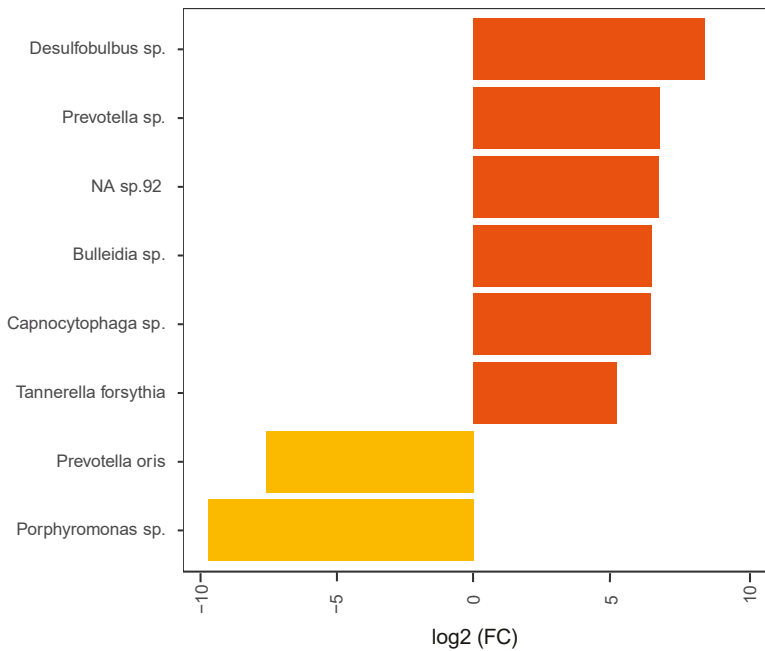


Figure 3. Most highly differentially abundant bacteria in plaque from patients with RA in relation to periodontitis severity. The graph demonstrates log₂ Fold Change (FC) for the most highly enriched and depleted bacteria in plaque from RA patients with no/mild and moderate/severe periodontitis. Negative log₂ FC indicate higher abundance in patients with no/mild periodontitis (light orange bars) and positive values indicate higher abundances in those with moderate/severe periodontitis (dark orange bars). NA, genome reference not available. The differences between the groups were analysed using DESeq2 [40]. *Adjusted *p* value < 0.05 was considered statistically significant.

4. Discussion

The objective of this study was to investigate the severity of periodontitis in relation to RA-associated clinical and immunological parameters, medication and comorbidities in patients with established RA. In this well-defined cohort, our results show that moderate/severe periodontitis is common in patients with RA, especially in ACPA-positive RA. In addition, we identified significantly enriched/differentially abundant bacteria in subgingival plaque of RA patients with moderate/severe periodontitis compared to those with no or mild periodontal disease. Moreover, the levels of both systemic and oral (saliva and GCF) inflammatory mediators were significantly higher in RA with moderate/severe periodontitis. Notably, the proliferation-inducing ligand APRIL (TNFSF13), a member of the TNF-receptor superfamily, was significantly increased in serum and saliva samples.

ACPAs are important diagnostic and prognostic serological markers in RA, and their presence is known to predict the development of RA [41]. In the current study, our results showed that ACPA positivity was significantly more common in RA patients with moderate/severe periodontitis when compared to those with no/mild form of disease. In agreement with our results, a more severe form of periodontitis (indicated by higher percentage of sites with alveolar bone loss) was reported in ACPA-positive patients with RA, in an American cohort [42]. Conversely, in a recent study, ACPA positivity only correlated with the gingival inflammation markers bleeding on probing and gingival index, but not with the severity of periodontitis assessed by CAL or PPD [43]. In our study, we found no differences in oral hygiene or gingival inflammation based on ACPA status. One possible

explanation for this might be that the ACPA-positive patients in our cohort were taking significantly more NSAIDs, which may reduce and mask the signs of gingival inflammation [44].

Using 16S rRNA gene sequencing, previous studies have investigated the subgingival microbiome of patients with RA and compared the results of subjects with osteoarthritis and healthy controls without periodontitis [45,46]. These studies did neither report subgingival profiles/compositions discriminating between RA and osteoarthritis [45], nor did they demonstrate differences between RA and controls without periodontitis with regard to the periodontal pathogens *P. gingivalis* or *A. actinomycetemcomitans* [46]. In our study, investigating the oral microbial composition in RA based on periodontitis severity, the subgingival microbial profile differed significantly between patients with moderate/severe and no/mild periodontitis. The species observed in higher abundance in moderate/severe periodontitis were *Desulfobulbus* sp., *Prevotella* sp., *NA* sp., *Bulleidia* sp., *Capnocytophaga* sp. and *Tannerella forsythia*, and two were more abundant in no/mild periodontitis (*Prevotella oris* and a *Porphyromonas* sp.). Similarly, in Norwegian RA cohort, the microbial composition of subgingival plaque also differed in relation to periodontal severity measures (such as gingival bleeding and PPD) [47]. However, in that study, RA disease status and medication (glucocorticosteroids) were found to be associated with different microbiome composition [47], in contrast to our study where no differences were found between no/mild and moderate/severe periodontitis group in terms of these potential confounding factors, therefore likely not affecting our results. Moreover, the prevalence of the periodontal pathogen *P. gingivalis*, assessed in 50 - 62% of the patients in our study, is in line with previous reports showing that this bacterium was present in 47% of patients with established RA and in 55% of newly-diagnosed RA patients [48]. We did not observe any significant differences in the prevalence of *P. gingivalis* in subgingival plaque based on the severity of periodontitis. In a previous study by Scher et al., *P. gingivalis* was reported to be more prevalent in newly-diagnosed treatment naïve RA patients with advanced periodontitis as compared to a corresponding group of RA without periodontitis [48]. This discrepancy could potentially be explained by the use of RA-medications, supported by the finding that DMARDs may modulate the microbiome in patients with RA [49]. It has been hypothesised that microbiome changes are partly driven by systemic inflammation and can be modulated by DMARDs, abating the inflammatory response [50]. Even though there were no differences in type of medications used between the groups in our study, we did not investigate the use of specific subgroups of DMARDs or biological DMARDs. Moreover, as proposed by Zhang et al., the differences in modulation of the oral microbiome could also be due to differences among RA patients [49], or possibly differences in response to RA-medication, which may explain why prevalence of *P. gingivalis* did not differ between RA patients with no/mild and moderate/severe periodontitis despite similar use of DMARDs therapy in the groups.

Inflammatory mediators play a central role in the pathogenesis of chronic inflammatory diseases such as periodontitis and RA, promoting autoimmunity and maintaining a chronic inflammation that collectively contribute to tissue and bone destruction [5,51]. Persistently increased levels of various pro-inflammatory cytokines (e.g., TNF- α and IL-6) have been well documented in RA and periodontitis, and several cytokine-targeting therapies are successfully used in RA treatment [52,53]. In the present study, the levels of sCD30 (TNFRSF8), sTNF-R1, gp130 (sIL-6R β), IL-19, IL-26 and MMP-1 were increased in serum, saliva or GCF samples from subjects with moderate/severe periodontitis as compared to no/mild disease. Interestingly, RA patients with moderate/severe periodontitis also had increased levels of APRIL both in serum and in saliva samples. APRIL, also known as TNFSF13, is a proliferation ligand and a member of the TNF-receptor superfamily [54]. This cytokine has together with BAFF an important role in the survival and maturation of B-cells [54], and could therefore potentially be important for the production of antibodies in RA. The overexpression of APRIL in serum has been reported in several autoimmune diseases including RA [54], and increased expression of APRIL mRNA has also been detected in gingival tissue from patients with periodontitis when compared to non-periodontitis controls [55]. However, to our knowledge this is the first study reporting significantly different levels of APRIL both in serum and saliva samples of RA patients with different

degrees of periodontal disease. Thus, given that overexpression of APRIL/BAFF is suggested to contribute to the autoimmune diseases [54,56], this system may be a key mediator involved in the link between RA and periodontitis. Only one protein, Chitinase 3-Like-1 (also known as YKL-40), was significantly reduced in saliva of patients with moderate/severe periodontitis. Although, this protein is a potential inflammatory biomarker in arthritis, its role as a marker for disease diagnosis is debated [57]. In RA, circulating levels of Chitinase 3-Like-1 reflect cartilage degradation and synovial inflammation [57,58], and in osteoarthritis this cartilage glycoprotein have been suggested as a potential target for treatment [59]. To our knowledge, this is the first study reporting the levels of Chitinase 3-Like-1 in saliva, both in RA and periodontitis. In serum, increased levels of this cytokine have previously been reported in both diseases and seem to correlate with disease activity at least in RA subjects [60,61]. The duration of treatment with RA medication, however, may have different effects (both increase and decrease) on the levels of Chitinase 3-Like-1 in patients with RA. Knudsen et al. showed that the serum levels of the protein is reduced after 4-, 8- and 12 weeks of methotrexate therapy, but not after 16 weeks [62]. Although the levels of serum and salivary cytokines do not always correlate [63], one explanation for the decreased levels of Chitinase 3-Like-1, observed in our study, might be due to potential differences in the duration of methotrexate therapy between the groups. Unfortunately, we were not able to investigate this due to lack of information about the duration of different RA therapies.

In this study, we did not observe any association between periodontitis severity and RA disease activity in terms of DAS28, the self-assessed health (HAQ-score) or in the levels of CRP (serum, saliva or GCF). In agreement with these findings, several studies report no impact of the severity of periodontitis on RA disease activity or on RA-associated serological markers [64–66]. For example, in a cohort of 100 RA patients and 112 matched controls, no association was found between RA disease activity (assessed by DAS28 score) and periodontitis severity [65]. In contrast, some studies have detected an association between periodontitis/alveolar bone loss and measurements of RA disease activity/severity such as DAS28, CRP, HAQ, tender joint count and/or joint space narrowing scores [42,67–69]. Three of these studies, conducted in the same cohort of 287 RA subjects, reported an association between periodontitis diagnosis/alveolar bone loss or self-reported “loose teeth” and higher DAS28-/HAQ-score and tender/swollen joint count [42,68,69]. The inconsistent results from different studies and the lack of association between periodontitis and RA may be due to RA medication. For example, DMARDs and biological DMARDs could be confounding factors potentially masking an association by decreasing RA disease activity or periodontitis severity [70–73]. In our study, however, there were no differences in type of medication used based on periodontitis, suggesting that the type of medication alone may not explain the lack of association between periodontitis and RA disease activity in this study. It is, however, plausible that the patients may respond differently to RA medication, or that different subtypes of DMARDs/biological DMARDs may affect periodontitis differently. In addition, the contrasting results between the different studies may also be due to the lack of uniformity in defining periodontitis [74].

One of the strengths of the current study is the well-characterised RA cohort and data set, including information on several potential confounding factors for RA and periodontitis, such as RA disease duration, BMI, comorbidities, type of RA medication used, smoking, alcohol consumption and education. In addition, the gender distribution also reflected the general RA population as the majority of the participants were women [75]. Furthermore, despite the limited number of patients in this pilot study (especially in the no/mild periodontitis group), our results still indicate that cigarette-exposure is associated with the ACPA-positive RA subset as well as with the severity of periodontitis, in agreement with previous findings [76,77]. A limitation of this study is the higher age of the patients with moderate/severe periodontitis compared to no/mild disease. Since age is known to be strongly correlated with periodontitis disease severity [76], this might potentially affect the results. On the other hand, no differences in age were detected between the groups based on ACPA status, showing an increased frequency of ACPA positivity among patients with moderate/severe periodontitis. Nevertheless, the results should be interpreted with caution due to limitations of the

study including small samples size and the lack of information about the duration of different RA medications. Furthermore, data about the levels of ACPA and RF were lacking, and we have instead used a positive/negative antibody classification following the ACR classifications criteria for RA. Additional studies using the levels of antibodies, particularly the fine specificities of anti-CCP/ACPA antibodies, in relation to the disease outcome could be relevant.

5. Conclusions

In conclusion, our findings demonstrate that patients with ACPA-positive RA have more severe forms of periodontitis, irrespective of DMARD therapy or the presence of subgingival *P. gingivalis*. Moreover, our data show a different subgingival microbial profile in RA patients with moderate/severe periodontitis *versus* no/mild periodontal disease. In addition, we also report, to our knowledge for the first time, that RA subjects with moderate/severe periodontitis have increased serum and salivary levels of the proliferation-inducing ligand APRIL. This cytokine, known to be important for B-cell survival and maturation, could potentially be involved in the association between RA and periodontitis, although additional studies including larger number of patients are required for confirmation of this finding.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/8/5/630/s1>, Table S1: Periodontal and dental characteristics in relation to periodontal diagnosis, Table S2: Characteristics of subjects with RA in relation to the ACPA status, Table S3: Most abundant bacteria in saliva of patients with RA in relation to the periodontal diagnosis, Table S4. Presence of subgingival *P. gingivalis* in relation to the periodontal diagnosis. Supplementary Methods.

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References

1. Fuggle, N.R.; Smith, T.O.; Kaul, A.; Sofat, N. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis. *Front Immunol.* **2016**, *7*, 80. [CrossRef] [PubMed]
2. Rosenstein, E.D.; Greenwald, R.A.; Kushner, L.J.; Weissmann, G. Hypothesis: The humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* **2004**, *28*, 311–318. [CrossRef] [PubMed]
3. Leech, M.T.; Bartold, P.M. The association between rheumatoid arthritis and periodontitis. *Best Pract. Res. Clin. Rheumatol.* **2015**, *29*, 189–201. [CrossRef]
4. Klareskog, L.; Catrina, A.I.; Paget, S. Rheumatoid arthritis. *Lancet* **2009**, *373*, 659–672. [CrossRef]
5. McInnes, I.B.; Schett, G. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* **2011**, *365*, 2205–2219. [CrossRef]
6. Bascones, A.; Noronha, S.; Gomez, M.; Mota, P.; Gonzalez Moles, M.A.; Villarroel Dorrego, M. Tissue destruction in periodontitis: Bacteria or cytokines fault? *Quintessence Int.* **2005**, *36*, 299–306.

7. Janssen, K.M.; Vissink, A.; de Smit, M.J.; Westra, J.; Brouwer, E. Lessons to be learned from periodontitis. *Curr. Opin. Rheumatol.* **2013**, *25*, 241–247. [[CrossRef](#)] [[PubMed](#)]
8. Bartold, P.M.; Marshall, R.I.; Haynes, D.R. Periodontitis and rheumatoid arthritis: A review. *J. Periodontol.* **2005**, *76*, 2066–2074. [[CrossRef](#)]
9. Stabholz, A.; Soskolne, W.A.; Shapira, L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontol 2000* **2010**, *53*, 138–153. [[CrossRef](#)]
10. Rutger Persson, G. Rheumatoid arthritis and periodontitis—Inflammatory and infectious connections. Review of the literature. *J. Oral Microbiol.* **2012**, *4*. [[CrossRef](#)]
11. Hajishengallis, G. Periodontitis: From microbial immune subversion to systemic inflammation. *Nat. Rev. Immunol.* **2015**, *15*, 30–44. [[CrossRef](#)]
12. Trouw, L.A.; Huizinga, T.W.; Toes, R.E. Autoimmunity in rheumatoid arthritis: Different antigens—Common principles. *Ann. Rheum. Dis.* **2013**, *72* (Suppl. 2), ii132–ii136. [[CrossRef](#)]
13. Klareskog, L.; Ronnelid, J.; Lundberg, K.; Padyukov, L.; Alfredsson, L. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu. Rev. Immunol.* **2008**, *26*, 651–675. [[CrossRef](#)]
14. van der Woude, D.; Catrina, A.I. HLA and anti-citrullinated protein antibodies: Building blocks in RA. *Best Pract. Res. Clin. Rheumatol.* **2015**, *29*, 692–705. [[CrossRef](#)]
15. Lundberg, K.; Wegner, N.; Yucel-Lindberg, T.; Venables, P.J. Periodontitis in RA—the citrullinated enolase connection. *Nat. Rev. Rheumatol.* **2010**, *6*, 727–730. [[CrossRef](#)]
16. Konig, M.F.; Abusleme, L.; Reinholdt, J.; Palmer, R.J.; Teles, R.P.; Sampson, K.; Rosen, A.; Nigrovic, P.A.; Sokolove, J.; Giles, J.T.; et al. Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci. Transl. Med.* **2016**, *8*, 369ra176. [[CrossRef](#)]
17. Wegner, N.; Wait, R.; Sroka, A.; Eick, S.; Nguyen, K.A.; Lundberg, K.; Kinloch, A.; Culshaw, S.; Potempa, J.; Venables, P.J. Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and alpha-enolase: Implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum.* **2010**, *62*, 2662–2672. [[CrossRef](#)]
18. Chen, H.H.; Huang, N.; Chen, Y.M.; Chen, T.J.; Chou, P.; Lee, Y.L.; Chou, Y.J.; Lan, J.L.; Lai, K.L.; Lin, C.H.; et al. Association between a history of periodontitis and the risk of rheumatoid arthritis: A nationwide, population-based, case-control study. *Ann. Rheum. Dis.* **2013**, *72*, 1206–1211. [[CrossRef](#)]
19. Lee, J.H.; Lee, J.S.; Park, J.Y.; Choi, J.K.; Kim, D.W.; Kim, Y.T.; Choi, S.H. Association of Lifestyle-Related Comorbidities With Periodontitis: A Nationwide Cohort Study in Korea. *Medicine (Baltimore)* **2015**, *94*, e1567. [[CrossRef](#)] [[PubMed](#)]
20. Arkema, E.V.; Karlson, E.W.; Costenbader, K.H. A prospective study of periodontal disease and risk of rheumatoid arthritis. *J. Rheumatol.* **2010**, *37*, 1800–1804. [[CrossRef](#)] [[PubMed](#)]
21. Eriksson, K.; Nise, L.; Kats, A.; Luttrupp, E.; Catrina, A.I.; Askling, J.; Jansson, L.; Alfredsson, L.; Klareskog, L.; Lundberg, K.; et al. Prevalence of Periodontitis in Patients with Established Rheumatoid Arthritis: A Swedish Population Based Case-Control Study. *PLoS ONE* **2016**, *11*, e0155956. [[CrossRef](#)]
22. Page, R.C.; Eke, P.I. Case definitions for use in population-based surveillance of periodontitis. *J. Periodontol.* **2007**, *78*, 1387–1399. [[CrossRef](#)] [[PubMed](#)]
23. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O., 3rd; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* **2010**, *62*, 2569–2581. [[CrossRef](#)] [[PubMed](#)]
24. Prevoo, M.L.; van't Hof, M.A.; Kuper, H.H.; van Leeuwen, M.A.; van de Putte, L.B.; van Riel, P.L. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum.* **1995**, *38*, 44–48. [[CrossRef](#)] [[PubMed](#)]
25. Fransen, J.; Creemers, M.C.; Van Riel, P.L. Remission in rheumatoid arthritis: Agreement of the disease activity score (DAS28) with the ARA preliminary remission criteria. *Rheumatology (Oxford)* **2004**, *43*, 1252–1255. [[CrossRef](#)]
26. Fries, J.F.; Spitz, P.; Kraines, R.G.; Holman, H.R. Measurement of patient outcome in arthritis. *Arthritis Rheum.* **1980**, *23*, 137–145. [[CrossRef](#)]
27. Cappelli, D.P.; Mobley, C.C. *Prevention in Clinical Oral Health Care*, 1st ed.; Elsevier Health Sciences: New York, NY, USA, 2007; p. 6.

28. Lundmark, A.; Johannsen, G.; Eriksson, K.; Kats, A.; Jansson, L.; Tervahartiala, T.; Rathnayake, N.; Akerman, S.; Klinge, B.; Sorsa, T.; et al. Mucin 4 and matrix metalloproteinase 7 as novel salivary biomarkers for periodontitis. *J. Clin. Periodontol.* **2017**, *44*, 247–254. [[CrossRef](#)]
29. Nickles, K.; Scharf, S.; Rollke, L.; Mayer, I.; Mayer, M.; Eickholz, P. Detection of subgingival periodontal pathogens—comparison of two sampling strategies. *Clin. Oral Investig.* **2016**, *20*, 571–579. [[CrossRef](#)]
30. Hensvold, A.H.; Frisell, T.; Magnusson, P.K.; Holmdahl, R.; Askling, J.; Catrina, A.I. How well do ACPA discriminate and predict RA in the general population: A study based on 12 590 population-representative Swedish twins. *Ann. Rheum. Dis.* **2017**, *76*, 119–125. [[CrossRef](#)]
31. Hugerth, L.W.; Wefer, H.A.; Lundin, S.; Jakobsson, H.E.; Lindberg, M.; Rodin, S.; Engstrand, L.; Andersson, A.F. DegePrime, a program for degenerate primer design for broad-taxonomic-range PCR in microbial ecology studies. *Appl. Environ. Microbiol.* **2014**, *80*, 5116–5123. [[CrossRef](#)]
32. Herlemann, D.P.; Labrenz, M.; Jurgens, K.; Bertilsson, S.; Waniek, J.J.; Andersson, A.F. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* **2011**, *5*, 1571–1579. [[CrossRef](#)]
33. Lundin, S.; Stranneheim, H.; Pettersson, E.; Klevebring, D.; Lundeberg, J. Increased throughput by parallelization of library preparation for massive sequencing. *PLoS ONE* **2010**, *5*, e10029. [[CrossRef](#)]
34. Nonnenmacher, C.; Dalpke, A.; Mutters, R.; Heeg, K. Quantitative detection of periodontopathogens by real-time PCR. *J. Microbiol. Methods* **2004**, *59*, 117–125. [[CrossRef](#)] [[PubMed](#)]
35. Eriksson, K.; Lonnblom, E.; Tour, G.; Kats, A.; Mydel, P.; Georgsson, P.; Hultgren, C.; Kharlamova, N.; Norin, U.; Jonsson, J.; et al. Effects by periodontitis on pristane-induced arthritis in rats. *J. Transl. Med.* **2016**, *14*, 311. [[CrossRef](#)] [[PubMed](#)]
36. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [[CrossRef](#)]
37. Cole, J.R.; Wang, Q.; Fish, J.A.; Chai, B.; McGarrell, D.M.; Sun, Y.; Brown, C.T.; Porras-Alfaro, A.; Kuske, C.R.; Tiedje, J.M. Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **2014**, *42*, D633–D642. [[CrossRef](#)]
38. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [[CrossRef](#)]
39. McMurdie, P.J.; Holmes, S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **2013**, *8*, e61217. [[CrossRef](#)]
40. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [[CrossRef](#)]
41. Catrina, A.I.; Svensson, C.I.; Malmstrom, V.; Schett, G.; Klareskog, L. Mechanisms leading from systemic autoimmunity to joint-specific disease in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **2017**, *13*, 79–86. [[CrossRef](#)]
42. Gonzalez, S.M.; Payne, J.B.; Yu, F.; Thiele, G.M.; Erickson, A.R.; Johnson, P.G.; Schmid, M.J.; Cannon, G.W.; Kerr, G.S.; Reimold, A.M.; et al. Alveolar bone loss is associated with circulating anti-citrullinated protein antibody (ACPA) in patients with rheumatoid arthritis. *J. Periodontol.* **2015**, *86*, 222–231. [[CrossRef](#)] [[PubMed](#)]
43. Choi, I.A.; Kim, J.H.; Kim, Y.M.; Lee, J.Y.; Kim, K.H.; Lee, E.Y.; Lee, E.B.; Lee, Y.M.; Song, Y.W. Periodontitis is associated with rheumatoid arthritis: A study with longstanding rheumatoid arthritis patients in Korea. *Korean J. Intern. Med.* **2016**, *31*, 977–986. [[CrossRef](#)]
44. Heasman, P.A.; Hughes, E.J. Drugs, medications and periodontal disease. *Br. Dent. J.* **2014**, *217*, 411–419. [[CrossRef](#)] [[PubMed](#)]
45. Mikuls, T.R.; Walker, C.; Qiu, F.; Yu, F.; Thiele, G.M.; Alfant, B.; Li, E.C.; Zhao, L.Y.; Wang, G.P.; Datta, S.; et al. The subgingival microbiome in patients with established rheumatoid arthritis. *Rheumatology (Oxford)* **2018**. [[CrossRef](#)]
46. Lopez-Oliva, I.; Paropkari, A.D.; Saraswat, S.; Serban, S.; Yonel, Z.; Sharma, P.; de Pablo, P.; Raza, K.; Filer, A.; Chapple, I.; et al. Dysbiotic Subgingival Microbial Communities in Periodontally Healthy Patients With Rheumatoid Arthritis. *Arthritis Rheumatol.* **2018**, *70*, 1008–1013. [[CrossRef](#)]
47. Beyer, K.; Zaura, E.; Brandt, B.W.; Buijs, M.J.; Brun, J.G.; Crielaard, W.; Bolstad, A.I. Subgingival microbiome of rheumatoid arthritis patients in relation to their disease status and periodontal health. *PLoS ONE* **2018**, *13*, e0202278. [[CrossRef](#)]

48. Scher, J.U.; Ubeda, C.; Equinda, M.; Khanin, R.; Buischi, Y.; Viale, A.; Lipuma, L.; Attur, M.; Pillinger, M.H.; Weissmann, G.; et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. *Arthritis Rheum.* **2012**, *64*, 3083–3094. [[CrossRef](#)] [[PubMed](#)]
49. Zhang, X.; Zhang, D.; Jia, H.; Feng, Q.; Wang, D.; Liang, D.; Wu, X.; Li, J.; Tang, L.; Li, Y.; et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat. Med.* **2015**, *21*, 895–905. [[CrossRef](#)]
50. Wells, P.M.; Williams, F.M.K.; Matey-Hernandez, M.L.; Menni, C.; Steves, C.J. 'RA and the microbiome: Do host genetic factors provide the link? *J. Autoimmun.* **2019**, *99*, 104–115. [[CrossRef](#)]
51. Yucel-Lindberg, T.; Bage, T. Inflammatory mediators in the pathogenesis of periodontitis. *Expert Rev. Mol. Med.* **2013**, *15*, e7. [[CrossRef](#)]
52. Kobayashi, T.; Yoshie, H. Host Responses in the Link Between Periodontitis and Rheumatoid Arthritis. *Curr. Oral Health Rep.* **2015**, *2*, 1–8. [[CrossRef](#)] [[PubMed](#)]
53. Thompson, C.; Davies, R.; Choy, E. Anti cytokine therapy in chronic inflammatory arthritis. *Cytokine* **2016**, *86*, 92–99. [[CrossRef](#)]
54. Vincent, F.B.; Saulep-Easton, D.; Figgett, W.A.; Fairfax, K.A.; Mackay, F. The BAFF/APRIL system: Emerging functions beyond B cell biology and autoimmunity. *Cytokine Growth Factor Rev.* **2013**, *24*, 203–215. [[CrossRef](#)] [[PubMed](#)]
55. Abe, T.; AlSarhan, M.; Benakanakere, M.R.; Maekawa, T.; Kinane, D.F.; Cancro, M.P.; Korostoff, J.M.; Hajishengallis, G. The B Cell-Stimulatory Cytokines BlyS and APRIL Are Elevated in Human Periodontitis and Are Required for B Cell-Dependent Bone Loss in Experimental Murine Periodontitis. *J. Immunol.* **2015**, *195*, 1427–1435. [[CrossRef](#)] [[PubMed](#)]
56. Shabgah, A.G.; Shariati-Sarabi, Z.; Tavakkol-Afshari, J.; Mohammadi, M. The role of BAFF and APRIL in rheumatoid arthritis. *J. Cell. Physiol.* **2019**. [[CrossRef](#)] [[PubMed](#)]
57. Kazakova, M.H.; Sarafian, V.S. *New Developments in the Pathogenesis of Rheumatoid Arthritis*; IntechOpen: London, UK, 2017; pp. 139–151.
58. Kazakova, M.; Batalov, A.; Deneva, T.; Mateva, N.; Kolarov, Z.; Sarafian, V. Relationship between sonographic parameters and YKL-40 levels in rheumatoid arthritis. *Rheumatol. Int.* **2013**, *33*, 341–346. [[CrossRef](#)]
59. Szychlinska, M.A.; Trovato, F.M.; Di Rosa, M.; Malaguarnera, L.; Puzzo, L.; Leonardi, R.; Castrogiovanni, P.; Musumeci, G. Co-Expression and Co-Localization of Cartilage Glycoproteins CHI3L1 and Lubricin in Osteoarthritic Cartilage: Morphological, Immunohistochemical and Gene Expression Profiles. *Int. J. Mol. Sci.* **2016**, *17*, 359. [[CrossRef](#)]
60. Coffman, F.D. Chitinase 3-Like-1 (CHI3L1): A putative disease marker at the interface of proteomics and glycomics. *Crit. Rev. Clin. Lab. Sci.* **2008**, *45*, 531–562. [[CrossRef](#)] [[PubMed](#)]
61. Keles, Z.P.; Keles, G.C.; Avci, B.; Cetinkaya, B.O.; Emingil, G. Analysis of YKL-40 acute-phase protein and interleukin-6 levels in periodontal disease. *J. Periodontol.* **2014**, *85*, 1240–1246. [[CrossRef](#)]
62. Knudsen, L.S.; Hetland, M.L.; Johansen, J.S.; Skjodt, H.; Peters, N.D.; Colic, A.; Grau, K.; Nielsen, H.J.; Ostergaard, M. Changes in plasma IL-6, plasma VEGF and serum YKL-40 during Treatment with Etanercept and Methotrexate or Etanercept alone in Patients with Active Rheumatoid Arthritis Despite Methotrexate Therapy. *Biomark. Insights* **2009**, *4*, 91–95. [[CrossRef](#)]
63. Williamson, S.; Munro, C.; Pickler, R.; Grap, M.J.; Elswick, R.K., Jr. Comparison of biomarkers in blood and saliva in healthy adults. *Nurs. Res. Pract.* **2012**, *2012*, 246178. [[CrossRef](#)]
64. Biyikoglu, B.; Buduneli, N.; Kardesler, L.; Aksu, K.; Oder, G.; Kutukculer, N. Evaluation of t-PA, PAI-2, IL-1beta and PGE(2) in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease. *J. Clin. Periodontol.* **2006**, *33*, 605–611. [[CrossRef](#)] [[PubMed](#)]
65. Joseph, R.; Rajappan, S.; Nath, S.G.; Paul, B.J. Association between chronic periodontitis and rheumatoid arthritis: A hospital-based case-control study. *Rheumatol. Int.* **2013**, *33*, 103–109. [[CrossRef](#)]
66. Kasser, U.R.; Gleissner, C.; Dehne, F.; Michel, A.; Willershausen-Zonchen, B.; Bolten, W.W. Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis Rheum.* **1997**, *40*, 2248–2251.
67. de Smit, M.J.; Brouwer, E.; Westra, J.; Nesse, W.; Vissink, A.; van Winkelhoff, A.J. Effect of periodontal treatment on rheumatoid arthritis and vice versa. *Ned Tijdschr Tandheelkd.* **2012**, *119*, 191–197. [[CrossRef](#)] [[PubMed](#)]

68. Coburn, B.W.; Sayles, H.R.; Payne, J.B.; Redman, R.S.; Markt, J.C.; Beatty, M.W.; Griffiths, G.R.; McGowan, D.J.; Mikuls, T.R. Performance of self-reported measures for periodontitis in rheumatoid arthritis and osteoarthritis. *J. Periodontol.* **2015**, *86*, 16–26. [[CrossRef](#)] [[PubMed](#)]
69. Mikuls, T.R.; Payne, J.B.; Yu, F.; Thiele, G.M.; Reynolds, R.J.; Cannon, G.W.; Markt, J.; McGowan, D.; Kerr, G.S.; Redman, R.S.; et al. Periodontitis and Porphyromonas gingivalis in patients with rheumatoid arthritis. *Arthritis Rheumatol.* **2014**, *66*, 1090–1100. [[CrossRef](#)] [[PubMed](#)]
70. Ortiz, P.; Bissada, N.F.; Palomo, L.; Han, Y.W.; Al-Zahrani, M.S.; Panneerselvam, A.; Askari, A. Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. *J. Periodontol.* **2009**, *80*, 535–540. [[CrossRef](#)] [[PubMed](#)]
71. Mayer, Y.; Balbir-Gurman, A.; Machtei, E.E. Anti-tumor necrosis factor-alpha therapy and periodontal parameters in patients with rheumatoid arthritis. *J. Periodontol.* **2009**, *80*, 1414–1420. [[CrossRef](#)]
72. Kobayashi, T.; Yokoyama, T.; Ito, S.; Kobayashi, D.; Yamagata, A.; Okada, M.; Oofusa, K.; Narita, I.; Murasawa, A.; Nakazono, K.; et al. Periodontal and serum protein profiles in patients with rheumatoid arthritis treated with tumor necrosis factor inhibitor adalimumab. *J. Periodontol.* **2014**, *85*, 1480–1488. [[CrossRef](#)]
73. Coat, J.; Demoersman, J.; Beuzit, S.; Cornec, D.; Devauchelle-Pensec, V.; Saraux, A.; Pers, J.O. Anti-B lymphocyte immunotherapy is associated with improvement of periodontal status in subjects with rheumatoid arthritis. *J. Clin. Periodontol.* **2015**, *42*, 817–823. [[CrossRef](#)] [[PubMed](#)]
74. Linden, G.J.; Lyons, A.; Scannapieco, F.A. Periodontal systemic associations: Review of the evidence. *J. Periodontol.* **2013**, *84*, S8–s19. [[CrossRef](#)] [[PubMed](#)]
75. Oliver, J.E.; Silman, A.J. Why are women predisposed to autoimmune rheumatic diseases? *Arthritis Res. Ther.* **2009**, *11*, 252. [[CrossRef](#)] [[PubMed](#)]
76. Page, R.C.; Beck, J.D. Risk assessment for periodontal diseases. *Int. Dent. J.* **1997**, *47*, 61–87. [[CrossRef](#)] [[PubMed](#)]
77. Klareskog, L.; Gregersen, P.K.; Huizinga, T.W. Prevention of autoimmune rheumatic disease: State of the art and future perspectives. *Ann. Rheum. Dis.* **2010**, *69*, 2062–2066. [[CrossRef](#)]



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Article

Superiority of a Treat-to-Target Strategy over Conventional Treatment with Fixed csDMARD and Corticosteroids: A Multi-Center Randomized Controlled Trial in RA Patients with an Inadequate Response to Conventional Synthetic DMARDs, and New Therapy with Certolizumab Pegol

Ruediger B. Mueller ^{1,2,3,*}, Michael Spaeth ⁴, Cord von Restorff ⁵, Christoph Ackermann ⁶, Hendrik Schulze-Koops ² and Johannes von Kempis ¹

¹ Division of Rheumatology and Immunology, Kantonsspital St. Gallen, 9007 St. Gallen, Switzerland; johannes.vonKempis@kssg.ch

² Division of Rheumatology and Clinical Immunology, Department of Internal Medicine IV, Ludwig-Maximilians-University Munich, 80336 Munich, Germany; Hendrik.Schulze-Koops@med.uni-muenchen.de

³ Division of Rheumatology, Medical University Department, Kantonsspital Aarau, CH-5001 Aarau, Switzerland

⁴ Division of Rheumatology, Spital Linth, 8730 Uznach, Switzerland; michael.spaeth@spital-linth.ch

⁵ Medical practice, 8708 Männedorf, Switzerland; vonrestorff@hin.ch

⁶ Medical practice, 9495 Triesen, Liechtenstein; ackermann.c@gmx.net

* Correspondence: Ruediger.Mueller@ksa.ch

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Abstract: Background: Treatment of rheumatoid arthritis (RA) includes the use of conventional (cs), biologic (b) disease-modifying anti-rheumatic drugs (DMARDs) and oral, intramuscularly, intravenous, or intraarticular (IA) glucocorticoids (GCs). In this paper, we analysed whether a treat-to-target (T2T) strategy optimizing csDMARD, oral, and IA-GC treatment as an adjunct new therapy to a new certolizumab pegol (CZP) therapy improves the effectiveness in RA patients. Methods: 43 patients with active RA (≥ 6 tender, ≥ 6 swollen joints, ESR ≥ 20 mm/h or CRP ≥ 7 mg/L) despite csDMARD treatment for ≥ 3 months and naïve to bDMARDs were randomized to CZP (200 mg/2 weeks after loading with 400 mg at weeks 0–2–4) plus a treat-to-target strategy (T2T, $n = 21$), or to CZP added to the established csDMARD therapy (fixed regimen, $n = 22$). The T2T strategy consisted of changing the baseline csDMARD therapy (1) SC-methotrexate (dose: 15 $\geq 20 \geq 25$ mg/week, depending on the initial dose) \geq leflunomide (20 mg/d) \geq sulphasalazine (2 \times 1000 mg/d) plus (2) oral GCs (prednisolone 20–15–12.5–10–7.5–5–2.5–0 mg/d tapered every five days) and (3) injections of ≤ 5 affected joints with triamcinolone. DMARD modification and an addition of oral GCs were initiated, depending on the achievement of low disease activity (DAS 28 < 3.2). The primary objective was defined as the ACR 50 response at week 24. Results: ACR 50 was achieved in 76.2% of the T2T, as compared to 36.4% of the fixed regimen patients ($p = 0.020$). ACR 20 and 70 responses were achieved in 90.5% and 71.4% of the T2T patients and 59.1% and 27.3% of the fixed regimen patients, respectively ($p = 0.045$ and $p = 0.010$, respectively). The adverse event rate was similar for both groups (T2T $n = 51$; fixed regimen $n = 55$). Conclusion: Treat-to-target management with the optimization of csDMARDs, oral, and IA-GCs of RA patients in parallel to a newly established CZP treatment was safe and efficacious in comparison to a fixed regimen of csDMARDs background therapy.

Keywords: rheumatoid arthritis; treat-to-target; certolizumab pegol; csDMARDs; glucocorticoids; intra-articular injections; DAS 28; ACR response; HAQ-DI

1. Introduction

Present treatment strategies for rheumatoid arthritis (RA) use conventional synthetic (cs), targeted synthetic (ts), and bDMARDs (biological disease modifying drugs). All of these classes of drugs have been shown to halt disease progression to a certain extent in clinical studies [1–7]. In general, therapy of RA is initiated with csDMARDs with or without concomitant glucocorticoids (GCs). If disease activity is not controlled under csDMARD treatment, ts or bDMARDs are added to treatment, as described, e.g., the EULAR (European League Against Rheumatism) guidelines [8]. In real life, a physician has more options than just adding the new ts/bDMARD. They can modify or optimize the therapy with concomitant csDMARDs, and oral or IA-GC (intraarticular glucocorticoids) can be added to the treatment regimen. Thus, a new therapeutic agent can be embedded in a whole strategy with parallel optimization of the csDMARD and GC treatment. In clinical studies with new compounds, on the other hand, the drug under examination is, in general, tested with a more or less fixed regimen of the pre-study csDMARDs, mostly methotrexate (MTX), and of GCs.

Strategic trials for the treatment of RA are rare [9–12]. In the TICORA (Tight Control of Rheumatoid Arthritis) trial [13], patients with early RA were treated, in the tight control arm, with a therapeutic strategy consisting of monthly visits, optimization of the therapeutic strategy (csDMARDs), and joint injections with GCs (triamcinolone acetonide). Not only was the efficacy, as measured by ACR (American College of Rheumatology) 20/50/70 responses, significantly higher in the patients in the tight control arm of the trial, as compared to the conventionally treated patients in the control arm of the study, but the rate of adverse events was also lower. A similar effect was repeated in the Camera study, where intensive treatment determined by a computerized decision program was superior to a conventional strategy [10]. This program forced the treating physician to increase the MTX dose and, as further escalation, add ciclosporine in early RA patients on a monthly basis, depending on the therapeutic response. This approach was shown to be superior to a conventional strategy with visits every three months. Goekoop-Ruiterman et al. demonstrated in the BeSt study that a treat-to-target approach was only superior with a step up to a bDMARD as compared to switching or adding more csDMARDs. Similarly, as shown in the Guepard study, the therapeutic efficacy under the guidance of tight control with MTX and step up to adalimumab was equally effective as compared to initial combination therapy of MTX and adalimumab after 12 months [14]. An ultrasound-guided step up of a therapeutic algorithm in early arthritis patients of the TaSer and the ARCTIC (Aiming for Remission in rheumatoid arthritis: a randomised trial examining the benefit of ultrasound in a Clinical Tight Control regimen) study did not lead to a significant amelioration of the clinical findings as compared to therapeutic escalation based on clinical assessment [11,12]. All these studies follow the idea of therapeutic escalation from cs to bDMARDs starting early in the course of the disease. In the EULAR recommendations, a therapeutic adjustment including the “optimization of csDMARDs dose or route of administration [15] or intra-articular injections of GCs” is recommended [8]. The European guidelines’ authors state that they did not want to “capture several current ways of GC application” independently of the dosage or route of application. In clinical trials, however, the doses of csDMARD are not always optimized. In the Oral Standard Study, the average dosage of MTX, which could not be increased during the trials, was 12.7 mg per week [16], and 16.5 mg per week in the Armada trial [17], suggesting that the dosage of csDMARD before and after the start of b or tsDMARDs had not been optimized. We hypothesize that the treating physicians may think that if one or more csDMARDs have failed, although the drug was not optimized, no additional effect of csDMARDs in the therapeutic algorithm can be expected. As in clinical trials, the doses of MTX are low; we estimate that csDMARDs are, in general, not optimized before or during a new b or tsDMARD treatment in real life, independently of what has been recommended by national or international organizations. During these trials [16,17], modification of the concomitant therapy with csDMARD and GCs was not allowed, as in almost every comparable trial.

Certolizumab pegol (CZP) is a PEGylated Fab’ fragment of a humanized anti-TNF (tumor necrosis factor) antibody with high affinity for TNF. CZP is an effective treatment, approved for moderate to

severe RA in many countries, when given in combination with MTX, and has been demonstrated to be effective and safe in the treatment of RA in several phase III trials [18–21]. In the Rapid 1 trial, the ACR 20/50/70 responses to CZP were 59%, 37%, and 21%, respectively, at week 24 (week 52 was comparable) in combination with fixed regimen MTX and GC [20]. In comparison, in the TICORA study, an ACR 20/50/70 response was found in 91%, 84%, and 71%, respectively, of the patients in the tight control arm at 18 months.

Trials to date, involving CZP and other biologic agents, have primarily been controlled trials with placebo comparisons. This trial design has resulted in a lack of data concerning the use of bDMARDs in a setting closer to real-life, where treatment strategies are more adaptive based on the patient's signs and symptoms. This trial was aimed at comparing the use of CZP in patients with moderate to severe RA, when administered in conjunction with an intensive, adaptive treatment program, versus a fixed regimen approach, as seen in the previous clinical trials. We hypothesized that an adaptive treat-to-target (T2T) strategy, in parallel to a new initiation of CZP, will lead to an improved outcome of RA patients with active disease despite csDMARD treatment, as compared to patients treated with a regimen of fixed csDMARDs and GC.

2. Methods

2.1. Patients

This study was conducted at four sites in Switzerland and Liechtenstein. Between March 2014 and December 2017, we recruited patients above 18 years of age suffering from RA and fulfilling the 2010 ACR/EULAR criteria [22] (NCT02293590). All patients had active disease despite csDMARD treatment prior to the baseline for \geq three months, at a stable dose for \geq 4 weeks for oral glucocorticoids \leq 10 mg daily prednisolone or equivalent and csDMARDs, defined by \geq 6 tender and \geq 6 swollen joint defined active disease out of the 66/68 joint count and an ESR (erythrocyte sedimentation rate) \geq 20 mm/h or a CRP (C reactive protein) \geq 7 mg/L. Key exclusion criteria included: previous treatment with a bDMARD; missing anti-conception in fertile patients; untreated, active, or latent bacterial (e.g., tuberculosis) or viral infections; or malignant diseases within the last five years.

This study was conducted in compliance with the ethical principles described in the Declaration of Helsinki and Good Clinical Practice. Approval of the Institutional Review Boards designated by each study site was obtained. All patients signed a written informed consent form.

2.2. Procedures

All patients were started on CZP (400 mg subcutaneously at weeks 0-2-4, and then 200 mg every two weeks to week 24). Patients were randomly assigned to either the T2T group or the group with the fixed regimen of the background treatment with csDMARDs and GCs. Both groups were started on CZP after randomization. Randomization at week 0 was performed centrally using the Secutrial system (interActive Systems GmbH, Berlin, Germany, also used as clinical research form). This system used for assigning eligible subjects to a treatment regimen was based on a predetermined schedule for minimization. Minimization was conducted with respect to DAS (disease activity score, based on 28 joints) $28 \geq 5.1$ vs. <5.1 , age ≥ 55 vs. <55 , and male vs. female gender.

Patients were reviewed for their disease activity at weeks 4, 8, 12, 18, and 24 to determine their treatment strategy and to document the proportion of patients classified as treatment responders. The efficacy assessments included the number of swollen and tender joints (66/68 joint count); DAS 28; the patient's global assessment of disease activity and pain; the physician's global assessment of disease activity; CRP, ESR, and HAQ-DI (health assessment questionnaire disability index) scores; and ACR 20/50/70 responses.

Efficacy as a basis for adaption of the therapeutic strategy during the trial (outlined below) was defined by a DAS 28 reduction of ≥ 1.2 since the last visit or achievement of LDA (low disease activity, $\text{DAS } 28 \leq 3.2$). Therapy was modified if these criteria were not fulfilled. In the case of treatment-emergent adverse events, the DMARD could be tapered stepwise.

2.3. Therapeutic Strategies

2.3.1. T2T csDMARD Strategy

At the study start and during the study, patients who did not reach a sufficient treatment response, as outlined in the previous chapter, or who developed treatment-emergent adverse events, were taken to the next step or drug according to the therapeutic algorithm. For example:

- Step 1: 15 mg MTX/week (MTX was always employed SC (subcutaneous) in the T2T arm);
- Step 2: 20 mg MTX/week;
- Step 3: 25 mg MTX/week;
- Step 4: 20 mg leflunomide/d;
- Step 5: sulphasalazine (target dose 2×1000 mg/d).

If a drug of this therapeutic algorithm had already been stopped before study entry because of intolerance, this drug was left out, and csDMARD treatment was directly continued with the next drug. This algorithm is illustrated in Figure 1.

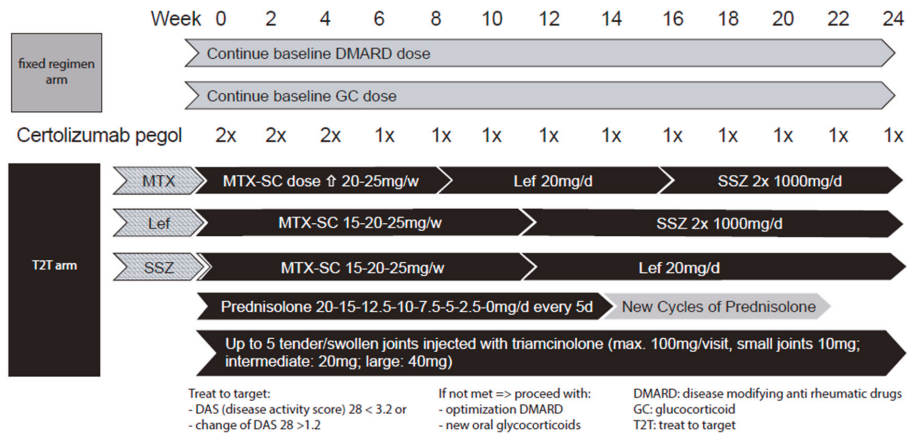


Figure 1. The treatment strategy arms: Flow diagram for treatment for escalation of disease-modifying anti-rheumatic therapy in RA patients. Patients were randomized into two arms: Fixed regimen (grey) or T2T (treat-to-target, black). Patients with both therapeutic arms were treated with certolizumab pegol (CZP). Fixed regimen arm: Baseline GCs and csDMARDs were continued at a stable dose throughout the study. T2T arm: Depending on the csDMARD used before the study (shown as a striped arrow), the csDMARD therapy was modified. In parallel, a cycle of oral GCs was initiated, and up to five tender and/or swollen joints were injected with triamcinolone. The response criteria for the patients in the T2T arm are listed below. If these response criteria were not met, therapy was modified according to the mechanism described, and a new cycle of oral GCs was initiated. Joint injections were always conducted independently of achieving the pre-set goal of the T2T strategy if they were tender and/or swollen. Lef, leflunomide; MTX-SC, subcutaneous methotrexate; SSZ, sulphasalazine.

2.3.2. T2T GC Strategy

At the study start, all T2T patients were started on an oral dose of prednisolone 20 mg once daily, which was then tapered down every five days at the following increments: 20 mg, 15.0 mg, 12.5 mg, 10 mg, 7.5 mg, 5 mg, 2.5 mg, and 0 mg once daily. If the DAS 28 had not improved by ≥ 1.2 or LDA

was not reached at the subsequent visit, prednisolone was started again at 20 mg once daily, followed by the same tapering schedule as noted above.

Starting at week 0, up to five synovitic joints had to be injected. The maximum cumulative triamcinolone dose was 100 mg/visit.

Doses of triamcinolone and lidocaine for joint injections were:

- Small joints: metacarpophalangeal (MCP), proximal interphalangeal (PIP), metatarsophalangeal joints (MTP): distal interphalangeal joints (DIP), sternoclavicular joint, acromioclavicular joint, tarsus, and distal interphalangeal joints of the feet (IP): 10 mg triamcinolone;
- Intermediate joints: carpus, elbow, ankle: 20 mg triamcinolone with 0.5 mL 1% lidocaine; big joints: knee, shoulder, hip: 40 mg triamcinolone with 4 mL 1% lidocaine.

If more than five synovitic joints were found to be painful and/or tender, the physician selected the joints mostly affecting daily life activities based on the patient's judgment.

2.3.3. Fixed Regimen Strategy

Patients continued to receive their stable weekly dose of csDMARD and GC (≤ 10 mg prednisolone or equivalent), as noted at study entry, for the duration of the study (24 weeks, Figure 1).

2.4. Primary Objective

The primary objective was to determine the number of patients exhibiting at least a 50% improvement of the disease activity, as determined by the ACR 50 response following 24 weeks of treatment of CZP, when given as part of an intensive adaptive treatment plan (T2T), in comparison to a therapy with fixed, continued, csDMARDs and GCs with unchangeable doses (fixed regimen).

2.5. Anti-Drug Antibodies

Sera from patients were collected at baseline and at four and 24 weeks of the study. Samples at weeks 4 (because a first exposure to the drug was needed to measure CZP levels) and 24 were used to measure CZP drug levels. Samples taken at baseline and at week 24 were analysed for anti-drug antibodies. Analyses were conducted at Sanquin Research (Department of Immunopathology, Amsterdam, The Netherlands), as previously described [23].

2.6. Data and Statistical Methods

2.6.1. Power Calculation

To detect an increase in ACR 50 from 37% assumed in the fixed regimen group [20] to 84% in the T2T group [13], 21 patients were required in each group, if a chi-square test was used, to achieve 90% power, if the two-sided significance level was 0.05.

2.6.2. Statistical Analyses

Demographic variables and baseline characteristics of the disease activity were summarized using descriptive statistics. For ACR 20/50/70; DAS 28; and HAQ-DI, the chi-square test was used. Missing values on the ACR responses were calculated as "not reached" (worst-case analysis). Missing values of the DAS 28 and HAQ-DI were not imputed for the calculations (per protocol).

The chi-square test compared the proportion of the two groups of patients in remission. Time to DAS 28, CDAI (clinical disease activity index), and Boolean defined remission was summarized using Kaplan-Meier curves and compared using the log-rank test. The cumulative corticosteroid dose was compared for the two patient groups using the Mann-Whitney *U*-test. Safety outcomes, including treatment-emergent adverse events, were summarized in a table by type and severity and reported for all patients receiving at least one dose of CZP. Anti-drug antibodies and CZP levels were measured at the last visit, using the Mann-Whitney *U*-test.

3. Results

3.1. Patient Demographics/Characteristics

We pre-screened 107 patients for the study (first patient in 27 March 2014; last patient out 22 December 2017); after exclusions and two withdrawn consents before randomization, 21 patients were assigned to the T2T, and 22 to the fixed regimen arm of the study. Of these, 19 patients (90.5%) in the T2T arm and 21 patients (95.5%) in the fixed regimen arm completed the 24-week study (Figure 2). Two patients dropped out of the T2T group: one because of an adverse event and one because the initial diagnosis of RA (inclusion criterion) had to be revised and a new primary diagnosis was established. One patient of the fixed regimen arm dropped out for persistent disease activity. Baseline characteristics and measures of disease activity in the two groups were balanced (Table 1). Mean DAS 28 (ESR) was 5.89 ± 0.98 for T2T and 6.16 ± 0.86 for fixed regimen patients. Small differences in baseline measures of disease activity were deemed not to be clinically relevant. Median RA duration (time from the first diagnosis) was approximately one year in both groups. Bone erosions on hands and/or feet were described in 26.3% of the T2T patients and 30.0% of the fixed regimen patients.

Table 1. Demographics and disease activity at screening.

| | T2T (n = 21) | Fixed Regimen (n = 22) |
|--|-------------------------|---|
| Age (a, mean) | 56.3 ± 15.4 | 56.8 ± 14.8 |
| Gender (% female) | 66.7% | 63.6% |
| BMI (Kg/m ² , mean) | 28.6 ± 4.4 | 28.7 ± 5.6 |
| ACPA pos. (%) | 47.6% | 47.6% |
| RF pos. (%) | 66.7% | 81.0% |
| Erosive disease (%; defined by treating physician) | 26.3% | 30.0% |
| Disease duration (a, median, range) | 0.99, 3 months–10 years | 0.85, 3 months–18 years |
| Concomittant DMARD at baseline | MTX 10 mg/w, n = 2 | MTX 10 mg/w: n = 2 |
| | MTX 15 mg/w, n = 9 | MTX 15 mg/w n = 6 |
| | MTX 20 mg/w, n = 4 | MTX 20 mg/w n = 3 |
| | MTX 25 mg/w, n = 3 | MTX 25 mg n = 5 |
| | Lef 20 mg/d, n = 3 | MTX + HCQ, n = 2 15 mg/w + 200 mg/d; 20 mg/w + 400 mg/d |
| Concomitant GC (mean dose *, number of patients) | 4.7 mg/d; n = 8 | SSZ n = 2 Lef n = 2 6.3 mg/d; n = 10 |
| Disease activity score (DAS 28) | 5.89 ± 0.98 | 6.16 ± 0.86 |
| Tender joint score (0–68) | 20.7 ± 10.3 | 23.2 ± 13.7 |
| Swollen joint score (0–66) | 18.9 ± 7.6 | 18.6 ± 10.9 |
| Pain score (0–100) | 65.3 ± 20.8 | 60.2 ± 21.0 |
| Patient global assessment | 70.1 ± 16.0 | 64.2 ± 16.9 |
| Physician global assessment (0–100) | 71.8 ± 8.8 | 67.0 ± 19.0 |
| C-reactive protein (mg/L) | 13.0 ± 16.2 | 17.1 ± 18.8 |
| Erythrocyte sedimentation rate (mm/h) | 28.7 ± 19.9 | 35.1 ± 25.2 |
| Health assessment questionnaire score * (0–3) | 0.84 ± 0.62 | 0.85 ± 0.64 |

* assessed on patients treated with glucocorticoids; Lef: leflunomide; SSZ: sulphasalazine; MTX: methotrexate; HCQ: hydroxychloroquine, T2T: treat to target, BMI: body mass index, ACPA: anti-citrullinated protein antibodies, RF: rheumatoid factor, pos.: positive, DMARD: disease modifying anti rheumatic drugs, GC: glucocorticoids, DAS: disease activity score.

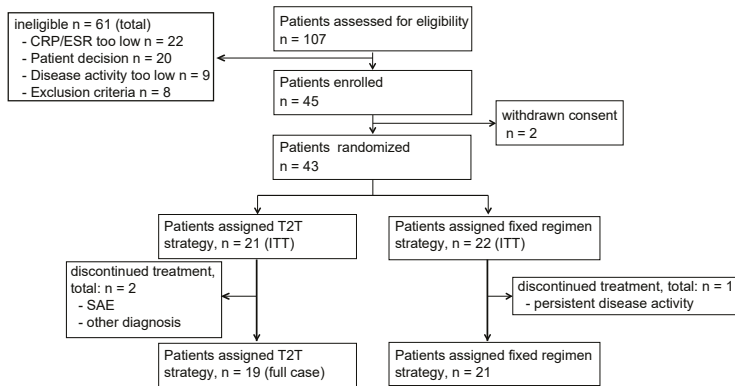


Figure 2. Patient disposition: T2T: treat-to-target, ITT: intention to treat, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, SAE: serious adverse event.

3.2. Clinical Responses

Treatment of RA with CZP plus T2T background therapy significantly reduced signs and symptoms as compared with CZP plus fixed regimen background therapy. At 24 weeks, the ACR 50 response rate (primary endpoint) was achieved in 16 out of 21 T2T patients (76.2%), as compared to eight out of 22 fixed regimen patients (36.4%; $\text{Chi}^2: 5.355, p = 0.020$, Figure 3A). ACR 20 and 70 responses were achieved in 90.5% and 71.4% of the T2T patients, and in 59.1% and 27.3% of the fixed regimen patients, respectively ($p = 0.045$ and $p = 0.010$, respectively, Figure 3A).

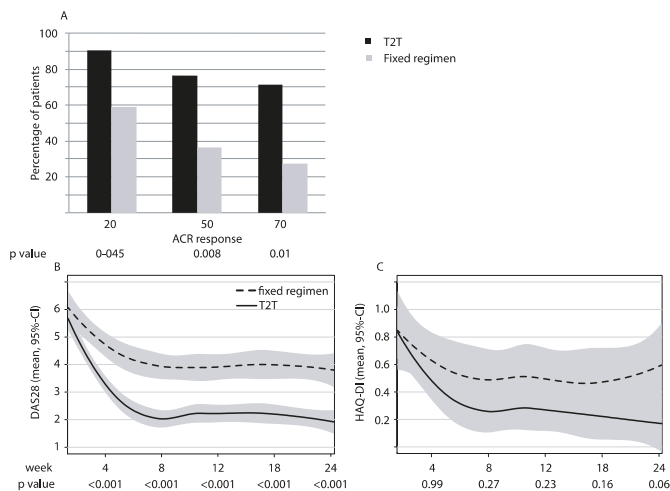


Figure 3. Response to treatment: (A) Percentage of patients achieving an ACR 20/50/70 response was demonstrated at week 24 (ITT analysis). T2T is shown in black, and fixed regimen in grey. (B) Average disease activity score in 28 joints (DAS 28) at baseline and during follow up until week 24. (C) Average health assessment questionnaire disability index (HAQ-DI) at baseline and during follow up until week 24. All data are shown for the two patient groups as means with the 95% confidence interval: T2T solid black line, and fixed regimen as a striped line; ACR: American college of rheumatology, ITT: intention to treat, T2T: treat to target.

The improvement in disease activity, as assessed by DAS 28, developed more rapidly within the first four weeks of treatment and remained after that at sustained lower levels until week 24 (Figure 3B) in the CZP plus T2T treated patients, as compared to the patients treated with the fixed regimen co-therapy. In more detail, DAS 28 levels decreased to 2.62 and 1.91 at weeks 4 and 24 in the T2T group, as compared to 4.21 and 3.77 in the fixed regimen group, respectively ($p < 0.001$ for all time points, Figure 3B).

In parallel, higher remission rates were found for CZP and T2T treated patients independently of the definition of remission employed. DAS-defined remission ($\text{DAS } 28 < 2.6$) was found in 68.4% vs. 28.6% (Log-rank test $p < 0.001$), CDAI defined remission ($\text{CDAI} \leq 2.8$) in 78.9% vs. 23.8% (Log-rank test $p < 0.001$), and Boolean remission in 47.4% vs. 19.1% (Log-rank test $p = 0.031$) of the T2T and fixed regimen therapy patients, respectively, at week 24 (data shown as Supplementary Materials).

3.3. Patient-Related Outcomes

The improvement in the HAQ-DI as a patient-related outcome developed more rapidly within the first four weeks of treatment, and remained after that at sustained lower levels until week 24 (Figure 3C) in the CZP plus T2T treated patients, as compared to the patients treated with the fixed regimen co-therapy. The mean reduction in HAQ-DI was markedly higher in the T2T group than in the fixed regimen group; however, significance was not reached (week 24: T2T 0.17 vs. fixed regimen 0.6, $p = 0.06$). Similarly, the changes in the patient’s pain score and in patient’s global assessment of disease activity were not significantly different at week 24 (Table 2).

Table 2. Improvement of ACR core components at week 24.

| | T2T | Fixed Regimen | <i>p</i> -Value |
|-------------------------------|--------|---------------|-----------------|
| Patient’s pain score | −53.34 | −50.51 | n.s. |
| Patient’s global assessment | −56.21 | −49.59 | n.s. |
| Physician’s global assessment | −64.62 | −31.74 | <0.001 |
| Tender joint count (68) | −19.67 | −9.9 | <0.001 |
| Swollen joint count (66) | −17.89 | −8.76 | <0.001 |
| ESR (mm/h) | −12.83 | −17.86 | <0.001 |
| CRP (mg/L) | −3.03 | −7.64 | <0.001 |
| HAQ-DI | −0.68 | −0.25 | n.s. |
| CDAI | −33.81 | −21.9 | <0.001 |

T2T: treat to target, n.s.: not significant, ESR: erythrocyte sedimentation rate, CRP: C reactive protein, HAQ-DI: health assessment questionnaire disability index, CDAI: clinical disease activity index.

3.4. Adverse Events

Of the 43 patients in this study, 18 (76.2%) patients in the T2T and 16 (72.7%) in the treatment fixed regimen reported at least one adverse event (AEs). The most common AEs were infections. Most reported AEs were of mild or moderate severity (98.1% T2T and 98.0% fixed regimen). Only three patients reported serious AEs (SAE: $n = 2$ T2T; $n = 1$ fixed regimen). The SAEs were: insufficiency of the adrenal gland, cardiovascular infarction (both SAEs were T2T), and bursitis infra-patellaris of the right knee (fixed regimen). All SAEs were resolved (Table 3). The most frequent AEs were infections. Thirteen T2T patients and 10 fixed regimen patients had infections during the study. In detail, we found bursitis ($n = 1$), a nail infection ($n = 1$), lip blisters ($n = 3$), pyelonephritis ($n = 1$), an upper respiratory infection ($n = 3$), and a urinary tract infection ($n = 1$) in fixed regimen patients and cutaneous mucositis ($n = 1$), parodontitis ($n = 1$), a gastrointestinal infection ($n = 1$), Herpes Labialis, ($n = 1$) mycosis ($n = 1$), pneumonia ($n = 1$), a skin infection ($n = 1$), and an upper airway infection ($n = 6$) in T2T patients.

Table 3. Adverse events.

| | Fixed Regimen | T2T |
|---|---------------|-------------|
| Total (n) | 51 | 55 |
| Patients with AEs (n) | 16 | 18 |
| Intensity (mild/moderate/severe, n) | 49/1/1 | 50/4/1 |
| SAE (n) | 1 * | 2 ** |
| Related (Not, unlikely/possibly/ probably/definitely, n) | 22/3/21/4/1 | 33/3/19/0/1 |
| Cardiovascular (n) | 2 | 7 |
| Dermatological (n) | 6 | 4 |
| Gastrointestinal (n) | 3 | 4 |
| General (n) | 1 | 4 |
| Hepatology (n) | 2 | 1 |
| Infection | 10 | 13 |
| Injection site reaction to CZP (n) | 4 | 3 |
| Injury (n) | 5 | 2 |
| Joint injection reaction (n) | - | 3 |
| Musculoskeletal (n) | 4 | 3 |
| Neurological (n) | 11 | 10 |
| Ophthalmological (n) | 1 | 1 |
| Psychology (n) | 2 | 1 |

n: number; (S) AE: (serious) adverse events; CZP: Certolizumab pegol; * bursitis infrapatellaris; ** insufficiency of adrenal gland, cardiovascular infarction; T2T: treat to target.

3.5. Cumulative Steroid Dose

For the T2T group, the cumulative oral corticosteroid dose was calculated by counting the number of GC cycles: One patient required three cycles of oral GC, four patients two cycles, and all the other T2T patients only one cycle. In total, an average of 471.2 mg prednisolone (SD (standard deviation) \pm 207.1) was used in the T2T patients. Additionally, 10.2 (mean, SD \pm 7.1) joints were injected with triamcinolone per T2T patient. Furthermore, 7.2 small, 1.7 intermediate, and 1.0 large joints were injected per patient. In total, a mean of 149.1 mg triamcinolone (SD \pm 109.3) was injected into the joints of T2T patients. This adds up to a cumulative average GC dose (equivalence dose prednisolone:triamcinolone = 1:1.2) in T2T patients of 666.9 mg (SD \pm 309.5) prednisolone/patient or equivalent [24] over the whole study period.

Among the fixed regimen patients, three patients were continuously treated with 10 mg prednisolone/d, two with 7.5 mg/d, seven with 5 mg/d, two with 2.5 mg/d, and eight without concomitant prednisolone. This adds up to a cumulative average GC dose in fixed regimen patients of 792.5 mg prednisolone/patient or equivalent (SD \pm 594.5) over the whole study period. The cumulative GC doses did not differ significantly between both groups (Mann-Whitney test, $p = 0.723$, Figure S1).

3.6. Therapeutic Changes of the csDMARD Protocol

Only two patients required two modifications of their baseline T2T csDMARD regimen. All other patient required only one optimization of the csDMARD. Two patients required a step up from 10 mg to 15 mg MTX/week, nine from 15 mg to 20 mg MTX/week, six from 20 to 25 mg MTX/week, two from 25 mg MTX/week to 20 mg leflunomide/d, three from 20 mg leflunomide/d to 15 mg MTX/week, and one from 20 mg leflunomide/d to 2 \times 1000 mg sulphasalazine/day.

3.7. ADA (Anti Drug Antibodies) Antibodies

Blood serum CZP levels were analysed at week 4 and after 24 weeks and for anti-CZP antibodies at baseline and after 24 weeks. Anti-CZP antibodies were not detectable at baseline, but were detected after 24 weeks of treatment in 8/19 patients of the T2T group (42.1%) and in 10/19 patients of the fixed-dose group (52.6%). This difference in frequencies was not significant ($\chi^2 = 0.106$, $p = 0.745$).

There was, however, a trend of lower anti-CZP antibodies levels in the T2T group (T2T: 139.1 ± 138.8 vs. fixed regimen: 197.1 ± 170.0 AU (arbitrary units)/mL, Mann-Whitney test, $p = 0.439$, Figure S2).

CZP levels decreased from week 4 (46.5 ± 19.4 mg/mL) to week 24 (29.5 ± 12.6 mg/mL, Wilcoxon signed-rank test, $p < 0.001$), but did not differ between treatment groups (Mann-Whitney test, week 4: $p = 0.857$, week 24: $p = 0.977$).

4. Discussion

Treat-to-target management with the optimization of csDMARDs, oral, and of IA-GC (T2T) in parallel to new CZP treatment was safe and led to a substantial decrease of disease activity, as compared to new CZP with a fixed regimen of csDMARDs and GCs, in RA patients with an inadequate response to previous csDMARDs.

The idea of treating to target has been around since the late 90s for the treatment of RA patients. The study employing a modification of csDMARDs and IA-GCs had much influence on how we treat RA to target nowadays [13]. The arrival of TNF antagonists and the other biologics had an even more profound impact on the treatment of RA. As mentioned in the introduction, the underlying principle of these T2T strategies is that if a class of drugs (e.g., csDMARDs) has failed to reach the target, mostly due to low disease activity or even remission, a step up to the next class of therapeutics (bDMARDs) is immediately necessary. Thus, an assumption is made that the csDMARD that has failed cannot add any additional efficacy to the treatment of this patient. The novelty of our protocol is that the therapeutic strategy is optimized by four parallel actions: first bDMARD (TNF antagonist), csDMARD with dose adjustment, and additional oral and IA-GCs. Adaptation of concomitant medication is generally not allowed in clinical phase II/III studies for a new compound in RA. The superiority of a combination of the treat-to-target idea, as suggested by the TICORA study, with the addition of a biologic treatment at a level that has never been reached for any biologic agents, in csDMARD-IR patients, is the real novelty of this study.

Over the last two decades, various compounds have been tested for efficacy and safety: combination therapy of csDMARDs, TNF antagonists, other biologic agents, and targeted synthetic DMARDs. Efficacy, as assessed by ACR response rates, was always comparable: 60% for ACR 20, 40% for ACR 50, and 20% for ACR 70, in MTX-incomplete, responding patients [16,20,25–27].

Every method available to improve an RA patient's health, i.e., csDMARD dose adjustment, in addition to oral and IA-GCs and bDMARD, should be applied to improve these response rates. It seems likely that the effects demonstrated here for a T2T strategy in combination with a parallel start of CZP can be generalized for all bDMARDs and tsDMARDs in csDMARD-IR patients. Whether a similar effectiveness could also be achieved after an inadequate response to b or tsDMARDs would have to be confirmed in other trials with a design similar to ours.

This publication has several limitations: Firstly, the differences found for efficacy (ACR response, DAS28, CDAI) could not be demonstrated at a significance level for HAQ-DI and other patient-related outcomes (PROs), but a strong tendency towards superiority of the T2T-regimen was found. Little is known about the response of HAQ-DI in a treat-to-target clinical trial setting. The only publication with a comparable design, where Hirano et al. demonstrated a reduction of HAQ-DI from 1.4 to 1.0 when following the Brazilian therapeutic recommendations, had no control group and the observation period was much longer [28]. As a consequence, we did not have any data to calculate the power for significant changes in HAQ-DI for the design of our study. Considering that 84% of the T2T patients achieved an HAQ-DI < 0.5 , and 65% of the patients with a fixed regimen, a sample size of two times 81 patients would have been necessary to reach a power of 0.8 with an alpha of 0.05. It has to be considered, that in the face of the impressive results regarding efficacy, we would have treated an additional 60 patients according to the fixed regimen protocol with a significantly lower clinical efficacy. In summary, we think that this would have been ethically controversial. Psychological effects of RA are well-described. Such phenomena may influence PROs [29,30]. Strand et al. [31] have described that PROs improve with CZP treatment. Interestingly, this improvement had the lowest correlation with

DAS 28 or ACR 20 response levels. It seems likely that regression towards the mean may be a reason why the PROs in this study did not reflect the outstanding results of the efficacy parameters [32].

Secondly, changes in radiographic progression were not analysed. The reasons for this were the sample size and that the study was only planned for 24 weeks. Concerning these two restrictions, we did not expect to find any differences in the radiographic progression during the study. The low rate of radiographic progression usually found in comparable patient populations was another reason not to consider it as an outcome parameter: an increase of 0.4 Sharp units over only 52 weeks, or 0.2 over 24 weeks, was found in patients treated with CZP and MTX in the 0.2 units for RAPID 2 and 0.4 for RAPID 1.

Thirdly, a double blind, randomized study is superior to an open study like ours. On the other hand, studies with the combined therapeutic treat-to-target approaches, especially involving IA-GCs, are difficult to blind. In the TICORA [13] and CAMERA [10] studies, which, like our study, also used joint injections, the two therapeutic arms were not blinded.

5. Conclusions

In conclusion, this study shows that a T2T approach in the treatment of RA patients with an inadequate response to csDMARDs, using the full armamentarium of the therapeutic possibilities, has a high potential to improve disease activity in parallel to the start of a bDMARD, in our case, CZP. We feel strongly that this approach is transferable to the start of other b or tsDMARDs. We are aware of the fact that designing clinical studies for the demonstration of effectivity/effectiveness of new drugs with dynamic background therapies is difficult. This, however, should not influence our daily approach to ameliorate the situation for our patients.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-0383/8/3/302/s1>, Figure S1: Cumulative GC (glucocorticoids) dose during the study period. The average cumulative GC dose was calculated separately for patients treated with T2T or with fixed regimen. The average cumulative GC dose was standardized for prednisolone or equivalent, Figure S2: (A) CZP (certolizumab pegol) levels and were measured at week 4 and week 24. (B) CZP anti-drug antibodies were measured at the baseline and week 24. Patients treated with CZP + T2T are shown in dark grey and CZP + fixed regimen in light grey. Data are shown as medians with the 25th and 75th percentile and the standard deviation as error bars. Outliers are implemented as dots; w: week, AU/mL: arbitrary units per milliliter, T2T: treat to target.

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References

1. Strand, V.; Cohen, S.; Schiff, M.; Weaver, A.; Fleischmann, R.; Cannon, G.; Fox, R.; Moreland, L.; Olsen, N.; Furst, D.; et al. Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Group. *Arch. Intern. Med.* **1999**, *159*, 2542–2550. [[CrossRef](#)] [[PubMed](#)]

2. Lipsky, P.E.; van der Heijde, D.M.; St Clair, E.W.; Furst, D.E.; Breedveld, F.C.; Kalden, J.R.; Smolen, J.S.; Weisman, M.; Emery, P.; Feldmann, M.; et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. *N. Engl. J. Med.* **2000**, *343*, 1594–1602. [[CrossRef](#)] [[PubMed](#)]
3. Kremer, J.M.; Genant, H.K.; Moreland, L.W.; Russell, A.S.; Emery, P.; Abud-Mendoza, C.; Szechinski, J.; Li, T.; Ge, Z.; Becker, J.C.; et al. Effects of abatacept in patients with methotrexate-resistant active rheumatoid arthritis: A randomized trial. *Ann. Intern. Med.* **2006**, *144*, 865–876. [[CrossRef](#)] [[PubMed](#)]
4. Keystone, E.; Emery, P.; Peterfy, C.G.; Tak, P.P.; Cohen, S.; Genoveses, M.C.; Dougados, M.; Burmester, G.R.; Greenwald, M.; Kvien, T.K.; et al. Rituximab inhibits structural joint damage in patients with rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitor therapies. *Ann. Rheum. Dis.* **2009**, *68*, 216–221. [[CrossRef](#)] [[PubMed](#)]
5. Nishimoto, N.; Hashimoto, J.; Miyasaka, N.; Yamamoto, K.; Kawai, S.; Takeuchi, T.; Murata, N.; van der Heijde, D.; Kishimoto, T. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): Evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial of tocilizumab. *Ann. Rheum. Dis.* **2007**, *66*, 1162–1167. [[CrossRef](#)] [[PubMed](#)]
6. Van der Heijde, D.; Tanaka, Y.; Fleischmann, R.; Keystone, E.; Kremer, J.; Zerbini, C.; Cardiel, M.H.; Cohen, S.; Nash, P.; Song, Y.W.; et al. Tofacitinib (CP-690,550) in patients with rheumatoid arthritis receiving methotrexate: Twelve-month data from a twenty-four-month phase III randomized radiographic study. *Arthritis Rheum.* **2013**, *65*, 559–570. [[CrossRef](#)] [[PubMed](#)]
7. Taylor, P.C.; Keystone, E.C.; van der Heijde, D.; Weinblatt, M.E.; Del Carmen Morales, L.; Reyes Gonzaga, J.; Yakushin, S.; Ishii, T.; Emoto, K.; Beattie, S.; et al. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N. Engl. J. Med.* **2017**, *376*, 652–662. [[CrossRef](#)] [[PubMed](#)]
8. Smolen, J.S.; Landewé, R.; Bijlsma, J.; Burmester, G.; Chatzidionysiou, K.; Dougados, M.; Nam, J.; Ramiro, S.; Voshaar, M.; van Vollenhoven, R.; 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*, 960–977. [[CrossRef](#)] [[PubMed](#)]
9. Goekoop-Ruiterman, Y.P.; de Vries-Bouwstra, J.K.; Allaart, C.F.; van Zeben, D.; Kerstens, P.J.; Hazes, J.M.; Zwinderman, A.H.; Roodman, H.K.; Han, K.H.; Westedt, M.L.; et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): A randomized, controlled trial. *Arthritis Rheum.* **2005**, *52*, 3381–3390. [[CrossRef](#)] [[PubMed](#)]
10. Verstappen, S.M.; Jacobs, J.W.; van der Veen, M.J.; Heurkens, A.H.; Schenk, Y.; ter Borg, E.J.; Blaauw, A.A.; Bijlsma, J.W. 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*, 1443–1449. [[CrossRef](#)] [[PubMed](#)]
11. Dale, J.; Stirling, A.; Zhang, R.; Purves, D.; Foley, J.; Sambrook, M.; Conaghan, P.G.; van der Heijde, D.; McConnachie, A.; McInnes, I.B.; et al. Targeting ultrasound remission in early rheumatoid arthritis: The results of the TaSER study, a randomised clinical trial. *Ann. Rheum. Dis.* **2016**, *75*, 1043–1050. [[CrossRef](#)] [[PubMed](#)]
12. Haavardsholm, E.A.; Aga, A.B.; Olsen, I.C.; Lillegraven, S.; Hammer, H.B.; Uhliq, T.; Fremstad, H.; Madland, T.M.; Lexberg, Å.; Haukeland, H.; et al. Ultrasound in management of rheumatoid arthritis: ARCTIC randomised controlled strategy trial. *BMJ* **2016**, *354*, i4205. [[CrossRef](#)] [[PubMed](#)]
13. Grigor, C.; Capell, H.; Stirling, A.; McMahon, A.D.; Lock, P.; Vallance, R.; Kincaid, W.; Porter, D. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): A single-blind randomised controlled trial. *Lancet* **2004**, *364*, 263–269. [[CrossRef](#)]
14. Soubrier, M.; Puéchal, X.; Sibilia, J.; Mariette, X.; Meyer, O.; Combe, B.; Flipo, R.M.; Mulleman, D.; Berenbaum, F.; Zarnitsky, C.; et al. Evaluation of two strategies (initial methotrexate monotherapy vs. its combination with adalimumab) in management of early active rheumatoid arthritis: Data from the GUEPARD trial. *Rheumatology (Oxford)* **2009**, *48*, 1429–1434. [[CrossRef](#)] [[PubMed](#)]
15. Visser, K.; van der Heijde, D. Optimal dosage and route of administration of methotrexate in rheumatoid arthritis: A systematic review of the literature. *Ann. Rheum. Dis.* **2009**, *68*, 1094–1099. [[CrossRef](#)] [[PubMed](#)]

16. Fleischmann, R.; Cutolo, M.; Genovese, M.C.; Lee, E.B.; Kanik, K.S.; Sadis, S.; Connell, C.A.; Gruben, D.; Krishnaswami, S.; Wallenstein, G.; et al. Phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) or adalimumab monotherapy versus placebo in patients with active rheumatoid arthritis with an inadequate response to disease-modifying antirheumatic drugs. *Arthritis Rheum.* **2012**, *64*, 617–629. [[CrossRef](#)] [[PubMed](#)]
17. Weinblatt, M.E.; Keystone, E.C.; Furst, D.E.; Moreland, L.W.; Weisman, M.H.; Birbara, C.A.; Teoh, L.A.; Fischkoff, S.A.; Chartash, E.K. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: The ARMADA trial. *Arthritis Rheum.* **2003**, *48*, 35–45. [[CrossRef](#)] [[PubMed](#)]
18. Strand, V.; Smolen, J.S.; van Vollenhoven, R.F.; Mease, P.; Burmester, G.R.; Hiepe, F.; Khanna, D.; Nikaï, E.; Coteur, G.; Schiff, M. Certolizumab pegol plus methotrexate provides broad relief from the burden of rheumatoid arthritis: Analysis of patient-reported outcomes from the RAPID 2 trial. *Ann. Rheum. Dis.* **2011**, *70*, 996–1002. [[CrossRef](#)] [[PubMed](#)]
19. Weinblatt, M.E.; Fleischmann, R.; Huizinga, T.W.; Emery, P.; Pope, J.; Massarotti, E.M.; van Vollenhoven, R.F.; Wollenhaupt, J.; Bingham, C.O., 3rd; Duncan, B.; et al. Efficacy and safety of certolizumab pegol in a broad population of patients with active rheumatoid arthritis: Results from the REALISTIC phase IIIb study. *Rheumatology (Oxford)* **2012**, *51*, 2204–2214. [[CrossRef](#)] [[PubMed](#)]
20. Keystone, E.; van der Heijde, D.; Mason, D., Jr.; Landewé, R.; Vollenhoven, R.V.; Combe, B.; Emery, P.; Strand, V.; Mease, P.; Desai, C.; et al. Certolizumab pegol plus methotrexate is significantly more effective than placebo plus methotrexate in active rheumatoid arthritis: Findings of a fifty-two-week, phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Arthritis Rheum.* **2008**, *58*, 3319–3329. [[CrossRef](#)] [[PubMed](#)]
21. Smolen, J.S.; Emery, P.; Ferraccioli, G.F.; Samborski, W.; Berenbaum, F.; Davies, O.R.; Koetse, W.; Purcaru, O.; Bennett, B.; Burkhardt, H. Certolizumab pegol in rheumatoid arthritis patients with low to moderate activity: The CERTAIN double-blind, randomised, placebo-controlled trial. *Ann. Rheum. Dis.* **2015**, *74*, 843–850. [[CrossRef](#)] [[PubMed](#)]
22. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O., 3rd; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **2010**, *69*, 1580–1588. [[CrossRef](#)] [[PubMed](#)]
23. Van Schie, K.A.; Hart, M.H.; de Groot, E.R.; Kruithof, S.; Aarden, L.A.; Wolbink, G.J.; Rispen, T. Response to: 'The antibody response against human and chimeric anti-TNF therapeutic antibodies primarily targets the TNF binding region' by Rinaudo-Gaujous et al. *Ann. Rheum. Dis.* **2015**, *74*, e41. [[CrossRef](#)] [[PubMed](#)]
24. Meikle, A.W.; Tyler, F.H. Potency and duration of action of glucocorticoids. Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *Am. J. Med.* **1977**, *63*, 200–207. [[CrossRef](#)]
25. Maini, R.N.; Taylor, P.C.; Szechinski, J.; Pavelka, K.; Bröll, J.; Balint, G.; Emery, P.; Raemen, F.; Petersen, J.; Smolen, J.; et al. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum.* **2006**, *54*, 2817–2829. [[CrossRef](#)] [[PubMed](#)]
26. Kremer, J.M.; Westhovens, R.; Leon, M.; Di Giorgio, E.; Alten, R.; Steinfeld, S.; Russell, A.; Dougados, M.; Emery, P.; Nuamah, I.F.; et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N. Engl. J. Med.* **2003**, *349*, 1907–1915. [[CrossRef](#)] [[PubMed](#)]
27. Edwards, J.C.; Szczepanski, L.; Szechinski, J.; Filipowicz-Sosnowska, A.; Emery, P.; Close, D.R.; Stevens, R.M.; Shaw, T. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N. Engl. J. Med.* **2004**, *350*, 2572–2581. [[CrossRef](#)] [[PubMed](#)]
28. De Andrade, N.P.B.; da Silva Chakr, R.M.; Xavier, R.M.; Viécili, D.; Correa, R.H.B.; de Oliveira Filho, C.M.; Brenol, C.V. Long-term outcomes of treat-to-target strategy in established rheumatoid arthritis: A daily practice prospective cohort study. *Rheumatol. Int.* **2017**, *37*, 993–997. [[CrossRef](#)] [[PubMed](#)]
29. Dickens, C.; Jackson, J.; Tomenson, B.; Hay, E.; Creed, F. Association of depression and rheumatoid arthritis. *Psychosomatics* **2003**, *44*, 209–215. [[CrossRef](#)] [[PubMed](#)]
30. Murphy, S.; Creed, F.; Jayson, M.I. Psychiatric disorder and illness behaviour in rheumatoid arthritis. *Br. J. Rheumatol.* **1988**, *27*, 357–363. [[CrossRef](#)] [[PubMed](#)]

31. Strand, V.; Mease, P.; Burmester, G.R.; Nikaï, E.; Coteur, G.; van Vollenhoven, R.; Combe, B.; Keystone, E.C.; Kavanaugh, A. Rapid and sustained improvements in health-related quality of life, fatigue, and other patient-reported outcomes in rheumatoid arthritis patients treated with certolizumab pegol plus methotrexate over 1 year: Results from the RAPID 1 randomized controlled trial. *Arthritis Res. Ther.* **2009**, *11*, R170. [[PubMed](#)]
32. Bland, J.M.; Altman, D.G. Regression towards the mean. *BMJ* **1994**, *308*, 1499. [[CrossRef](#)] [[PubMed](#)]



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Article

Prevalence and Fracture Risk of Osteoporosis in Patients with Rheumatoid Arthritis: A Multicenter Comparative Study of the FRAX and WHO Criteria

Sang Tae Choi ^{1,†}, Seong-Ryul Kwon ^{2,†}, Ju-Yang Jung ³, Hyoun-Ah Kim ³, Sung-Soo Kim ⁴, Sang Hyon Kim ⁵, Ji-Min Kim ⁵, Ji-Ho Park ¹ and Chang-Hee Suh ^{3,*}

¹ Division of Rheumatology, Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul 06973, Korea; beconst@cau.ac.kr (S.T.C.); pjh853@hanmail.net (J.-H.P.)

² Division of Rheumatology, Department of Internal Medicine, Inha University College of Medicine, Incheon 22332, Korea; rhksr@inha.ac.kr

³ Department of Rheumatology, Ajou University School of Medicine, Suwon 16499, Korea; serinne20@hanmail.net (J.-Y.J.); nakhada@naver.com (H.-A.K.)

⁴ Division of Rheumatology, Department of Internal Medicine, Ulsan University College of Medicine, Gangneung Asan Hospital, Gangneung 25440, Korea; drkiss@ulsan.ac.kr

⁵ Division of Rheumatology, Department of Internal Medicine, Keimyung University College of Medicine, Daegu 41931, Korea; mdkim9111@hanmail.net (S.H.K.); okjimin@hanmail.net (J.-M.K.)

* Correspondence: chsuh@ajou.ac.kr; Tel.: +82-10-8860-1534

† Sang Tae Choi and Seong-Ryul Kwon contributed equally to this work.

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Abstract: (1) Background: We evaluated the prevalence and fracture risk of osteoporosis in patients with rheumatoid arthritis (RA), and compared the fracture risk assessment tool (FRAX) criteria and bone mineral density (BMD) criteria established by the World Health Organization (WHO). (2) Methods: This retrospective cross-sectional study, which included 479 RA patients in 5 hospitals, was conducted between January 2012 and December 2016. The FRAX criteria for high-risk osteoporotic fractures were calculated including and excluding the BMD values, respectively. The definition of high risk for fracture by FRAX criteria and BMD criteria by WHO was 10-year probability of $\geq 20\%$ for major osteoporotic fracture or $\geq 3\%$ for hip fracture, and T score ≤ -2.5 or Z score ≤ -2.0 , respectively. (3) Results: The mean age was 61.7 ± 11.9 years. The study included 426 female patients (88.9%), 353 (82.9%) of whom were postmenopausal. Osteoporotic fractures were detected in 81 (16.9%) patients. The numbers of candidates for pharmacological intervention using the FRAX criteria with and without BMD and the WHO criteria were 226 (47.2%), 292 (61%), and 160 (33.4%), respectively. Only 69.2%–77% of the patients in the high-risk group using the FRAX criteria were receiving osteoporosis treatments. The following were significant using the WHO criteria: female (OR 3.55, 95% CI 1.46–8.63), age (OR 1.1, 95% CI 1.08–1.13), and BMI (OR 0.8, 95% CI 0.75–0.87). Glucocorticoid dose (OR 1.09, 95% CI 1.01–1.17), age (OR 1.09, 95% CI 1.06–1.12), and disease duration (OR 1.01, 95% CI 1–1.01) were independent risk factors for fracture. (4) Conclusions: The proportion of RA patients with a high risk of osteoporotic fractures was 33.4%–61%. Only 69.2%–77% of candidate patients were receiving osteoporotic treatments while applying FRAX criteria. Independent risk factors for osteoporotic fractures in RA patients were age, the dose of glucocorticoid, and disease duration.

Keywords: osteoporosis; fracture; fracture risk assessment tool; rheumatoid arthritis

1. Introduction

Osteoporosis is one of the most well-known complications in patients with rheumatoid arthritis (RA). RA is a disease that presents a state of chronic inflammation that is known to cause an increase in osteoclastic differentiation and an inhibition of osteogenesis [1]. Furthermore, the treatment with glucocorticoids then increases the imbalance which already existed due to the disease. Moreover, this association may be due to a lack of mobility and frequent occurrences of RA during menopause as well as systemic inflammation of RA and the use of corticosteroids [1–3]. The prevalence of osteoporosis in patients with RA was reported to be approximately twice as high as in the general population [4]. The frequency of osteoporosis in patients with RA has been reported to be 6.3% to 36.3% in the hip, and 12.3% to 38.9% in the spine [4–6]. Compared with controls, the fracture risk in patients with RA also increased for the hip (relative risk (RR): 2) and spine (RR: 2.4) [7]. Moreover, hip and vertebral osteoporotic fractures are known to be associated with an immediate and long-term (up to 20 years) increased risk of mortality [8]. Excess mortality during the first year after a hip fracture ranged from 8.4% to 36%, and the risk of mortality following hip fracture was estimated to be more than 2 times higher than that of the general population [9]. Therefore, it is very important to accurately assess the risk of osteoporotic fractures in RA patients.

The fracture risk assessment tool (FRAX) criteria and the bone mineral density (BMD) criteria established by the World Health Organization (WHO) are widely used for the risk assessment of osteoporotic fractures. The WHO criteria, using BMD measured by dual-energy X-ray absorptiometry (DXA), are the most widely used in the diagnosis of osteoporosis [10]. The management guidelines for the prevention and treatment of osteoporosis, developed by an expert committee of the National Osteoporosis Foundation (NOF), are also based on BMDs [11]. In 2008, a WHO task force introduced the FRAX tool to evaluate the 10-year probability for hip and major osteoporotic fractures [12]. The FRAX model contains various risk factors for osteoporotic fractures including country, age, sex, weight, height, smoking, previous fracture, family history of fracture, glucocorticoid treatment, alcohol intake, and BMD, if available [13]. In particular, RA is the only disease risk factor in the FRAX model, even though the input for RA is just a dichotomous variable.

In clinical settings, physicians determine the proper medications to prevent osteoporotic fractures based on these criteria [11,14]. However, when assessing the risk of osteoporotic fractures in patients with RA, the relevance and benefits among the three assessment methods (WHO osteoporosis criteria and FRAX criteria with BMD and without BMD) have not been clearly studied. Therefore, in this multicenter study, we aimed to evaluate the incidence among a high-risk group for osteoporotic fracture and to identify the risk factors of osteoporotic fractures in patients with RA by comparing these criteria. We also examined the extent of treatments for osteoporosis among patients in need of osteoporosis treatment.

2. Experimental Section

2.1. Study Population

In this retrospective cross-sectional study, we assessed 479 Korean patients with RA in 5 university hospitals between January 2012 and December 2016. All recruited patients were over the age of 18 and satisfied the 1987 American College of Rheumatology (ACR) criteria or the 2010 ACR/European League Against Rheumatism (EULAR) criteria for RA [15,16]. Recorded data using a medical chart review included age, sex, body mass index (BMI), menopausal status, hormone supplement therapy in postmenopausal women, fracture history, history of parental hip fracture, daily alcohol intake, smoking status, autoantibody status, erythrocyte sediment rate (ESR), C-reactive protein (CRP), and the presence of secondary osteoporosis. The standard of BMI was 25 kg/m² using validated BMI categorization for the Korean population [17]. Therapeutic medication lists for the treatment of RA including current glucocorticoid use, cumulative glucocorticoid dose, and conventional and biological disease-modifying anti-rheumatic drugs, as well as pharmacological intervention for osteoporosis, such as bisphosphonate,

selective estrogen receptor modulators (SERM), vitamin D and calcium, were also obtained. The study was approved by the Institutional Review Board (IRB) of each Hospital (C2015163 (1621), 2015-09-026, AJIRB-MED-MDB-15-285, 3-32100191-AB-N-01, and DSMC2015-12-017-007). Informed consent was waived by the IRB.

2.2. Evaluation of Osteoporosis by BMD Criteria

Candidates for pharmacological interventions to prevent osteoporosis were assessed using the WHO osteoporosis criteria [10]. All BMD measurements were done using the same technique. The BMD of the lumbar vertebrae (L1–L4) and both hips were measured using DXA (GE Lunar, Madison, WI, USA). *t*- and *z*-scores were calculated with the referent BMD of 5 hospitals. According to the WHO criterion, patients with osteoporosis and osteopenia were defined having a value of the *t*-score that was -2.5 or less, and from -2.5 to -1 , respectively, for postmenopausal women or men ≥ 50 years old. For the evaluation of premenopausal women or men < 50 years old, *z*-scores ≤ -2 were considered as osteoporosis.

2.3. Osteoporotic Fracture Risk Evaluation by FRAX

The 10-year probability of major osteoporotic and hip fractures was calculated by the FRAX tool, based on medical chart reviews and questionnaires. The FRAX criteria for high risk of osteoporotic fracture were defined as a 10-year probability of $\geq 20\%$ for major osteoporotic fracture or $\geq 3\%$ for hip fracture. We calculated two kinds of FRAX values using the Korean model (<http://www.shef.ac.uk/FRAX/tool.aspx?country=25>); FRAX with BMD was calculated including the femur neck BMD (g/cm^2) value.

2.4. Risk Factors for Fracture

Information regarding previous fractures was obtained through patient self-reporting questionnaires during routine clinic visits and spinal x-ray evaluations. Potential factors associated with osteoporotic fractures were collected by patient interviews, physical examinations, and laboratory tests. Patient baseline characteristics, including clinical variables, RA medications, and osteoporosis-related factors, were investigated at enrollment. The dose of glucocorticoid was calculated on the basis of prednisolone. Glucocorticoid use was grouped as ≥ 5 mg/day prednisolone equivalent and < 5 mg/day prednisolone-equivalent. Candidates for pharmacological interventions preventing osteoporotic fracture were identified using the WHO osteoporosis or the FRAX criteria. We evaluated osteoporotic medications, such as bisphosphonate and SERM, to obtain the ratio of high-risk patients currently undergoing osteoporosis treatments.

2.5. Statistical Analysis

All measurements were expressed as means \pm SD. Values that were not normally distributed were represented as medians (IQR). A Student's *t*-test was used for the comparison of mean values. A Pearson's χ^2 test was used to compare the differences between categorical variables for analyzing correlations. Multiple linear analysis, including glucocorticoid dose, age, sex, BMI, smoking, and disease duration, was used to identify factors affecting the FRAX values and BMD scores. Glucocorticoid dose, age, BMI and disease duration were continuous variables in this model. A multivariable logistic regression analysis using glucocorticoid dose, age, sex, BMI, and disease duration was performed to obtain the respective odds ratios, and all the variables except sex were continuous variables in this model. In all analyses, a *p* value of < 0.05 was considered statistically significant. All statistical analyses were conducted using the SPSS version 20 (IBM Corp, Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics of the Study Participants

The baseline characteristics of the participants are summarized in Table 1. The data of baseline characteristics in Table 1 were based on the time of BMD test, except for rheumatoid factor and anti-citrullinated protein antibody. The average age of the 479 subjects was 61.5 ± 11.5 years. Study participants included 426 female patients (88.9%) with 353 (82.9%) being postmenopausal. The median disease duration was 53 (33–72) months. The median ESR and CRP levels were 15 (7–29) mm/h and 0.18 (0.05–0.79) mg/L, respectively. The proportion of patients who were using glucocorticoids was 92.3% (442/479), and the median dose was 2.5 mg/day prednisolone-equivalent. The number of patients with biological disease-modifying anti-rheumatic drug (DMARD) use was 56 (11.7%); 13 (2.7%) etanercept, 10 (2.1%) infliximab, 9 (2.9%) adalimumab, 3 (0.6%) golimumab, 11 (2.3%) tocilizumab, 6 (1.3%) abatacept, 2 (0.4%) rituximab, and 2 (0.4%) tofacitinib. A total of 262 (54.7%) patients were receiving osteoporosis treatments: 189 (39.5%) patients were treated with bisphosphonate, and 98 (20.5%) were using SERM. Among all the participants, 81 (16.9%) patients (7 male and 74 postmenopausal females; mean age of 69.5 ± 9.3 years) had fractures, all of which were vertebral.

Table 1. Baseline characteristics of the study participants.

| Variables | RA Patients (N = 479) |
|---------------------------------------|-----------------------|
| Age (year) | 61.8 ± 11.5 |
| Sex (female) | 426 (88.9%) |
| Menopause | 353 (82.9%) |
| Weight (kg) | 55.4 (49.2–62.8) |
| Height (cm) | 155.3 (150.1–160) |
| BMI (kg/m ²) | 23.1 (20.9–25) |
| Smoking | 33 (6.9%) |
| Alcohol | 41 (8.6%) |
| Disease duration (month) | 53 (33–72) |
| 1987 ACR criteria | 377 (78.7%) |
| 2010 ACR/EULAR criteria | 418 (87.2%) |
| RF titer (IU/mL) | 67.2 (24–165.2) |
| ACPA titer (U/mL) | 78 (0.4–179.1) |
| RF positive | 380 (79.3%) |
| ACPA positive | 295 (61.6%) |
| ESR (mm/h) | 15 (7–29) |
| CRP (mg/L) | 0.18 (0.05–0.79) |
| Medications | |
| Glucocorticoid | 442 (92.3%) |
| Prednisolone-equivalent dose (mg/day) | 2.5 (1.25–5) |
| NSAID | 341 (71.2%) |
| Biologics | 56 (11.7%) |
| Vitamin D | 228 (47.6%) |
| Calcium | 195 (40.7%) |
| Proton pump inhibitor | 123 (25.7%) |
| Treatment for osteoporosis | 262 (54.7%) |
| Bisphosphonate | 189 (39.5%) |
| SERM | 98 (20.5%) |
| Vertebral fracture | 81 (16.9%) |
| Age (year) | 69.5 ± 9.3 |
| Male | 7 (8.6%) |
| Postmenopausal woman | 74 (91.4%) |

BMI, body mass index; ACR, American college of rheumatology; EULAR, European League Against Rheumatism; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NSAID, non-steroidal anti-inflammatory drug; SERM, selective estrogen receptor modulator. Values were represented as mean ± standard deviation. Values that were not normally distributed are represented as medians (IQR).

Table 2. Candidates for pharmacological treatment using the FRAX criteria with and without BMD and the WHO osteoporosis criteria in patients with rheumatoid arthritis.

| Patient Groups | FRAX with BMD | FRAX without BMD | Osteoporosis of the WHO | p Value ¹ | p Value ² | p Value ³ |
|--------------------------|---------------|------------------|-------------------------|----------------------|----------------------|----------------------|
| Overall (n = 479) | 226 (47.2%) | 292 (61%) | 160 (33.4%) | <0.001 | <0.001 | <0.001 |
| Men (n = 53) | 17 (32.1%) | 27 (50.9%) | 10 (18.9%) | 0.006 | 0.070 | <0.001 |
| Women (n = 426) | 209 (49.1%) | 265 (62.2%) | 150 (35.2%) | <0.001 | <0.001 | <0.001 |
| Premenopausal (n = 56) | 3 (5.4%) | 2 (3.6%) | 1 (1.8%) | 0.659 | 0.322 | 0.568 |
| Postmenopausal (n = 370) | 206 (55.7%) | 263 (71.1%) | 149 (40.3%) | <0.001 | <0.001 | <0.001 |

FRAX, fracture risk assessment tool; BMD, bone mineral density; WHO, World Health Organization. ¹ FRAX with BMD vs. FRAX without BMD. ² FRAX with BMD vs. osteoporosis.

³ FRAX without BMD vs. osteoporosis.

3.2. Distribution of Candidates for Pharmacological Intervention Using the FRAX and the WHO Osteoporosis Criteria

The number of patients with osteoporosis according to the WHO criteria was 160 (33.4%), and the number of candidates for pharmacological intervention using the FRAX criteria with and without BMD was 226 (47.2%) and 292 (61%), respectively. There were significant differences among these three groups (Table 2). When men, women, and postmenopausal women were analyzed separately, the number of candidates for pharmacological intervention was the highest for the FRAX criteria without BMD, followed by the FRAX criteria with BMD, and finally, the WHO osteoporosis criteria (Table 2). The numbers of candidates for pharmacological intervention of women were higher than those of men using the FRAX criteria with BMD ($p = 0.014$) and when using the WHO osteoporosis criteria ($p = 0.011$); however, there were no significant differences when using the FRAX criteria without BMD ($p = 0.077$). The proportion of patients with a BMD < -1.0 was 216/226 (95.6%) among those eligible for FRAX criteria with BMD, and 265/292 (90.8%) for FRAX without BMD.

3.3. Proportion of Patients Receiving Osteoporosis Treatment in the High-Risk Group According to the FRAX Criteria and the WHO Osteoporosis Criteria

Table 3 shows the proportion of the patients currently receiving osteoporosis treatments (bisphosphonate or SERM) in the high-risk group, based on the FRAX criteria with or without BMD and the WHO osteoporosis criteria. Of the patients in the high-risk group using the FRAX criteria with BMD, only 77% were receiving osteoporosis treatments, which was similar to the percentages in sex and menopause subgroup analyses (men, 64.7%; women, 78%; postmenopausal women, 78.6%). The proportion of patients undergoing osteoporosis treatment among the high-risk group using the FRAX criteria without BMD was similar (overall, 69.2%; men, 55.6%; woman, 70.6%; postmenopausal women, 71.1%, respectively). However, when applying the WHO osteoporosis criteria, more than 90% of patients were receiving osteoporosis treatments in all patient groups or subgroups (overall, 91.3%; men, 90%; woman, 91.3%; postmenopausal women, 91.3%).

Table 3. Current osteoporosis treatments in high-risk groups according to the FRAX criteria and the WHO osteoporosis criteria.

| Patient Groups | FRAX with BMD | FRAX without BMD | WHO Osteoporosis |
|----------------|-----------------|------------------|------------------|
| Overall | 174/226 (77%) | 202/292 (69.2%) | 146/160 (91.3%) |
| Men | 11/17 (64.7%) | 15/27 (55.6%) | 9/10 (90%) |
| Women | 163/209 (78%) | 187/265 (70.6%) | 137/150 (91.3%) |
| Premenopausal | 0/3 (0%) | 0/2 (0%) | 1/1 (100%) |
| Postmenopausal | 163/206 (78.6%) | 187/263 (71.1%) | 136/149 (91.3%) |

FRAX, fracture risk assessment tool; BMD, bone mineral density; WHO, World Health Organization.

3.4. Fracture Risk for the FRAX Criteria, the WHO Osteoporosis Criteria and Fractures

Table 4 shows which dichotomous factors were related to the FRAX criteria with BMD and without BMD, the WHO criteria, and incidence of fractures. Patients with high fracture risk by FRAX with BMD were more likely to be female, especially postmenopausal women (female, $p = 0.019$; menopause, $p < 0.001$), with alcohol use, glucocorticoid use, and proton pump inhibitor use ($p = 0.042$, $p = 0.007$, $p = 0.018$, and $p < 0.001$, respectively), and also with lower BMI ($<25 \text{ kg/m}^2$) ($p = 0.042$). Patients with high fracture risk by FRAX without BMD had lower BMI and longer disease duration, and were more likely to be menopausal women, alcohol users, glucocorticoid users and proton pump users than patients who were not at high risk by FRAX without BMD ($p = 0.004$, $p = 0.007$, $p < 0.001$, $p = 0.005$, $p < 0.001$, and $p < 0.001$, respectively).

The results of frequency comparison for WHO osteoporosis criteria were similar to those for the FRAX criteria with BMD except for glucocorticoid use (female, $p = 0.017$; menopause, $p < 0.001$;

BMI < 25 kg/m², *p* < 0.001; alcohol use, *p* = 0.002; proton pump inhibitor use, *p* = 0.008). Biologic use, seropositivity, and ESR elevation did not show any significant difference for all the criteria.

The number of patients meeting the FRAX criteria with BMD was significantly higher in the higher dose with ≥5 mg/day prednisolone group in contrast to the lower dose with < 5mg/day prednisolone group (76/127; 59.8% vs. 136/307; 44.3%, *p* = 0.003).

Multiple linear analyses were implemented to identify factors affecting the FRAX criteria and BMD scores (Table 5). The 10-year probability percentage for hip fractures and that for major osteoporotic fractures in the FRAX with BMD, the 10-year probability percentage for major osteoporotic fractures in the FRAX without BMD, and total hip BMD scores, were significantly associated with glucocorticoid dose, age, female sex, BMI, and disease duration. However, the 10-year probability percentage for hip fractures in the FRAX without BMD was only associated with age and BMI.

Multivariable logistic regression analyses were performed to identify the fracture risk for the FRAX criteria and the WHO osteoporosis criteria, and fractures in patients with RA (Table 6). Glucocorticoid dose (odds ratio (OR) 1.15, 95% confidence interval (CI) 1.03–1.28, *p* = 0.016), age (OR 1.17, CI 1.13–1.2, *p* < 0.001), female sex (OR 4.22, CI 1.8–9.9, *p* = 0.001), and BMI (OR 0.92, CI 0.85–0.99, *p* = 0.002) were independent fracture risk for the FRAX criteria with BMD. Age (OR 1.65, CI 1.47–1.84, *p* < 0.001), female sex (OR 8.23, CI 2.27–29.88, *p* = 0.001), and BMI (OR 0.6, CI 0.5–0.72, *p* < 0.001) were associated with the FRAX criteria without BMD. Likewise, age (OR 1.1, CI 1.08–1.14, *p* < 0.001), female sex (OR 3.55, CI 1.46–8.63, *p* = 0.005), and BMI (OR 0.8, CI 0.75–0.87, *p* < 0.001) were associated fracture risk for the WHO criteria. Furthermore, independent risk factors for fracture are glucocorticoid dose (OR 1.09, 95% CI 1.01–1.17, *p* = 0.02), age (OR 1.09, 95% CI 1.06–1.12, *p* < 0.001) and disease duration (OR 1.01, 95% CI 1–1.01, *p* = 0.002).

Table 4. Comparison of the frequencies for the high-risk of osteoporotic fractures according to FRAX criteria and WHO osteoporosis criteria.

| Variables | FRAX Criteria with BMD (% <i>p</i> Value) | FRAX Criteria without BMD (% <i>p</i> value) | WHO Criteria (% <i>p</i> Value) | Fracture (% <i>p</i> Value) |
|----------------------------|--|---|------------------------------------|--------------------------------|
| Sex (female) | 48.6%, 0.019 | 62.2%, 0.077 | 35.2%, 0.017 | 17.6%, 0.457 |
| Menopause | 57.2%, <0.001 | 71.1%, <0.001 | 41.9%, <0.001 | 21%, <0.001 |
| BMI < 25 kg/m ² | 50%, 0.042 | 64.9%, 0.004 | 39%, <0.001 | 17.3%, 0.868 |
| Disease duration > 2 years | 48.9%, 0.098 | 63.7%, 0.007 | 35.1%, 0.081 | 18.6%, 0.063 |
| Seropositivity | 46.5%, 0.970 | 61.4%, 0.170 | 35.3%, 0.118 | 17.5%, 0.129 |
| ESR elevation | 52.5%, 0.052 | 65.4%, 0.100 | 37.4%, 0.196 | 16.9%, 0.950 |
| Smoking | 30.3%, 0.092 | 45.5%, 0.109 | 21.2%, 0.209 | 18.2%, 0.855 |
| Alcohol use | 24.4%, 0.007 | 36.6%, 0.005 | 9.8%, 0.002 | 10.3%, 0.239 |
| Glucocorticoid use | 49%, 0.018 | 63.8%, <0.001 | 33.9%, 0.532 | 17.3%, 0.81 |
| Biologics use | 46%, 0.785 | 60%, 0.378 | 40%, 0.137 | 20.8%, 0.574 |
| PPI use | 62.6%, <0.001 | 77.2%, <0.001 | 43.1%, 0.008 | 19%, 0.524 |

FRAX, fracture risk assessment tool; BMD, bone mineral density; WHO, World Health Organization; BMI, body mass index; ESR, erythrocyte sedimentation rate; PPI, proton pump inhibitor.

Table 5. Multiple linear risk analyses using the FRAX criteria and the WHO osteoporosis criteria.

| | Variable | β | <i>p</i> Value | Adjusted <i>R</i> ² |
|--|---------------------|---------|----------------|--------------------------------|
| FRAX with BMD % 10-year probability for hip fracture | Constant | -13.464 | | 0.309 |
| | Glucocorticoid dose | 0.418 | <0.001 | |
| | Age | 0.254 | <0.001 | |
| | Female | 3.63 | <0.001 | |
| | BMI | -0.271 | 0.001 | |
| | Smoking | 2.912 | 0.012 | |
| | Disease duration | 0.01 | 0.114 | |
| FRAX with BMD % 10-year probability for major osteoporotic fracture | Constant | -24.341 | | 0.407 |
| | Glucocorticoid dose | 0.599 | <0.001 | |
| | Age | 0.386 | <0.001 | |
| | Female | 7.203 | <0.001 | |
| | BMI | -0.163 | 0.097 | |
| | Smoking | 2.289 | 0.109 | |
| | Disease duration | 0.025 | 0.001 | |
| FRAX without BMD % 10-year probability for hip fracture | Constant | -8.455 | | 0.326 |
| | Glucocorticoid dose | 0.179 | 0.219 | |
| | Age | 0.475 | <0.001 | |
| | Female | 0.515 | 0.75 | |
| | BMI | -0.641 | <0.001 | |
| | Smoking | -0.399 | 0.832 | |
| | Disease duration | 0.004 | 0.722 | |
| FRAX without BMD % 10-year probability for major osteoporotic fracture | Constant | -26.833 | | 0.630 |
| | Glucocorticoid dose | 0.36 | <0.001 | |
| | Age | 0.547 | <0.001 | |
| | Female | 7.863 | <0.001 | |
| | BMI | -0.411 | <0.001 | |
| | Smoking | 1.85 | 0.122 | |
| | Disease duration | 0.025 | <0.001 | |

Table 5. Cont.

| | Variable | β | <i>p</i> Value | Adjusted <i>R</i> ² |
|---------------------------|---------------------|---------|----------------|--------------------------------|
| BMD score mean L-spine | Constant | 0.705 | | 0.322 |
| | Glucocorticoid dose | -0.037 | 0.054 | |
| | Age | -0.055 | <0.001 | |
| | Female | -0.485 | 0.024 | |
| | BMI | 0.097 | <0.001 | |
| | Smoking | -0.102 | 0.638 | |
| | Disease duration | 0.004 | 0.001 | |
| BMD score total hip | Constant | 1.444 | | 0.444 |
| | Glucocorticoid dose | -0.054 | 0.001 | |
| | Age | -0.047 | <0.001 | |
| | Female | -1.072 | <0.001 | |
| | BMI | 0.111 | <0.001 | |
| | Smoking | 0.018 | 0.926 | |
| | Disease duration | -0.004 | <0.001 | |

FRAX, fracture risk assessment tool; BMD, bone mineral density; WHO, World Health Organization; BMI, body mass index.

Table 6. Multivariable logistic regression analyses for high-risk of fracture of FRAX criteria and WHO osteoporosis criteria, and fractures in patients with rheumatoid arthritis.

| | FRAX with BMD Criteria | | FRAX without BMD Criteria | | WHO Criteria | | Fracture | |
|--------------------------|------------------------|---------|---------------------------|---------|-------------------|---------|-------------------|---------|
| | OR (95% CI) | p Value | OR (95% CI) | p Value | OR (95% CI) | p Value | OR (95% CI) | p Value |
| Constant | 0 | | 0 | | 0.09 | | 0 | |
| Glucocorticoid dose (mg) | 1.15 (1.03, 1.28) | 0.016 | 1.03 (0.94, 1.12) | 0.537 | 1.05 (0.98, 1.14) | 0.187 | 1.09 (1.01, 1.17) | 0.02 |
| Age (year) | 1.17 (1.13, 1.2) | <0.001 | 1.65 (1.47, 1.84) | <0.001 | 1.1 (1.08, 1.13) | <0.001 | 1.09 (1.06, 1.12) | <0.001 |
| Female | 4.22 (1.8, 9.9) | 0.001 | 8.23 (2.27, 29.88) | 0.001 | 3.55 (1.46, 8.63) | 0.005 | 1.81 (0.58, 4.01) | 0.395 |
| BMI (kg/m ²) | 0.92 (0.85, 0.99) | 0.02 | 0.6 (0.5, 0.72) | <0.001 | 0.8 (0.75, 0.87) | <0.001 | 1.02 (0.95, 1.11) | 0.471 |
| Disease duration (month) | 1 (1, 1.01) | 0.189 | 1.01 (1, 1.02) | 0.121 | 1 (1, 1.01) | 0.093 | 1.01 (1, 1.01) | 0.002 |

FRAX, fracture risk assessment tool; BMD, bone mineral density; WHO, World Health Organization; OR, Odds Ratio; 95% CI, 95% confidence intervals; BMI, body mass index.

4. Discussion

In this study, we compared both the WHO and the FRAX criteria to estimate the risk of osteoporotic fractures and to determine candidates for pharmacological treatments. As a result, 33.4% of patients were eligible for the WHO osteoporosis criteria, 47.2% for the FRAX criteria with BMD, and 61% for the FRAX criteria without BMD, with statistically significant differences in each high-risk group for osteoporotic fractures (Table 2). When we performed subgroup analyses separated by men, women, and postmenopausal women, all except premenopausal women showed a similar tendency.

We found that there was a significant difference between the WHO criteria and the FRAX criteria with or without BMD, as well as between the FRAX criteria with BMD and those without BMD, which is different from the previous assertion that there is a high degree of agreement [12,18]. A significant difference in the number of high-risk groups for osteoporotic fractures according to the criteria applied is a serious problem, since both the FRAX and WHO criteria are widely used in clinical practice, and drugs used to prevent osteoporotic fractures have several side effects [11,12,14]. Therefore, it is very important to determine which of the three criteria should be used. To accomplish this, we had to determine the objective standard in order to evaluate all three criteria; however, it was not easy to set standards to which everyone could agree. Ultimately, the goal of osteoporosis treatment is to prevent fractures, so in order to select the best criteria, it may be advantageous to compare the incidences of actual fractures in each criterion. Unfortunately, our study was only a retrospective cross-sectional study, and patients with actual fracture histories were confined to compression fractures of the spine, thereby limiting the prevalence of actual fractures used to evaluate the best criteria.

Recently, a study comparing the FRAX without BMD and BMD alone was published [19]. In that study, the FRAX probability of a major osteoporotic fracture in 50-year-old women was approximately two-fold higher than that for women of the same age, but with an average BMD. The authors concluded that intervention thresholds based on BMD alone do not optimally target women at higher fracture risk than age-matched individuals without clinical risk factors [19]. However, the FRAX with BMD was not evaluated in that study, and there is no comparative study in RA patients. As shown in our study, the WHO criteria may have less actual risk reflected in high-risk disease such as RA [7]. Conversely, the FRAX value may be artificially elevated because RA itself is regarded as one of the major risk factors in FRAX calculations [12].

Table 3 shows that the number of patients actually receiving pharmacological treatments for osteoporosis (bisphosphonate or SERM) was only 69.2%–77% based on the FRAX with or without BMD. Interestingly, more than 90% of patients were receiving medication for osteoporosis when the WHO criteria were applied. This is probably due to the fact that insurance, covering the treatment of osteoporosis in Korea, is based on the WHO criteria.

Osteoporotic fracture, especially hip fracture, can increase mortality rate, so there are attempts to further expand the definition of osteoporosis. For example, while osteoporosis has traditionally been diagnosed based on *t*-score of less than -2.5 , osteoporosis can be clinically diagnosed if there is a low-trauma fracture in the absence of other metabolic bone disease, independent of the BMD (*t*-score) value [14]. However, there is no clear evidence that the WHO criteria reflect osteoporotic fractures better than the FRAX criteria. Considering that the FRAX criteria are calculated with BMD values and various clinical aspects of osteoporosis patients, the importance of FRAX criteria in predicting the risk of osteoporotic fracture is increasingly emphasized [19,20], making the decision to treat osteoporosis merely on the basis of WHO osteoporosis criteria problematic. Furthermore, as shown in Table 2, the number of candidates for pharmacological intervention based on the WHO osteoporosis criteria is less than those based on the FRAX criteria; therefore, candidates in need of pharmacological treatment might be missed or their numbers underestimated when applying the WHO criteria. Alternately, we may also consider the new criteria suggested by the National Bone Health Alliance, that osteoporosis may be diagnosed in patients with osteopenia and increased fracture risk using FRAX country-specific thresholds [21]. This is a kind of compromise between FRAX criteria and WHO criteria. In our study, however, almost all patients who met the FRAX criteria had a BMD of -1.0 or less (FRAX criteria with

BMD, 95.6%; FRAX criteria without BMD, 90.8%). This result implies that applying the above criteria gives almost the same result as applying the FRAX criteria at least in patients with RA.

Therefore, further study is needed on how to apply the FRAX and WHO criteria in diagnosing and treating osteoporosis, or how to combine these two criteria. In addition, since FRAX criteria with BMD and without BMD may be different, as we have seen in our study, which of these tools is better must be clarified in the future research. Ultimately, osteoporosis treatment should aim for precision medicine. In order to do this, it is necessary to evaluate and approve the risk factors of individual patients. In particular, considering that there is a high risk of osteoporosis in RA patients and that the risk of osteoporotic fracture may be overestimated by FRAX tool in RA patients, more prospective studies involving large populations are required to clarify which assessment tool can precisely estimate osteoporotic fractures in patients with RA.

Nevertheless, it is clear that more than half of postmenopausal women with RA are included in the high-risk group for osteoporotic fractures. The numbers of the high-risk group for osteoporotic fractures in RA patients with postmenopausal woman were higher than those in total RA patients, regardless of which criteria were applied (WHO criteria, 40.3% vs. 33.4%; FRAX criteria with BMD, 55.7% vs. 47.2%; FRAX without BMD, 71.1% vs. 61%, respectively) (Table 2).

The independent risk factors for pharmacological treatments to prevent osteoporotic fractures were female sex, menopause, alcohol use, glucocorticoid use, and proton pump inhibitor use (Table 4). In multiple linear analyses, female sex, older age, lower BMI, longer disease duration, and glucocorticoid dose were associated with a higher probability of osteoporotic fractures (Table 5). In multivariable logistic regression analyses, the OR of female sex was 3.55 and those of age and BMI were 1.1 and 0.8, respectively for the WHO osteoporosis criteria (Table 6). These findings agreed with previous studies [11]. Although smoking has been known to be a major risk factor in many studies [11,22], it was not a statistically significance factor in our study, which was probably due to the fact that the majority of patients enrolled in our study were postmenopausal women with a smoking rate of only 6.9%. Our results showed that the use of biologics, seropositivity, and the levels of acute phase reactants were not associated with an increased risk of osteoporotic fractures. Regarding the use of glucocorticoids, the number of patients meeting the FRAX criteria with BMD was higher in the higher dose with ≥ 5 mg/day prednisolone group. The OR of prednisolone dose was 1.15 in the FRAX criteria with BMD and 1.09 for fractures. These results suggest that no use of glucocorticoid is important in RA patients for the prevention of osteoporotic fractures, and if used, glucocorticoid use should be at a dosage of <5 mg/day.

There are some limitations to this study. The first is that it was conducted not on *all* RA patients, but on RA patients who had a BMD. There may be many patients who had been tested for BMD, given that BMD is not a routine evaluation in RA patients, which could cause a selection bias in this study. The prevalence of the high-risk group of osteoporotic fractures in patients with RA was 33.4%–61.0% in this study, which was higher than the prevalence of osteoporosis in the general population of the United States and Korea [23,24]. However, these results may be overestimated considering selection bias. There are some studies dealing with the incidence and risk factors of osteoporotic fractures in patients with RA [25–27]. In a study conducted using the WHO criteria for RA postmenopausal patients, 46.8% of the patients were considered to have osteoporosis (t -score ≤ -2.5) [25]. However, in another study using the FRAX criteria for RA patients, in which the population of this study was similar to our study population, only 17.4% of the patients were reported to meet the FRAX criteria for pharmacological interventions [27]. Therefore, a large prospective study of all RA patients is needed to more accurately evaluate the prevalence of the high-risk group of osteoporotic fractures in patients with RA.

Another limitation is that only bisphosphonate and SERM were evaluated as treatments for osteoporosis. Recently, new drugs such as teriparatide and denosumab are being used to treat osteoporosis [11,28,29], but there is no data evaluating these drugs in this study. Nevertheless, this study has important implications, in that bisphosphonate and SERM are still widely used as

primary drugs in the treatment of osteoporosis [11,14]. Another limitation is that 88.9% of patients enrolled in this study were female. The final limitation is that our study did not measure vitamin D levels. Lower levels of vitamin D, which are known in addition to regulating bone homeostasis, regulate the differentiation and activities of immune cells [30]. So, vitamin D deficiency can stimulate inflammatory status influencing disease status other than increasing bone erosion. Moreover, mutation and polymorphism in vitamin D metabolism genes are associated with serum 25(OH)D levels [30]. Therefore, these factors might have been associated with the results of our study.

5. Conclusions

The proportion of RA patients with a high risk of osteoporotic fractures was 33.4% for WHO criteria, 47.2% for FRAX criteria with BMD, and 61.0% for FRAX without BMD. Only 69.2%–77% of candidate patients were receiving osteoporotic treatments when applying the FRAX criteria, in contrast to 91.3% when applying the WHO criteria. Older age, higher doses of glucocorticoid, and longer disease duration were independent risk factors for osteoporotic fractures in patients with RA.

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References

1. Kleyer, A.; Schett, G. Arthritis and bone loss: A hen and egg story. *Curr. Opin. Rheumatol.* **2014**, *26*, 80–84. [[CrossRef](#)] [[PubMed](#)]
2. Orstavik, R.E.; Haugeberg, G.; Mowinckel, P.; Hoiseth, A.; Uhlig, T.; Falch, J.A.; Halse, J.I.; McCloskey, E.; Kvien, T.K. Vertebral deformities in rheumatoid arthritis: A comparison with population-based controls. *Arch. Intern. Med.* **2004**, *164*, 420–425. [[CrossRef](#)] [[PubMed](#)]
3. Durward, G.; Non Pugh, C.; Ogunremi, L.; Wills, R.; Cottee, M.; Patel, S. Detection of risk of falling and hip fracture in women referred for bone densitometry. *Lancet* **1999**, *354*, 220–221. [[CrossRef](#)]
4. Haugeberg, G.; Uhlig, T.; Falch, J.A.; Halse, J.I.; Kvien, T.K. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: Results from 394 patients in the Oslo County Rheumatoid Arthritis register. *Arthritis Rheum.* **2000**, *43*, 522–530. [[CrossRef](#)]
5. Sinigaglia, L.; Nervetti, A.; Mela, Q.; Bianchi, G.; Del Puente, A.; Di Munno, O.; Frediani, B.; Cantatore, F.; Pellerito, R.; Bartolone, S.; et al. A multicenter cross sectional study on bone mineral density in rheumatoid arthritis. Italian Study Group on Bone Mass in Rheumatoid Arthritis. *J. Rheumatol.* **2000**, *27*, 2582–2589. [[PubMed](#)]
6. Guler-Yuksel, M.; Bijsterbosch, J.; Goekoop-Ruiterman, Y.P.; de Vries-Bouwstra, J.K.; Runday, H.K.; Peeters, A.J.; de Jonge-Bok, J.M.; Breedveld, F.C.; Dijkmans, B.A.; Allaart, C.F.; et al. Bone mineral density in patients with recently diagnosed, active rheumatoid arthritis. *Ann. Rheum. Dis.* **2007**, *66*, 1508–1512. [[CrossRef](#)]
7. van Staa, T.P.; Geusens, P.; Bijlsma, J.W.; Leufkens, H.G.; Cooper, C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. *Arthritis Rheum.* **2006**, *54*, 3104–3112. [[CrossRef](#)]
8. Sattui, S.E.; Saag, K.G. Fracture mortality: Associations with epidemiology and osteoporosis treatment. *Nat. Rev. Endocrinol.* **2014**, *10*, 592–602. [[CrossRef](#)]
9. Abrahamsen, B.; van Staa, T.; Ariely, R.; Olson, M.; Cooper, C. Excess mortality following hip fracture: A systematic epidemiological review. *Osteoporos. Int.* **2009**, *20*, 1633–1650. [[CrossRef](#)]
10. Report of a WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. *World Health Organ. Tech. Rep. Ser.* **1994**, *843*, 1–129.

11. Cosman, F.; de Beur, S.J.; LeBoff, M.S.; Lewiecki, E.M.; Tanner, B.; Randall, S.; Lindsay, R. Clinician's guide to prevention and treatment of osteoporosis. *Osteoporos. Int.* **2014**, *25*, 2359–2381. [CrossRef] [PubMed]
12. Kanis, J.A.; Johnell, O.; Oden, A.; Johansson, H.; McCloskey, E. FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos. Int.* **2008**, *19*, 385–397. [CrossRef] [PubMed]
13. World Health Organization. Fracture Risk Assessment Tool (FRAX). Available online: <http://www.shef.ac.uk/FRAX> (accessed on 5 December 2016).
14. Camacho, P.M.; Petak, S.M.; Binkley, N.; Clarke, B.L.; Harris, S.T.; Hurley, D.L.; Kleerekoper, M.; Lewiecki, E.M.; Miller, P.D.; Narula, H.S.; et al. American association of clinical endocrinologists and American college of endocrinology clinical practice guidelines for the diagnosis and treatment of postmenopausal osteoporosis—2016. *Endocr. Pract.* **2016**, *22*, 1–42. [CrossRef] [PubMed]
15. Arnett, F.C.; Edworthy, S.M.; Bloch, D.A.; McShane, D.J.; Fries, J.F.; Cooper, N.S.; Healey, L.A.; Kaplan, S.R.; Liang, M.H.; Luthra, H.S.; et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* **1988**, *31*, 315–324. [CrossRef] [PubMed]
16. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O., 3rd; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; Combe, B.; et al. 2010 rheumatoid arthritis classification criteria: An American college of rheumatology/european league against rheumatism collaborative initiative. *Ann. Rheum. Dis.* **2010**, *69*, 1580–1588. [CrossRef] [PubMed]
17. Korean Statistical Information Service. Available online: <http://kosis.kr/eng/> (accessed on 14 November 2018).
18. Gadam, R.K.; Schlauch, K.; Izuora, K.E. Frax prediction without BMD for assessment of osteoporotic fracture risk. *Endocr. Pract.* **2013**, *19*, 780–784. [CrossRef] [PubMed]
19. Johansson, H.; Azizieh, F.; Al Ali, N.; Alessa, T.; Harvey, N.C.; McCloskey, E.; Kanis, J.A. FRAX- vs. T-score-based intervention thresholds for osteoporosis. *Osteoporos. Int.* **2017**, *28*, 3099–3105. [CrossRef]
20. Kanis, J.A.; Harvey, N.C.; Cooper, C.; Johansson, H.; Oden, A.; McCloskey, E.V. A systematic review of intervention thresholds based on FRAX: A report prepared for the National Osteoporosis Guideline Group and the International Osteoporosis Foundation. *Arch. Osteoporos.* **2016**, *11*, 25. [CrossRef]
21. Siris, E.S.; Adler, R.; Bilezikian, J.; Bolognese, M.; Dawson-Hughes, B.; Favus, M.J.; Harris, S.T.; Jan de Beur, S.M.; Khosla, S.; Lane, N.E.; et al. The clinical diagnosis of osteoporosis: A position statement from the National Bone Health Alliance Working Group. *Osteoporos. Int.* **2014**, *25*, 1439–1443. [CrossRef]
22. Yoon, V.; Maalouf, N.M.; Sakhaee, K. The effects of smoking on bone metabolism. *Osteoporos. Int.* **2012**, *23*, 2081–2092. [CrossRef]
23. Looker, A.C.; Melton, L.J., 3rd; Harris, T.B.; Borrud, L.G.; Shepherd, J.A. Prevalence and trends in low femur bone density among older US adults: NHANES 2005–2006 compared with NHANES III. *J. Bone Miner. Res.* **2010**, *25*, 64–71. [CrossRef] [PubMed]
24. Ha, Y. Epidemiology of osteoporosis in Korea. *J. Korean Med. Assoc.* **2016**, *59*, 836–841. [CrossRef]
25. Lee, J.H.; Sung, Y.K.; Choi, C.B.; Cho, S.K.; Bang, S.Y.; Choe, J.Y.; Hong, S.J.; Jun, J.B.; Kim, T.H.; Lee, J.; et al. The frequency of and risk factors for osteoporosis in Korean patients with rheumatoid arthritis. *BMC Musculoskelet. Disord.* **2016**, *17*, 98. [CrossRef] [PubMed]
26. Kim, D.; Cho, S.K.; Choi, C.B.; Jun, J.B.; Kim, T.H.; Lee, H.S.; Lee, J.; Lee, S.S.; Yoo, D.H.; Yoo, W.H.; et al. Incidence and risk factors of fractures in patients with rheumatoid arthritis: An Asian prospective cohort study. *Rheumatol. Int.* **2016**, *36*, 1205–1214. [CrossRef] [PubMed]
27. Lee, J.H.; Suh, Y.S.; Koh, J.H.; Jung, S.M.; Lee, J.J.; Kwok, S.K.; Ju, J.H.; Park, K.S.; Park, S.H. The risk of osteoporotic fractures according to the FRAX model in Korean patients with rheumatoid arthritis. *J. Korean Med. Sci.* **2014**, *29*, 1082–1089. [CrossRef] [PubMed]
28. Neer, R.M.; Arnaud, C.D.; Zanchetta, J.R.; Prince, R.; Gaich, G.A.; Reginster, J.Y.; Hodsmann, A.B.; Eriksen, E.F.; Ish-Shalom, S.; Genant, H.K.; et al. Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N. Engl. J. Med.* **2001**, *344*, 1434–1441. [CrossRef] [PubMed]

29. Cummings, S.R.; San Martin, J.; McClung, M.R.; Siris, E.S.; Eastell, R.; Reid, I.R.; Delmas, P.; Zoog, H.B.; Austin, M.; Wang, A.; et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N. Engl. J. Med.* **2009**, *361*, 756–765. [[CrossRef](#)]
30. Bellavia, D.; Costa, V.; De Luca, A.; Maglio, M.; Pagani, S.; Fini, M.; Giavaresi, G. Vitamin D Level Between Calcium-Phosphorus Homeostasis and Immune System: New Perspective in Osteoporosis. *Curr. Osteoporos. Rep.* **2016**. [[CrossRef](#)]



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Article

Sleep Quality in Patients with Rheumatoid Arthritis and Associations with Pain, Disability, Disease Duration, and Activity

Igor Grabovac¹, Sandra Haider^{1,*}, Carolin Berner², Thomas Lamprecht³, Karl-Heinrich Fenzl³, Ludwig Erlacher^{2,3}, Michael Quittan^{4,5} and Thomas E. Dorner¹

¹ Department of Social and Preventive Medicine, Centre for Public Health, Medical University of Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria; igor.grabovac@meduniwien.ac.at (I.G.); thomas.dorner@meduniwien.ac.at (T.E.D.)

² Department of Rheumatology and Osteology, Kaiser Franz Josef Hospital, Sozialmedizinisches Zentrum Süd, Kundrastrasse 3, 1100 Vienna, Austria; carolin.berner@gmx.net (C.B.); ludwig.erlacher@wienkav.at (L.E.)

³ Karl Landsteiner Institute for Autoimmune Diseases and Rheumatology, Kundrastrasse 3, 1100 Vienna, Austria; t.lamprecht@sportunion.at (T.L.); karlhfenzl@gmail.com (K.-H.F.)

⁴ Karl Landsteiner Institute for Remobilisation and Functional Health, Kundrastrasse 3, 1100 Vienna, Austria; michael.quittan@wienkav.at

⁵ Department of Physical Medicine and Rehabilitation, Kaiser-Franz-Josef-Hospital, Sozialmedizinisches Zentrum Süd, Kundrastrasse 3, 1100 Vienna, Austria

* Correspondence: sandra.a.haider@meduniwien.ac.at; Tel.: +431-40160-34895; Fax: +431-40160-934882

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Abstract: We aimed to assess the subjective sleep quality in patients with rheumatoid arthritis (RA) and its correlation with disease activity, pain, inflammatory parameters, and functional disability. In a cross-sectional study, patients with confirmed RA diagnosis responded to a questionnaire (consisting of socio-demographic data, the Health Assessment Questionnaire Disability Index, and the Medical Outcome Study Sleep Scale). Disease activity was assessed with the Clinical Disease Activity Index, and pain levels using the visual analogue scale. In addition, inflammatory markers (C-reactive protein, interleukin-6, and tumor necrosis factor alpha) were analyzed. Ninety-five patients were analyzed, predominantly female, with an average age of 50.59 (9.61) years. Fifty-seven percent reported non-optimal sleep duration, where functional disability (92.7% vs. 69.8%; $p = 0.006$) and higher median pain levels (3.75 (2.3–6.0) vs. 2.5 (2.0–3.5); $p = 0.003$) were also more prevalent. No differences in sociodemographic variables, disease duration or activity, inflammatory parameters, or use of biological and corticosteroid therapy were observed. The multivariate regression analysis showed that more intense pain was associated with a lower likelihood of optimal sleep (odds ratio (OR) = 0.68, 95% confidence interval (CI) 0.47–0.98, $p = 0.038$). Patients with RA report a high prevalence of non-optimal sleep, which is linked to pain level. Clinicians need to be aware of this issue and the potential effects on health and functional status.

Keywords: rheumatoid arthritis; sleep; sleep disorders; pain

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by changes in the synovium followed by joint swelling, pain, cartilage and bone destruction, and subsequent systemic inflammation [1]. The exact etiology is still unknown; however, 50% of the risk is attributable to genetics [2]. The incidence of RA has proven difficult to determine due to the wide variety of symptoms with which patients seek medical help and the associated delay in seeking medical help. However, the prevalence of RA has been reported at around 1% globally, with some countries showing reduced

prevalence [3]. Overall, the disease is more prevalent in women and has been found to increase in prevalence with age, with the highest rates found in women older than 65 years [2]. Patients with RA have a higher mortality and morbidity burden, reduced quality of life, and higher disability [4].

As in other pain conditions, issues of sleep disturbance are of major concern in RA patients, who often report problems with poor sleep quality, issues with falling asleep, as well as feeling unrested and fatigued after sleep [5–8]. These subjective findings have been supported by polysomnographic studies where, compared to healthy controls, patients with RA showed lower overall sleep efficiency and more awakenings [9]. Sleep problems in people with chronic illness are associated with a variety of problems, including lower quality of life, psychological issues, cognitive decline, as well as higher morbidity and mortality [10]. In addition, patients with RA have reported that issues with sleep are of high personal importance [6].

A commonly reported symptom of RA is fatigue, which is described as an overwhelming feeling and different from normal tiredness, and is a multifactorial phenomenon associated with sleep issues, pain, depression, and functional limitations [11–13]. RA patients that experience fatigue, pain, and depression also have higher levels of physical disability. Several studies have indicated that pain and depression, as well as sleep quality through its relationship with pain and fatigue, are associated with functional disability [7].

Studies have shown that most sleep issues experienced in patients with RA stem from pain. In particular, difficulties with falling asleep and feeling tired after sleep have been found to be associated with lower pain threshold, pain severity, depression, and inflammation in patients with RA [14–17]. Pain has also been found to predict sleep disturbance over time, even without sleep issues affecting pain. Both pain and sleep issues were found to be associated with depression after a 2-year follow-up [18]. Interestingly, poor clinical management and increased disease activity of RA were found to be associated with lower daytime sleepiness (but also, as expected, with sleep problems), which may be explained by pain-related alertness [19]. However, the exact mechanisms of disease activity and sleep issues are not known. Sleep problems may also be of concern for clinicians working with RA patients, as reduced sleepiness was found in studies of patients taking certain biopharmaceutical therapies [17,20]. For instance, studies on abatacept have shown a positive influence on some aspects of sleep quality [21].

Sleep quality and its associations with disease activity parameters has not yet been researched in Austria. This paper presents the results of a larger cross-sectional study in patients with RA. The complete study protocol was previously published [22]. The aim of the present study was to determine the prevalence of problems with sleep and its association with disease activity, pain levels, inflammatory parameters, and functional disability in patients with RA.

2. Materials and Methods

2.1. Participants

One hundred participants visiting the rheumatology outpatient clinic of the Kaiser Franz Josef Hospital in Vienna during their regularly scheduled visits were recruited for the purposes of this study [22]. Inclusion criteria were: age between 18 and 65 years; and fulfilled the RA diagnostic criteria according to the 2010 European League Against Rheumatism (EULAR) classification at the time of inclusion [23]. Additionally, patients with severe comorbidities (fibromyalgia, cancer, severe cardiovascular illness) as well as those that could affect handgrip strength measurements, and patients who refused or were not able to sign the informed consent were excluded from the study. The study took place from November 2015 to August 2016.

2.2. Methods

In this monocentric cross-sectional study, patients were approached during their regularly scheduled visits to a rheumatology outpatient clinic, and if they fitted the inclusion criteria, were asked

to participate in the study. After informed consent was given, the participants were asked to fill out a questionnaire, after which measurements were taken.

2.3. Questionnaire

The questionnaire was made up of four parts and had 49 items in total—multiple choice and open-end questions—and took around 10 min to finish. The questionnaire was designed for self-reporting, with measurements of disease activity undertaken by a member of the study team. The questionnaire was provided in several languages, including German, English, and Turkish, and one with a combination of both Serbian and Croatian.

2.3.1. Socio-Demographic Data

This part consisted of multiple choice and open-end questions and was made up of 13 items covering socio-demographic characteristics of the sample (age, sex, marital status, education level, and current occupation). Additional questions on disease duration, as well as current therapy and comorbidities, were also asked.

2.3.2. Functional Disability

The Health Assessment Questionnaire Disability Index (HAQ-DI) was used to assess the patients' self-reported functional disability. This validated instrument consists of 20 questions divided into eight categories of functioning: dressing, rising, eating, walking, hygiene, reach, grip, and usual activities. The overall functional disability index is given as a final score, with values between 0 (no functional disability) and 3 (severe functional disability) [24].

2.3.3. Sleep Quality

Quality of sleep was assessed using the Medical Outcome Study Sleep Scale (MOS-SS). This questionnaire is recommended for use in RA patients and is made up of 12 items regarding the patient's sleep over the last 4 weeks. MOS-SS is a self-report questionnaire that scores six different sleep dimensions as scales and two additional indices: sleep disturbance (four items), daytime somnolence (three items), snoring (one item), awakening short of breath or with a headache (1 item), sleep adequacy (two items), and quantity of sleep (one item, which is not scored, but is an average number of hours spent asleep over the past 4 weeks). Optimal sleep is an added dichotomized variable derived from the quantity of sleep, where sleep is considered optimal if the reported duration is between 7 and 8 h, otherwise it is non-optimal. All items in the MOS-SS, except for quantity of sleep, are given a numerical score, and the result is the sum of the individual scores, with the minimum value being 0 and the maximum 100. Higher scores indicate more of the named dimension, i.e., more sleep problems. The two indices—sleep problem indices I and II—are derived from several items, with sleep problem index I being derived from six items and sleep problem index II from nine items of the MOS-SS. As with the other items, higher values indicate more sleep problems [25]. Patients were also asked about their use of sleeping pills or pain medication.

2.4. Measurements

2.4.1. Disease Activity

Overall disease activity was measured by the Clinical Disease Activity Index (CDAI), which is a widely used and validated instrument. The CDAI score is derived as a sum of the subscales (Swollen 28-Joint Count, Tender 28-Joint Count, Patient Global Disease Activity, and Evaluator's Global disease Activity). Scores ≤ 2.8 are considered as remission, >2.8 and ≤ 10 as low disease activity, >10 and ≤ 22 as moderate, and >22 as high disease activity [26]. For the purposes of our study, we grouped the participants into two categories: remission and low disease activity in one group, and moderate and high disease activity as the other group.

2.4.2. Pain Intensity

Pain was assessed using the visual analogue scale (VAS), ranging from 0 to 10, whereby a higher number indicates a higher pain intensity [27].

2.4.3. Inflammatory Parameters

The inflammatory parameters of C-reactive protein (CRP; mg/dL), interleukin-6 (IL-6; pg/mL), and tumor necrosis factor alpha (TNF-alpha; ng/mL) were obtained through analysis of the patients' blood samples at the Department of Laboratory Medicine of Kaiser Franz Josef Hospital, where the research was conducted on the same day.

2.5. Statistical Analysis

Kolmogorov-Smirnov tests, Shapiro-Wilk tests, and histogram analysis were employed to determine the data distribution. Descriptive statistics were recorded for each variable, with the quantitative variables shown as mean values and standard deviation, and in the case of a non-normal distribution, as median and 25–75 percentile values. Differences between groups were calculated with T-tests or Mann-Whitney U-tests, depending on the data distribution. Chi-square tests were used for the differences between categorical variables. Spearman's rank order correlation was used for the possible correlations between the dimensions of sleep quality, as derived from the MOS-SS, and the other scores (age, disease duration, pain, CDAI score and HAQ-DI score). A multivariate logistical regression analysis was performed in order to assess the characteristics that were associated with the MOS-SS-derived dichotomized variable of optimal sleep (reported sleep between 7 and 8 h was deemed "optimal", while reported sleep under 7 h and more than 8 h was deemed "non-optimal"). Variables which showed significant correlations in the univariate correlation analysis were included in the multivariate logistic regression model. All *p*-values under 0.05 were considered statistically significant. The analysis was performed using SPSS for Windows version 24.0 software (IBM, Armonk, NY, USA).

2.6. Ethics Approval

The study complied with Good Clinical Practice standards and the Helsinki Declaration. The study was approved by the Ethical Committee of the City of Vienna (number: EK 15-173-0915).

3. Results

A total of 140 patients that fulfilled the inclusion criteria were approached and asked to participate. However, 14 (10%) were not interested to take part, 6 (4.3%) declined due to time constraints, 4 (2.9%) didn't speak any of the languages the surveys were available in and 22 (15.7%) did not complete the necessary measurements, which left 95 (67.9%) patients available for analysis. Approximately two-thirds of the study population were female, with an age range from 22 to 65 years. The mean duration of the disease in our population was around 9 years, with 80% receiving therapy with disease-modifying anti-rheumatic drugs (DMRAD). According to the HAQ-DI score, 21.4% of the participants had a level of functional disability, and the median pain score was 3.0 (2.0–5.0) out of 10. Differences according to optimal sleep duration were found in pain intensity, level of functional disability, and use of non-steroidal anti-inflammatory drugs (NSAID) and disease-modifying drugs. Additional information about the study participants is provided in Table 1.

Table 1. Sociodemographic and disease-related variables stratified by optimal sleep duration.

| Variable | Total (n = 95) | Non-Optimal Sleep Duration (n = 54) | Optimal Sleep Duration (n = 41) | P |
|---|-------------------|-------------------------------------|---------------------------------|-------|
| Age; mean (SD) | 50.59 (9.61) | 49.98 (9.14) | 51.39 (10.25) | 0.482 |
| Sex | | | | |
| Male | 32.6% | 33.3% | 31.7% | 0.867 |
| Female | 67.4% | 66.7% | 68.3% | |
| Relationship status | | | | |
| In a relationship | 73.7% | 77.8% | 68.3% | 0.298 |
| Not in a relationship | 26.3% | 22.2% | 31.7% | |
| Education level | | | | |
| Primary level | 15.8% | 13.0% | 19.5% | 0.434 |
| Secondary level | 72.6% | 77.8% | 65.9% | |
| Tertiary level | 11.6% | 9.3% | 14.6% | |
| Employment status | | | | |
| Employed | 61.1% | 63.0% | 58.5% | 0.661 |
| Unemployed | 38.9% | 37.0% | 41.5% | |
| Disease duration in months; median (Q₂₅–Q₇₅) | 72.0 (36.0–141.0) | 78.0 (36.0–144.0) | 60.0 (28.50–138.0) | 0.452 |
| Pain intensity; median (Q₂₅–Q₇₅) | 3.0 (2.0–5.0) | 3.75 (2.3–6.0) | 2.5 (2.0–3.5) | 0.003 |
| Functional disability | | | | |
| No disability | 79.8% | 69.8% | 92.7% | 0.006 |
| Disability | 20.2% | 30.2% | 7.3% | |
| Disease activity | | | | |
| Remission | 29.5% | 29.6% | 30.8% | 0.726 |
| Low | 33.7% | 31.5% | 38.5% | |
| Moderate | 26.3% | 27.8% | 25.6% | |
| High | 8.4% | 11.1% | 5.1% | |
| Inflammatory parameters | | | | |
| CRP (mg/dL); median (Q ₂₅ –Q ₇₅) | 3.20 (1.10–6.70) | 3.05 (1.07–7.05) | 2.70 (1.00–5.55) | 0.594 |
| TNF-α (g/mL); median (Q ₂₅ –Q ₇₅) | 1.60 (0.56–2.35) | 1.65 (0.54–2.89) | 1.60 (0.64–2.32) | 0.884 |
| IL-6 (pg/mL); median (Q ₂₅ –Q ₇₅) | 3.89 (1.98–7.91) | 4.47 (2.04–9.67) | 3.44 (1.72–6.18) | 0.179 |
| Therapy | | | | |
| Disease-modifying drugs | 81.1% | 72.2% | 92.7% | 0.012 |
| Biologicals | 43.2% | 50.0% | 34.1% | 0.122 |
| Corticosteroid | 16.8% | 18.5% | 14.6% | 0.616 |
| Non-steroidal anti-inflammatory | 14.7 | 7.4% | 24.4% | 0.021 |
| Other medication | 55.8% | 55.6% | 56.1% | 0.958 |

SD = standard deviation. Differences between groups were calculated with the T-test, Mann-Whitney U test dependent on data distribution. Chi-square test was used for differences between categorical variables.

Overall, our patients reported non-optimal sleep duration in 56.8% of cases, with a mean duration of sleep over the past 4 weeks of 6.5 (1.3) h. The median score of sleep problem index I was 30.0, with the score of sleep problem index II being slightly higher at 32.2 points. The highest reported median value of the six items was in sleep adequacy, which indicates that our patients, in general, feel well rested and have enough sleep during the night. Furthermore, most of the participants did not take pain medication or sleeping pills. Other results regarding the MOS-SS score and the other sleep quality variables are presented in Table 2. In terms of disease activity, patients with moderate and high disease activity reported fewer hours asleep in comparison to patients with low disease activity or in remission (6.7 (1.2) vs. 6.0 (1.2); $p = 0.009$).

Table 2. Sleep characteristics of the study participants.

| Sleep-Related Variables | n = 95 |
|---|------------------|
| Sleep disturbance median (Q ₂₅ –Q ₇₅) | 32.5 (15.0–51.2) |
| Snoring; median (Q ₂₅ –Q ₇₅) | 40.0 (20.0–60.0) |
| Shortness of breath or headache; median (Q ₂₅ –Q ₇₅) | 20.0 (0.0–30.0) |
| Sleep adequacy; median (Q ₂₅ –Q ₇₅) | 60.0 (30.0–80.0) |
| Somnolence; median (Q ₂₅ –Q ₇₅) | 26.6 (13.3–46.6) |
| Sleep problem index I; median (Q ₂₅ –Q ₇₅) | 30.0 (16.6–46.6) |
| Sleep problem index II; median (Q ₂₅ –Q ₇₅) | 32.2 (18.3–47.8) |
| Optimal sleep | |
| Yes | 43.2% |
| No | 56.8% |
| Pain medication for sleep | |
| Daily | 8.2% |
| Up to 3 times a week | 7.2% |
| More than 3 times a week | 5.2% |
| Up to 3 times a month | 12.4% |
| Never | 67.0% |
| Sleeping pills | |
| Daily | 9.3% |
| Up to 3 times a week | 2.1% |
| More than 3 times a week | 1.0% |
| Up to 3 times a month | 4.1% |
| Never | 83.5% |
| Hours asleep; mean (SD) | 6.5 (1.3) |

SD = standard deviation.

The relationship between the sleep domains of the MOS-SS and age, disease duration in months, pain intensity, disease activity, and functional disability scores was investigated, as seen in Table 3. Pain intensity was the only variable that was correlated with all the MOS-SS domains, with the highest coefficient being a medium positive correlation with sleep problem index II ($r = 0.406$; $p < 0.001$). Overall, the strongest relationship was found between functional disability and sleep problem index II ($r = 0.516$; $p < 0.001$). Disease activity had significant small to medium positive correlations with sleep disturbance, snoring, shortness of breath or snoring, and sleep problem indices I and II, while a significant negative correlation was observed between sleep duration and the sleep adequacy domains of the MOS-SS, as seen in Table 3.

Table 3. Spearman’s rank order correlation of variables that correlate with the sleep domains.

| Variable | Hours Asleep | Sleep Disturbance | Snoring | Awakening Short of Breath or with Headache | Sleep Adequacy | Sleep Somnolence | Sleep Problem Index I | Sleep Problem Index II |
|----------------------|--------------|-------------------|---------|--|----------------|------------------|-----------------------|------------------------|
| Age | 0.077 | −0.007 | 0.204 * | −0.012 | 0.043 | −0.027 | −0.050 | −0.040 |
| Disease duration | −0.177 | 0.201 * | −0.037 | 0.134 | −0.066 | 0.184 | 0.173 | 0.149 |
| Pain intensity (VAS) | −0.350 ** | 0.379 ** | 0.228 * | 0.279 ** | −0.297 ** | 0.305 ** | 0.374 ** | 0.406 ** |
| CDAI Score | −0.304 ** | 0.324 ** | 0.210 * | 0.249 * | −0.275 ** | 0.148 | 0.293 ** | 0.322 ** |
| HAQ-DI Score | 0.247 * | 0.459 ** | 0.090 | 0.298 ** | −0.436 ** | 0.390 ** | 0.495 ** | 0.516 ** |

* $p < 0.005$; ** $p < 0.001$; VAS: visual analogue scale, CDAI: Clinical Disease Activity Index, HAQ-DI: Health Assessment Questionnaire Disability Index.

According to the multivariate logistical regression analysis, pain intensity was the only variable that was significantly associated with optimal sleep duration, where more severe pain was associated with a reduced likelihood of optimal sleep, as seen in Table 4.

Table 4. Logistic regression model of variables associated with optimal sleep duration.

| Variable | OR | 95% CI | p |
|------------------|------|-----------|-------|
| Age | 1.03 | 0.99–1.09 | 0.162 |
| Pain VAS scale | 0.68 | 0.47–0.98 | 0.038 |
| HAQ-DI score | 0.53 | 0.19–1.45 | 0.215 |
| CDAI score | 1.02 | 0.95–1.11 | 0.544 |
| Disease duration | 1.00 | 0.99–1.01 | 0.756 |

OR: odds ratio, 95% CI: 95% confidence interval, VAS: visual analogue scale, CDAI: Clinical Disease Activity Index, HAQ-DI: Health Assessment Questionnaire Disability Index.

4. Discussion

Our study showed that, in an investigated sample of patients with RA, problems with sleep are common, with 56.8% of the participants reporting non-optimal sleep duration. Interestingly, the MOSS-SS sleep adequacy scale showed high results, meaning that our participants were, overall, satisfied with their sleep quality. Studies have shown that up to 70% of patients with RA suffer from problems with sleep, ranging from difficulty falling asleep to difficulty maintaining sleep or suffering from daytime sleepiness [6,28]. Several studies found that higher disease activity is associated with sleep problems [9,29,30]. A study by Wolfe et al. [8] showed that sleep disturbance could be attributed to RA in up to 42% of cases, linking sleep disturbance to pain, mood, and disease activity. Similarly, a Korean study found decreasing subjective sleep quality as the disease activity was increasing [30]. Conversely, studies have also found that reducing the active inflammatory disease and the arthritic process have a positive effect on sleep quality [31]. However, a connection between disease activity and sleep quality has not been universally reported in studies. For example, Hirsch et al. [5] reported overall disturbed sleep in patients with RA, but found no association with inflammatory disease activity. These results have often been questioned as the study population was very small (only 19 patients), which may be the reason for the lack of association. Our study, although five times larger in terms of the sample size, also found no difference in sleep quality in patients with different disease activity, as shown in Table 1. In terms of the relationship between the CDAI scores and the sleep domains of the MOS-SS, seen in Table 3, only low to moderate correlations were found. The only significant difference in sleep characteristics between patients with different disease activity was found in sleep duration, where patients in the moderate/high disease activity group reported shorter sleep duration.

The mechanism of how disease activity influences sleep quality is not completely clear; however, most studies have suggested the connection with joint stiffness and pain. In a study by Wolfe and Michaud [8], pain was shown to be one of the most common underlying reasons leading to problems with sleep, with a recent study of Austrian patients with chronic pain also reporting notable sleep disturbance [32]. Chronic pain and associated sleep issues are a risk for developing depressive symptoms in patients with RA, which in turn may have an additional influence as depression has been reported as being a predictive factor for poor quality of sleep [15,19]. In our study population, pain levels were generally low; however, there was a significant difference based on reported optimal sleep duration, as seen in Table 1, where lower pain levels were found in those patients who reported optimal sleep duration. The multivariate logistic regression model showed pain intensity to be a predictor for non-optimal sleep in our population, as shown in Table 4. Furthermore, pain was found to be correlated with all the domains of sleep quality in the MOS-SS, having a negative relationship with hours spent sleeping and the sleep adequacy domain. More pain intensity is positively correlated with sleep disturbance, snoring and shortness of breath, somnolence, and both sleep problem indices. Recent evidence shows that lower sleep quality lowers the threshold of pain and increases the pain intensity in patients with RA [9,16,19].

The relationship between RA therapy and medication used for sleep quality is ambivalent. Some studies have reported the use of biological medication as having a positive effect on sleep

quality, with longitudinal observations of anti-TNF substances also reporting greater sleep quality improvements in patients on abatacept in comparison to methotrexate. A trial investigating the effectiveness of indomethacin on sleep quality showed indomethacin to be superior to placebo in a questionnaire-based study. However, polysomnographic studies investigating sleep patterns in patients using NSAIDs showed no changes in sleep patterns or sleep quality in patients with RA. In our study, as presented in Table 1, patients who reported optimal sleep duration were found to more often use disease-modifying drugs and NSAIDs, which is probably connected to their analgesic effect. More research, especially blinded longitudinal studies, should be done to investigate the effects of medication on sleep quality of RA patients, as most cross-sectional studies have reported no connection [8,30,33].

Functional disability was found to be associated with sleep quality, as patients with RA often experience difficulties with the activities of daily living, which was found to be associated with fatigue and sleep disturbance. In addition, disability often leads to greater depression, which in turn leads to sleep disturbance. A relationship between pain, depression, sleep, and functional disability has been shown in numerous studies, which may mean that there is a casual relationship, as pain, fatigue, and depression inhibit normal daily productivity, causing sleep disorders and disability, which then contribute to pain, fatigue, depression, and disability [8,9,33]. In our study, patients who were categorized as having functional disability reported significantly non-optimal sleep duration, as seen in Table 1. However, the HAQ-DI score showed no association with optimal sleep in the multivariate logistic regression model and, interestingly, was found to have a positive relationship with sleep duration. Furthermore, functional disability was found to be significantly correlated with somnolence, shortness of breath, sleep adequacy, sleep disturbance, and both sleep problem indices as shown in Table 3.

The relationship between age and sleep quality in patients with RA is unclear, but some studies have reported lower sleep quality in older patients [30,33]. Our study showed only a small positive correlation with age and snoring, but no correlation with other dimensions of the MOS-SS as seen in Table 3. Other studies have often failed to report an association between age and sleep problems in patients with RA [8,19]. This was confirmed by our logistic regression model, where there was no significant association found between optimal sleep duration and age.

Finally, the study limitations need to be addressed. The cross-sectional study design does not allow for causal conclusions on the relationships between variables. Longitudinal studies need to be performed in order to examine the possible casual associations. Secondly, although the MOS-SS is recommended for use in RA, it refers to a time frame of the previous 4 weeks, which may affect the results due to recall bias. Additionally, we haven't reported on the body mass index (BMI) which is also a potential confounder. Finally, as patients were recruited to the study by their physicians, it is possible that the results are an underestimation due to reporting bias. The relatively small sample size might prevent the generalizability of the study results, as well as the higher proportion of women; however, this is to be expected in a RA patient population. Furthermore, our population consisted of men and women of working age, which may also contribute to the reduced sleep problems.

Given our results and the noted associations of pain and non-optimal sleep in patients with RA it is important to alert the clinical community working with RA patients. Sleep as well as pain assessments should be systematically included in the clinical assessments [34]. However, such monitoring will not be effective in reducing the burden of pain and sleep problems in RA patients, unless appropriate treatment is also not provided. Albeit research indicates the associations of pain and sleep disturbances, pharmacotherapy for these comorbidities receives little attention, and there is yet to be a consensus or evidence based treatment algorithms [35]. Additionally, a recent meta-analysis indicated that non-pharmacological sleep treatments (including physiotherapy, meditation, massage, sleep restriction therapy and sleep scheduling, imagery exercises, and others) were associated with large improvements in sleep quality [36]. In conclusion, there is a high frequency of non-optimal sleep duration in patients with RA of working age; however, these patients are mostly satisfied with their overall

sleep quality. Our study further shows the association between higher pain levels and non-optimal sleep. Physicians working with RA patients need to be aware of the sleep issues in this population and include pharmacological and psychological interventions as these may have a positive effect on sleep quality, and in turn on psychological wellbeing, physical wellbeing, and functional disability. Future studies could focus on sleep quality in newly diagnosed patients, as well as longitudinal studies need to be implemented in order to see if improvements in sleep contribute to reduction of depression, pain, and functional disability.

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References

1. McInnes, I.B.; Schett, G. The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* **2011**, *365*, 2205–2219. [[CrossRef](#)] [[PubMed](#)]
2. Scott, D.L.; Wolfe, F.; Huizinga, T.W. Rheumatoid arthritis. *Lancet* **2010**, *376*, 1094–1108. [[CrossRef](#)]
3. Gibofsky, A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *Am. J. Manag. Care* **2012**, *18*, S295–S302. [[PubMed](#)]
4. Kiltz, U.; van der Heijde, D. Health-related quality of life in patients with rheumatoid arthritis and in patients with ankylosing spondylitis. *Clin. Exp. Rheumatol.* **2009**, *27*, S108–S111. [[PubMed](#)]
5. Hirsch, M.; Carlander, B.; Verge, M.; Tafti, M.; Anaya, J.M.; Billiard, M.; Sany, J. Objective and subjective sleep disturbances in patients with rheumatoid arthritis. *Arthritis Rheum.* **1994**, *37*, 41–49. [[CrossRef](#)] [[PubMed](#)]
6. Kirwan, J.; Heiberg, T.; Hewlett, S.; Hughes, R.; Kvien, T.; Ahlmen, M.; Boers, M.; Minnock, P.; Saag, K.; Shea, B.; et al. Outcomes from the patient perspective workshop at OMERACT 6. *J. Rheumatol.* **2003**, *30*, 868–872. [[PubMed](#)]
7. Luyster, F.S.; Chasens, E.R.; Wasko, M.C.; Dunbar-Jacob, J. Sleep quality and functional disability in patients with rheumatoid arthritis. *J. Clin. Sleep Med.* **2011**, *7*, 49–55. [[PubMed](#)]
8. Wolfe, F.; Michaud, K.; Li, T. Sleep disturbance in patients with rheumatoid arthritis: Evaluation by medical outcomes study and visual analog sleep scales. *J. Rheumatol.* **2006**, *33*, 1942–1951. [[PubMed](#)]
9. Mahowald, M.W.; Mahowald, M.L.; Bundlie, S.R.; Ytterberg, S.R. Sleep fragmentation in rheumatoid arthritis. *Arthritis Rheum.* **1989**, *32*, 974–983. [[CrossRef](#)] [[PubMed](#)]
10. Kripke, D.F.; Garfinkel, L.; Wingard, D.L.; Klauber, M.R.; Marler, M.R. Mortality associated with sleep duration and insomnia. *Arch. Gen. Psychiatry* **2002**, *59*, 131–136. [[CrossRef](#)] [[PubMed](#)]
11. Hewlett, S.; Cockshott, Z.; Byron, M.; Kitchen, K.; Tipler, S.; Pope, D.; Hehir, M. Patients' perceptions of fatigue in rheumatoid arthritis: Overwhelming, uncontrollable, ignored. *Arthritis Rheum.* **2005**, *53*, 697–702. [[CrossRef](#)] [[PubMed](#)]
12. Loppenthin, K.; Esbensen, B.A.; Jennum, P.; Ostergaard, M.; Tolver, A.; Thomsen, T.; Midtgaard, J. Sleep quality and correlates of poor sleep in patients with rheumatoid arthritis. *Clin. Rheumatol.* **2015**, *34*, 2029–2039. [[CrossRef](#)] [[PubMed](#)]
13. Pollard, L.C.; Choy, E.H.; Gonzalez, J.; Khoshaba, B.; Scott, D.L. Fatigue in rheumatoid arthritis reflects pain, not disease activity. *Rheumatology (Oxford)* **2006**, *45*, 885–889. [[CrossRef](#)] [[PubMed](#)]
14. Cakirbay, H.; Bilici, M.; Kavakci, O.; Cebi, A.; Guler, M.; Tan, U. Sleep quality and immune functions in rheumatoid arthritis patients with and without major depression. *Int. J. Neurosci.* **2004**, *114*, 245–256. [[CrossRef](#)] [[PubMed](#)]

15. Lee, Y.C.; Chibnik, L.B.; Lu, B.; Wasan, A.D.; Edwards, R.R.; Fossel, A.H.; Helfgott, S.M.; Solomon, D.H.; Clauw, D.J.; Karlson, E.W. The relationship between disease activity, sleep, psychiatric distress and pain sensitivity in rheumatoid arthritis: A cross-sectional study. *Arthritis Res. Ther.* **2009**, *11*, R160. [[CrossRef](#)] [[PubMed](#)]
16. Power, J.D.; Perruccio, A.V.; Badley, E.M. Pain as a mediator of sleep problems in arthritis and other chronic conditions. *Arthritis Rheum.* **2005**, *53*, 911–919. [[CrossRef](#)] [[PubMed](#)]
17. Vgontzas, A.N.; Zoumakis, E.; Lin, H.M.; Bixler, E.O.; Trakada, G.; Chrousos, G.P. Marked decrease in sleepiness in patients with sleep apnea by etanercept, a tumor necrosis factor- α antagonist. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 4409–4413. [[CrossRef](#)] [[PubMed](#)]
18. Nicassio, P.M.; Wallston, K.A. Longitudinal relationships among pain, sleep problems, and depression in rheumatoid arthritis. *J. Abnorm. Psychol.* **1992**, *101*, 514–520. [[CrossRef](#)] [[PubMed](#)]
19. Westhovens, R.; Van der Elst, K.; Matthys, A.; Tran, M.; Gilloteau, I. Sleep problems in patients with rheumatoid arthritis. *J. Rheumatol.* **2014**, *41*, 31–40. [[CrossRef](#)] [[PubMed](#)]
20. Zamarron, C.; Maceiras, F.; Mera, A.; Gomez-Reino, J.J. Effect of the first infliximab infusion on sleep and alertness in patients with active rheumatoid arthritis. *Ann. Rheum. Dis.* **2004**, *63*, 88–90. [[CrossRef](#)] [[PubMed](#)]
21. Wells, G.; Li, T.; Tugwell, P. Investigation into the impact of abatacept on sleep quality in patients with rheumatoid arthritis, and the validity of the mos-sleep questionnaire sleep disturbance scale. *Ann. Rheum. Dis.* **2010**, *69*, 1768–1773. [[CrossRef](#)] [[PubMed](#)]
22. Berner, C.; Erlacher, L.; Quittan, M.; Fenzl, K.H.; Dorner, T.E. Workability and muscle strength in patients with seropositive rheumatoid arthritis: Survey study protocol. *JMIR Res. Protoc.* **2017**, *6*, e36. [[CrossRef](#)] [[PubMed](#)]
23. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O.; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 rheumatoid arthritis classification criteria: An american college of rheumatology/european league against rheumatism collaborative initiative. *Arthritis Rheum.* **2010**, *62*, 2569–2581. [[CrossRef](#)] [[PubMed](#)]
24. Fries, J.F.; Spitz, P.; Kraines, R.G.; Holman, H.R. Measurement of patient outcome in arthritis. *Arthritis Rheum.* **1980**, *23*, 137–145. [[CrossRef](#)] [[PubMed](#)]
25. Smith, M.T.; Wegener, S.T. Measures of sleep: The insomnia severity index, medical outcomes study (MOS) sleep scale, pittsburgh sleep diary (PSD), and pittsburgh sleep quality index (PSQI). *Arthritis Rheum.* **2003**, *49*, S184–S196. [[CrossRef](#)]
26. Anderson, J.; Caplan, L.; Yazdany, J.; Robbins, M.L.; Neogi, T.; Michaud, K.; Saag, K.G.; O'Dell, J.R.; Kazi, S. Rheumatoid arthritis disease activity measures: American college of rheumatology recommendations for use in clinical practice. *Arthritis Care Res.* **2012**, *64*, 640–647. [[CrossRef](#)] [[PubMed](#)]
27. Hawker, G.A.; Mian, S.; Kendzerska, T.; French, M. Measures of adult pain: Visual analog scale for pain (VAS pain), numeric rating scale for pain (NRS pain), mcgill pain questionnaire (MPQ), short-form McGill pain questionnaire (SF-MPQ), chronic pain grade scale (CPGS), short form-36 bodily pain scale (SF-36 BPS), and measure of intermittent and constant osteoarthritis pain (ICOAP). *Arthritis Care Res. (Hoboken)* **2011**, *63*, S240–S252. [[PubMed](#)]
28. Drewes, A.M.; Nielsen, K.D.; Hansen, B.; Taagholt, S.J.; Bjerregard, K.; Svendsen, L. A longitudinal study of clinical symptoms and sleep parameters in rheumatoid arthritis. *Rheumatology (Oxford)* **2000**, *39*, 1287–1289. [[CrossRef](#)]
29. Crosby, L.J. Factors which contribute to fatigue associated with rheumatoid arthritis. *J. Adv. Nurs.* **1991**, *16*, 974–981. [[CrossRef](#)] [[PubMed](#)]
30. Son, C.N.; Choi, G.; Lee, S.Y.; Lee, J.M.; Lee, T.H.; Jeong, H.J.; Jung, C.G.; Kim, J.M.; Cho, Y.W.; Kim, S.H. Sleep quality in rheumatoid arthritis, and its association with disease activity in a Korean population. *Korean J. Intern. Med.* **2015**, *30*, 384–390. [[CrossRef](#)] [[PubMed](#)]
31. Wells, G.; Li, T.; Maxwell, L.; Maclean, R.; Tugwell, P. Responsiveness of patient reported outcomes including fatigue, sleep quality, activity limitation, and quality of life following treatment with abatacept for rheumatoid arthritis. *Ann. Rheum. Dis.* **2008**, *67*, 260–265. [[CrossRef](#)] [[PubMed](#)]
32. Keilani, M.; Crevenna, R.; Dorner, T.E. Sleep quality in subjects suffering from chronic pain. *Wien. Klin. Wochenschr.* **2018**, *130*, 31–36. [[CrossRef](#)] [[PubMed](#)]

33. Sariyildiz, M.A.; Batmaz, I.; Bozkurt, M.; Bez, Y.; Cetincakmak, M.G.; Yazmalar, L.; Ucar, D.; Celepkolu, T. Sleep quality in rheumatoid arthritis: Relationship between the disease severity, depression, functional status and the quality of life. *J. Clin. Med. Res.* **2014**, *6*, 44–52. [[PubMed](#)]
34. Stubbs, B.; Vancampfort, D.; Thompson, T.; Veronese, N.; Carvalho, A.F.; Solmi, M.; Mugisha, J.; Schofield, P.; Matthew Prina, A.; Smith, L.; et al. Pain and severe sleep disturbance in the general population: Primary data and meta-analysis from 240,820 people across 45 low- and middle-income countries. *Gen. Hosp. Psychiatry* **2018**, *53*, 52–58. [[CrossRef](#)] [[PubMed](#)]
35. Doufas, A.G.; Panagiotou, O.A.; Ioannidis, J.P. Concordance of sleep and pain outcomes of diverse interventions: An umbrella review. *PLoS ONE* **2012**, *7*, e40891. [[CrossRef](#)] [[PubMed](#)]
36. Tang, N.K.; Lereya, S.T.; Boulton, H.; Miller, M.A.; Wolke, D.; Cappuccio, F.P. Nonpharmacological treatments of insomnia for long-term painful conditions: A systematic review and meta-analysis of patient-reported outcomes in randomized controlled trials. *Sleep* **2015**, *38*, 1751–1764. [[CrossRef](#)] [[PubMed](#)]



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Review

Current Therapeutic Options in the Treatment of Rheumatoid Arthritis

Birgit M. Köhler, Janine Günther, Dorothee Kaudewitz and Hanns-Martin Lorenz *

Internal Medicine 5, Division of Rheumatology, University Hospital Heidelberg, 69120 Heidelberg, Germany

* Correspondence: Hannes.Lorenz@med.uni-heidelberg.de; Tel.: +49-6221-56-8008; Fax: +49-6221-56-6824

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Abstract: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints. Untreated RA leads to a destruction of joints through the erosion of cartilage and bone. The loss of physical function is the consequence. Early treatment is important to control disease activity and to prevent joint destruction. Nowadays, different classes of drugs with different modes of action are available to control the inflammation and to achieve remission. In this review, we want to discuss differences and similarities of these different drugs.

Keywords: Rheumatoid Arthritis; therapy; DMARD; MTX; Tumor Necrosis Factor-Alpha Inhibitors

1. Introduction

Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the synovial membrane. Untreated RA can lead to progressive joint destruction, resulting in disability, poor quality of life, and increased mortality. About 1% of the population is affected, and the disease onset generally occurs between 30 years and 50 years of age, with a higher incidence in women.

The therapy is complex and includes different classes of drugs with different routes of application but also nonpharmacologic interventions. The most important are patient education followed by exercise and physical and occupational therapy. Because of an increased risk of coronary atherosclerosis, efforts should be made to reduce risk factors such as smoking, hyperlipidemia, hypertension, and obesity.

To relieve pain and swelling fast and to gain control of the inflammation, glucocorticoids (GC) are used widely in acute disease flares either orally or as intraarticular injections. Oral GC is for short-term use (up to 3–4 month) only and should be tapered to prevent side effects as soon as possible [1]. To control inflammation in the long run, Disease Modifying Anti-Rheumatic Drugs (DMARD) to spare GC are needed. Nowadays, there are a bunch of opportunities that can be challenges or chances.

The treatment of patients with RA aims to relieve pain and to control inflammation, and the final goal is to achieve remission or at least low disease activity for all patients. In this context, the European League Against Rheumatism (EULAR) has composed 10 international recommendations on how to treat patients [2]. An algorithm based on the EULAR recommendations is shown in Figure 1. In 2010, an international committee developed the treat-to-target (T2T) initiative. The centerpiece of this initiative is the shared decision-making and regular patient reevaluation that targets remission or at least low disease activity (LDA) [3].

By now, there is evidence from different studies that the T2T principle is superior, and it forms part of the treatment guidelines of the European League Against Rheumatism and the American College of Rheumatology. The core principles of T2T are shown in Table 1. Even more important is that an early start of therapy is required in order to achieve optimal outcomes.

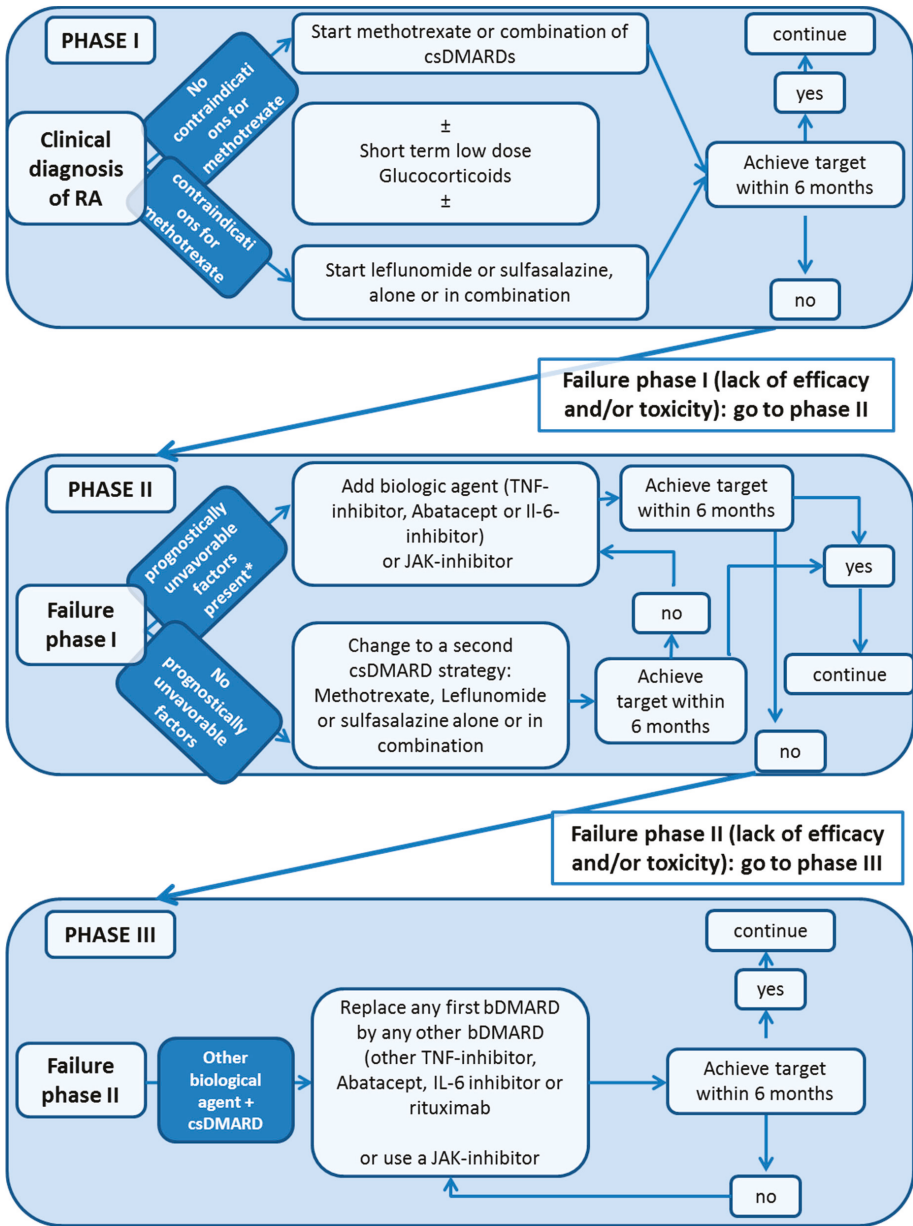


Figure 1. Algorithm adapted from the 2016 European League Against Rheumatism (EULAR) recommendations on rheumatoid arthritis (RA) management. bDMARD, biological; bsDMARD, biosimilar DMARDs; csDMARDs, conventional synthetic DMARDs; DMARDs disease modifying antirheumatic drug; IL, Interleukin; MTX, methotrexate; TNF, tumor necrosis factor; tsDMARDs, targeted synthetic DMARDs.

Table 1. Overarching principles of the T2T strategy (modified after Smolen J.S., et al. *Ann Rheum Dis* 2016;75:3–15). [3].

| Overarching Principles of the T2T Strategy | |
|--|---|
| 1 | Basis for the treatment is a shared decision making between patient and doctor |
| 2 | Major treatment goals are: maximization of quality of life, normalisation of function and participation in social and professional life |
| 3 | The elimination of inflammation is essential to achieve the treatment goals |
| 4 | Outcomes in rheumatoid arthritis are improved by implementing T2T |

2. Treatment Guidelines: Conventional Synthetic DMARD (csDMARD)

As soon as the diagnosis of rheumatoid arthritis is made, a treatment with a csDMARD should be started [4].

A controlled comparison between csDMARDs for the first-line therapy does not exist; however, within this group, methotrexate should be the first choice because, for this drug, most clinical experience exists in monotherapy and as a combination partner with other DMARDs [5]. Methotrexate is usually started at a dose of 15 mg/week and can be stepwise increased up to 25 mg/week. The combination with glucocorticoids is recommended [3]. Due to the decreased bioavailability, a subcutaneous way of administration is recommended [5].

The induction of remission with a combination of conventional synthetic DMARDs at this stage is not superior to methotrexate monotherapy; however, these combinations are associated with more adverse events and a higher rate of drug discontinuation [4,6]. Patients with a higher baseline disease activity and Rheumatoid factor (RF)-positive patients have an increased risk of methotrexate (MTX) failure due to inefficacy [6].

If MTX cannot be used, e.g., due to intolerance or contraindications, leflunomide (20 mg/week) or sulfasalazine (2 g/day) should be started. In a placebo-controlled randomized controlled trial (RCT), both substances showed a similar efficacy [7].

If by week 12 after the start of MTX therapy no adequate response is achieved or no remission is reached with optimum doses after 24 weeks, the therapy should be adjusted [5]. To find the best individual treatment strategy, patients should be categorized using prognostic markers. Poor prognostic markers such as the presence of autoantibodies, early joint damage, and high disease activity are associated with rapid disease progression; therefore, a biologic DMARD or a targeted synthetic DMARD should be added at this stage [4]. In the absence of poor prognostic markers and with moderate disease activity, a second csDMARD should be added to the therapy [5].

2.1. MTX

Unlike targeted DMARDs, conventional synthetic DMARDs came into clinical practice based on empiric observations and their mechanisms of action are still incompletely understood [4].

The mode of action of high-dose methotrexate via the depletion of thymidine and purine residues and cell-cycle arrest at S1 are well-known [8]. However, this mechanism does not seem to play a major role in the clinical effect of low-dose MTX, as folate co-therapy does not result in a loss of clinical benefit. Methotrexate has pleiotropic therapeutic effects on various immune cells and mediators, resulting in an overall dampening of the inflammatory response. The main mode of action of low-dose MTX in rheumatoid arthritis is thought to be via the potentiation of adenosine signaling. Adenosine acts as a paracrine signaling agent via four distinct purinergic G-protein-coupled receptors, which in rheumatoid arthritis are overexpressed. In addition to downregulating the production of tumor necrosis factor (TNF) and NF-κB, adenosine might be one of the main mediators of the downregulation of the activation and proliferation of T-lymphocytes, creating an immunotolerant environment [8].

Side effects of MTX are usually dependent on dose, mode of application, and duration of methotrexate therapy. Unlike high-dose MTX, the side effects of low-dose MTX are rarely

life-threatening and can often be relieved by substituting folate. Common side effects of low-dose MTX are hematologic abnormalities (thrombocytopenia and leucopenia), stomatitis, gastrointestinal problems (e.g., anorexia, loose stools, nausea, or stomach upset), elevation of liver enzymes, or central nervous system symptoms like fatigue or headache [9]. The substitution of folate significantly lowers the risk for side effects like hepatotoxicity (relative risk reduction of 77%) and decreases the number of serious adverse events (by 61%) [5,10]. If side effects occur, substituting folate acid regularly and gradually increasing the dose up to 5 mg daily can help to control the symptoms. Overall, fewer than 5% of patients have to stop using methotrexate because of adverse events [4].

MTX therapy can, in many cases, be safely continued perioperatively; however, a potentially decreased kidney function in this setting should be taken into account. In addition, to decrease the risk of pneumonia, the treatment should be paused if pulmonary comorbidities exist. At high doses (25 mg/week), a temporary dose reduction should be considered [11].

MTX exposure during pregnancy can induce multiple congenital deformities. Therefore, MTX therapy during pregnancy is not recommended. MTX should be withdrawn prophylactically 3 months before conception. Daily folate supplementation should be continued antenatal and throughout pregnancy. At the present stage of knowledge, it is not clear whether MTX transiently influences male fertility and sperm DNA integrity [12].

2.2. Leflunomide

The primary mechanism of action of leflunomide (LEF) is the reversible inhibition of the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), the rate limiting step in the de novo synthesis of pyrimidines. Activated lymphocytes expand their pyrimidine pool by approximately eightfold during proliferation. Therefore, the inhibition of DHODH prevents activated lymphocytes from moving from G1 to the S phase, hence triggering apoptosis [13]. The leflunomide effect seems to be rather lymphocyte-specific on other cells, can reuptake pyrimidines, and can thereby overcome the DHODH blockade.

The rate of discontinuation due to side effects is similar with methotrexate [14]. Potential side effects include diarrhea and nausea as well as the elevation of liver enzymes. The changes in liver function are generally reversible with dose reduction or a discontinuation of the drug, but in rare cases, hepatotoxicity can be severe. However, transaminase elevation mainly occurs if other comorbidities contributing to hepatotoxicity, e.g., concomitant non-steroidal anti-inflammatory drugs (NSAID) or MTX therapy, previous or concurrent alcohol abuse, or viral or autoimmune hepatitis, are present [15]. A small percentage of patients with RA develop hypertension when taking LEF; therefore, blood pressure monitoring is recommended during treatment [14].

The active metabolite of leflunomide is detectable in plasma until 2 years after the discontinuation of the drug; therefore, a discontinuation of leflunomide perioperatively is generally not recommended. Only at a high risk of infection or if a greater intervention is planned, a wash out with cholestyramine should be initiated as recommended [11].

Leflunomide is contraindicated during pregnancy. Safe contraception during therapy in both women and men is recommended. Before conception, leflunomide must be withdrawn and a washout should be carried out until the drug is undetectable in the blood [12].

2.3. Sulfasalazine

Sulfasalazine SSZ was specifically designed in 1938 for the treatment of rheumatoid arthritis. The idea behind the drug was to combine an antibacterial and an anti-inflammatory agent [16]. Sulfasalazine is effective in the treatment of rheumatoid arthritis; however, the mode of action is incompletely understood. The main pharmacological effects of SSZ include effects on the gut bacterial flora, on inflammatory cell functions, and on immunological processes [16]. Several plausible mechanisms of action have been observed *in vitro*, such as the inhibition of NF- κ B and osteoclast formation via modulatory effects on the receptor activators of NF- κ B (RANK), osteoprotegerin (OPG),

and RANK-ligand. In addition, SASP can inhibit tumor necrosis factor (TNF)-alpha expression and may reduce the secretion of inflammatory cytokines such as interleukin (IL)-8 as well as may suppress B-cell function [17]. An additional mechanism that has been suggested is the increased production of adenosine at sites of inflammation similar to the mode of action of methotrexate.

Adverse reactions, including idiosyncratic (e.g., hypersensitivity-/immune-related) and dose-related effects, are common with sulfasalazine, especially gastrointestinal, central nervous system, cutaneous, and hematologic adverse effects. The withdrawal rate for adverse events is about 25%, two thirds of which are due to gastrointestinal and central nervous system toxicity. If dose-related side effects occur, treatment can be paused for a week and after a resolution of the symptoms, the treatment can be restarted at a lower dose [18]. However, if idiosyncratic effects like skin reactions, hepatitis, pneumonitis, or hematologic side effects like agranulocytosis and hemolytic anemia occur, immediate discontinuation of the drug is necessary; patients with this type of adverse effect should not be rechallenged with the drug [18].

As sulfasalazine only has a short half-life of about 4–5 h and only a minimal immunosuppressive effect, it can usually be continued perioperatively. If there is a risk of interaction or a potential additive hepatotoxic effect with medication used preoperatively, sulfasalazine can be paused on the day of the operation [11].

If treatment of rheumatoid arthritis is required during pregnancy, SSZ is an acceptable therapeutic option, as the continuation of SSZ during pregnancy is very unlikely to cause fetal harm [12,19]. To increase safety, a concomitant folate supplementation before and throughout pregnancy is advised and the dose of SSZ should not exceed 2 g per day to prevent neutropenia in the newborns. SSZ can cause reduced male fertility; however, spermatogenesis recovers at about 2–3 months after a withdrawal of the drug [12].

3. Biologic DMARDs (bDMARD)

According to the EULAR guidelines, a bDMARD should be considered if remission or LDA is not achieved with the first DMARD strategy, if poor prognostic factors (i.e., high acute phase reactant levels, high swollen joint counts, or the presence of early erosions) exist, or if the patient responds inadequately to MTX and/or other csDMARD strategies.

Before starting a therapy with bDMARD, active or latent infections with hepatitis or tuberculosis must be ruled out. Patients, if possible, should be brought up to date with all immunizations before initiating therapy. Blood cell counts and liver and kidney function also need to be evaluated prior to treatment.

3.1. Tumor Necrosis Factor-Alpha Inhibitors (TNFi)

Until now, five TNFi were available. Although all anti-TNF drugs bind TNF- α , there are differences in their molecular structures, their administration regimens, and their modes of action.

The first preclinical studies using antibodies against TNF- α were performed in animal models of sepsis in 1985. About 6 years later, Keffer et al. provided the first evidence that TNF plays a role in developing arthritis [20]. In 1994, *The Lancet* published the first RCT showing that the blockade of a specific cytokine can be an effective treatment in patients with rheumatoid arthritis [21]. Since then, antibodies against TNF-alpha have gotten an important cornerstone in the treatment of RA and changed the lives of our patients.

Nowadays, we could not think of a world without antibodies against TNF- α or other cytokines. For our patients, this changed the game.

The first TNF- α -inhibitor, approved in 2000 for the therapy of RA, was the chimeric murine/human IgG1 monoclonal antibody Infliximab (IFX) that binds to both soluble and membrane-bound TNF- α [21]. The administration is intravenous every 8 weeks at a dosage between 3–5 mg/kg. Another TNF-inhibitor also approved in 2000 is Etanercept (ETN), a recombinant fusion protein compound of the soluble TNF-alpha receptor linked to the Fc portion of human IgG. ETN binds to the TNF receptor, preventing

TNF-mediated cellular responses. ETN is administered subcutaneously at a dose of 25 mg twice a week or 50 mg weekly [22,23]. The third inhibitor approved in 2003 by the European Medicines Agency (EMA) is Adalimumab (ADA) [24]. It is a recombinant human IgG1 monoclonal antibody that binds to soluble and membrane-bound TNF- α with a high affinity. It is administered by subcutaneous injection once every 2 weeks. Golimumab (GOL) is a human IgG1 monoclonal antibody neutralizing both soluble and membrane-bound TNF- α . GOL was approved in 2010 and is administered as a subcutaneous injection at an initial dose of 50 mg every 4 weeks that can be increased to 100 mg if there is no response after 4 doses (in patients with a body weight > 100 kg) [25]. Certolizumab Pegol (CZP) was approved by the EMA in 2007. CZP is a recombinant humanized Fab' fragment of a TNF antibody coupled to polyethylene glycol (PEG) that prolongs its plasma half-life to approximately 2 weeks. It has an initial loading dose of 400 mg every 2 weeks for 6 weeks, followed by 200 mg every 2 weeks [26,27].

Only certolizumab, adalimumab, and etanercept are approved as monotherapy as well as in combination with methotrexate [27–31].

Adverse effects include skin reactions characterized by itching, pain, and redness at the site of medication injection. Such injection site reactions characteristically occur during the first weeks of treatment. For intravenously administered agents, acute infusion reactions can occur with hypotension, bronchospasm, wheezing, and/or urticaria. Acute reactions may, in some cases, represent immunoglobulin-E-mediated type I reactions. The majority of acute infusion reactions that occur are anaphylactoid reactions and not immunoglobulin E mediated. These reactions can be managed by just reducing the rate of infusion [32,33].

However, delayed infusion reactions can also occur, and they are associated with skin rash, diffuse joint pains, myalgia, and fatigue and sometimes accompanied by fever. Delayed reactions may represent mild type III (immune complex-mediated) reactions [34]. It has been shown that the formation of Anti-monoclonal antibodies may lead to a greater risk of infusion reactions and also may limit the long-term efficacy of the drug. It has also been shown that the use of concomitant immunomodulators prior to starting a TNF- α -inhibitor is effective in reducing antibody production and therefore decreasing immunogenicity [32,34].

As TNF-alpha is a key player of the immune system during infections, this treatment has been associated with an increased risk of serious infections. These include bacterial infections (particularly pneumonia), herpes zoster, tuberculosis, and opportunistic infections [35]. As mentioned above, screening for latent tuberculosis infections should be performed before the initiation of TNF-alpha inhibitor therapy. If there is an indication of latent tuberculosis, a treatment for latent tuberculosis should be initiated before starting therapy with a TNF-alpha inhibitor [36]. Another side effect occurring in a few cases is neutropenia. Other cytopenias are uncommon [37]. The association between TNF-alpha inhibitors and demyelinating diseases remains uncertain. However, anti-TNF-alpha agents should generally be avoided in patients with established diseases that are associated with demyelination and should be discontinued directly in any patient with suspected demyelination [38]. Treating patients with NYHA III or IV cardiac failure is not recommended [2,39]. However, data regarding cardiac failure in patients treated with anti-TNF-alpha agents are inconclusive [40]. While Kwon et al. reported 38 patients who developed new-onset heart failure and 9 patients who experienced heart failure exacerbation after therapy [39], others report a reduced risk of cardiovascular events in patients treated with TNF-alpha inhibitors [40].

Pregnancy

Observational studies and case reports of women exposed to TNF inhibitors during pregnancy suggest that their pregnancy outcomes, including rates of preterm birth, spontaneous miscarriage, and congenital anomalies, are similar to those in women with RA who have not received a TNFi [41].

Complete immunoglobulin G (IgG) antibodies—maternal as well as therapeutic—cross placenta via active transport facilitated by the neonatal fragment crystallizable (Fc) receptor on the placenta.

IgG1 is the most effectively transported of the four subclasses of IgG (G1–G4) [42]. This is relevant to adalimumab, golimumab, and infliximab which are complete IgG1 anti-TNF. Etanercept is comprised of the Fc domain of human IgG1 fused with the extracellular ligand binding domain of human tumor necrosis factor receptor-2. Transplacental transport via the neonatal Fc receptor would theoretically be plausible. In contrast to the complete IgG1 anti-TNF antibodies certolizumab pegol differs structurally as it is a humanized PEG (polyethylene glycol)-ylated antibody Fab' fragment lacking the IgG1 Fc portion. Without the Fc portion it should, in theory, not be transported actively across the placenta [43]. So passive diffusion would be the only option for any detectable concentrations in exposed infants. This was shown in two trials. Based on these trials the EMA has approved a label change for CZP, making it the first anti-TNF for potential use in women with chronic rheumatic disease during both pregnancy and breastfeeding [44].

TNFi may have different structures, morphology, pharmacokinetic properties, and activity, but their clinical efficiency is comparable. If drug survival and safety are similar or different is still a matter of debate.

3.2. Interleukin 1 Inhibitor

Anakinra was first approved in the US in 2001 and in Europe in March 2002. It is a recombinant human IL-1 receptor antagonist with a short half-life (4–6 h), administered subcutaneously at a dose of 100 mg once a day. It was first developed for use in RA and showed some effects in early trials. A big systematic review of the literature in 2009 showed only a moderate effectiveness [45,46]. Anakinra plays not a very great role in RA therapy; today, it is much more effective in the therapy of auto-inflammatory diseases, gout, or polyserositis [45–50].

Adverse effects include injection site reactions characterized by itching, pain, and redness at the site of medication injection and lasting days to weeks. Between one and ten percent of people have severe infections, decreased white blood cells, or decreased platelets [51].

Pregnancy

There have been no adequate studies for the safety of Anakinra in Pregnancy, although several case reports of women with adult-onset Still's disease treated through pregnancy resulted in healthy neonates [52].

4. Interleukin 6 and Interleukin 6 Receptor Inhibitors

Interleukin-6 (IL-6) was identified in 1986 as a key cytokine in the pathogenesis of RA with proinflammatory activity. It is able to enhance the production of acute-phase proteins involved in the systemic inflammation process. Tocilizumab (TCZ) is the first humanized recombinant IgG1 monoclonal antibody that binds to both the soluble and membrane-bound IL-6 receptor, blocking its action and leading to the decrease of the inflammatory response cascade. Its half-life (10–13 days) allows its administration intravenously (8 mg/kg) every 4 weeks. A subcutaneous formulation of TCZ (162 mg/week) has been approved, with an efficacy and safety profile comparable to intravenous administration [52,53]. A second agent, Sarilumab is a human immunoglobulin G1 anti-interleukin-6 (IL-6) receptor monoclonal antibody that blocks IL-6 from binding to membrane-bound and soluble IL-6 receptor alpha [54].

Events of gastrointestinal (GI) perforations have been reported in Phase III clinical trials, primarily as complications of diverticulitis, including generalized purulent peritonitis, lower GI perforation, fistula, and abscess. Tocilizumab or Sarilumab should be used with caution in patients who may be at increased risk for GI perforation. Patients presenting with new-onset abdominal symptoms should be evaluated promptly for the early identification of GI perforation. It is important to keep in mind that, during IL-6 blockade, C-reactive protein or other acute phase reactants will increase slowly and less pronounced [55,56].

Other side effects are neutropenia or thrombocytopenia as well as hyperlipidemia. Also, serious infections and liver enzyme elevations were observed and can require dose adjustments or drug discontinuation [56].

4.1. *Pregnancy*

Based upon animal data, tocilizumab may cause fetal harm, but there are no adequate studies of its effects on human pregnancy to allow an assessment of its risk. There are a few case series available. No teratogenic effects were observed. In a case series of 50 pregnancies exposed to tocilizumab with known outcomes, 36 resulted in live births, while there were five low-birthweight infants born and one case of neonatal asphyxia [57,58].

4.2. *CD80/86-CD 28 Inhibitor*

Abatacept is a fusion protein constituted by an immunoglobulin fused to the extracellular domain of cytotoxic T-lymphocyte antigen 4 (CTLA-4). This is a molecule that binds with a high affinity to the CD80/86 ligand on antigen-presenting cells. Therefore, abatacept is able to block the interaction between the antigen-presenting cell's CD80/86 ligand and the CD28 ligand on the T cell [59]. This results in decreased T cell proliferation and cytokine production. Abatacept is administered intravenously once every 4 weeks or subcutaneously once a week [60,61].

Adverse effects including cases of hypersensitivity and anaphylaxis or anaphylactoid reactions have been reported with iv administration. As in other bDMARDs, serious infections (including tuberculosis and sepsis) have been reported, particularly in patients receiving concomitant immunosuppressive therapy [59].

The use of CTLA-4 Inhibitors due to its T cell inhibition affect defenses against malignancies. As compared to the general population, an increased risk of lymphoma has been noted in clinical trials; however, rheumatoid arthritis has been previously associated with an increased rate of lymphoma. Abatacept itself seems not to further increase this risk [62].

Pregnancy

Abatacept is not teratogenic in animals, but there are no adequate studies of its effects on human pregnancy to allow an assessment of its risk.

5. Anti-CD 20 Antibody

Rituximab (RTX) was initially developed for the treatment of hematologic malignancies. Since 2006, RTX is approved for the therapy of RA refractory to a combination therapy of anti-TNF-alpha, an MTX [60]. RTX is a chimeric murine-human monoclonal antibody that binds to the CD20 membrane receptor, leading to the depletion of circulating B cells. It is also able to inhibit the activation of T cells that produce proinflammatory cytokines. A cycle of RTX consists in 1000 mg by intravenous infusion, followed by a second 1000-mg intravenous infusion two weeks later. This is then repeated every 6 months. Different studies showed that particularly patients who are autoantibody-positive benefit from RTX [63,64].

According to results of different studies, patients benefit more from switching to RTX when an initial TNFi was discontinued due to inefficacy.

One of the common side effects is an infusion reaction especially during the first infusion, occurring in up to 30 to 45 percent of patients. Symptoms include headache, fever, skin rash, dyspnea, hypotension, nausea, rhinitis, pruritus, and mild angioedema [65].

Repeated courses of RTX are associated with an increasing risk of hypogammaglobinemia. A meta-analysis in 2009 found no increase in serious infections associated with the use of rituximab with or without MTX compared with MTX plus a placebo, but other studies have found that repeated courses may be associated with a higher rate of serious infections. The risk of serious infections seems to increase with age [65].

Pregnancy

There is only limited information about the use of RTX during pregnancy. Rituximab has been detected in high concentrations in umbilical cord blood. There are case reports available, but in most cases, the mother took RTX due to hematologic malignancies. Congenital anomalies were not reported. As for all babies indirectly exposed to immunosuppressant biologics during pregnancy, immunization with live vaccines should be postponed until the age of 6 months [66].

6. Targeted Synthetic DMARDS: JAK-Inhibitors

Recently, with the Janus-Kinase (JAK) Inhibitors, a new group of drugs for the treatment of rheumatoid arthritis was introduced. These targeted, synthetic DMARDS (tsDMARDS) are effective in inflammatory diseases by intracellularly blocking tyrosine kinase [67].

The JAK are cytoplasmic protein tyrosine kinases that are critical for signal transduction to the nucleus from the common gamma chain of the plasma membrane receptors for interleukin (IL)-2, -4, -7, -9, -15, and -21. JAK are receptor-associated intracellular proteins, with a tyrosine-kinase component. They are important downstream mediators of many pro-inflammatory cytokines, e.g., interferons or interleukin 6. Once a ligand binds to its receptor, the intracellular kinase is phosphorylated, which further leads to the phosphorylation and activation of the signal transducer and activator of transcription (STAT)-pathway. Accordingly, blocking these enzymes with a targeted molecule affects multiple inflammatory pathways [65,66]. How JAKs transmit signals from cytokines and thereby activates gene transcription is shown in Figure 2.

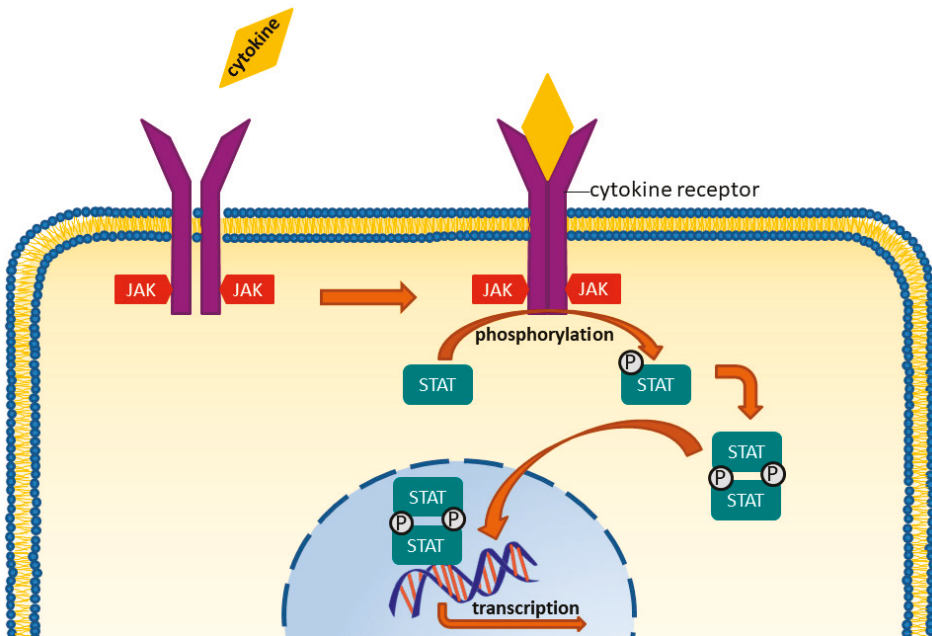


Figure 2. Cytokines bind to the receptors, which then undergo a change in their configuration into a dimeric structure, thereby activating their ability to work as a kinase. Consequently, they phosphorylate the STAT molecules (Signal Transducer and Activator of Transcription). Phosphorylated STATs also form dimers that travel to the cell core, where they activate transcription processes, which further fuel inflammatory processes.

Pharmacological aspects: JAK-inhibitors are referred to as small molecules, meaning that these compounds carry a low molecular weight and bind to a macromolecular target, altering their activity and function [68,69].

To date, two JAK-inhibitors are approved for therapeutic use in rheumatoid arthritis: Tofacitinib and Baricitinib.

Tofacitinib was approved by the FDA (Food and Drug Administration) in the US in 2012. In 2017, the European Medicines Agency (EMA) approved the drug for use in the European Union. Tofacitinib is an inhibitor of JAK 1 and 3, with only little affinity to JAK 2 and Tyrosine-Kinase 2. It has been shown effective in moderate and severe rheumatoid arthritis in monotherapy or in combination with methotrexate. Several phase III clinical trials showed efficacy in DMARD naïve patients as well as in patients with insufficient responses to csDMARDs and even bDMARDs. Tofacitinib is taken orally twice daily with a dosage of 5 mg. The elimination is mostly hepatic; therefore, an adaption to impaired kidney function is not necessary up to a GFR > 30 mL/min. Consequently, the treatment of patients with end-stage renal failure with a GFR < 30 mL/min with Tofacitinib is possible with reduced dosage [70,71].

The second JAK-inhibitor currently approved is Baricitinib which is an inhibitor of JAK 1 and 2. It was approved by the FDA as well as the EMA in 2017. Baricitinib can be administered as monotherapy or in combination with methotrexate. Several phase III clinical trials showed efficacy in the treatment of DMARD naïve patients as well as in patients with insufficient responses to csDMARDs or even bDMARDs [72–75].

Baricitinib is taken orally once daily. There are two dosages available: 4 mg or 2 mg. The elimination for Baricitinib is mostly renal, which makes it necessary to reduce the dosage for patients with impaired kidney function (estimated GFR 30–60 mL/min) from 4 mg to 2 mg daily. For patients with an estimated GFR < 30 mL/min, the use of Baricitinib is not recommended [2].

According to the EULAR-Guidelines, both JAK-Inhibitors can be used in rheumatoid arthritis once a therapy with csDMARDs has been insufficient or had to be discontinued due to adverse events. Both Tofacitinib and Baricitinib have been shown to be more efficient than a placebo in the treatment of rheumatoid arthritis; both could show non-inferiority compared with the TNF-inhibitor Adalimumab [2,71,73].

Side Effects

Tofacitinib: Increased susceptibility to infections including especially herpes zoster are the most common side effects of Tofacitinib as well as headaches, hypertension, nausea, and diarrhoea. Elevated levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, and liver enzymes have also been reported. In a post-marketing study, higher dosages of Tofacitinib showed an increased risk of deep vein thrombosis and pulmonary embolism. The recommended dosage of 5 mg twice daily should, therefore, not be exceeded [76,77].

Baricitinib: The most common side effect of Baricitinib was an increase of cholesterol levels. Elevated cholesterol levels return to normal once the medication is paused or be treated with statins effectively. This has been seen with other immunosuppressants as well. Other side effects include upper respiratory tract infections and nausea. Other infections, such as herpes zoster or pneumonia are also associated to Baricitinib. Patients treated with Baricitinib (0.3%) show significant neutropenia [78,79].

Screening pre-treatment: Active or latent infections with hepatitis or tuberculosis should be ruled out before initiating treatment with JAK-inhibitors. Blood cell counts and liver and kidney function also need to be evaluated prior to treatment.

Screening during treatment: neutrophil levels, lymphocyte levels, hemoglobin, and kidney and liver function should be screened; 8–12 weeks after the initiation of treatment, HDL, LDL, overall cholesterol, and triglycerides should also be screened.

Contraindications: JAK-inhibitors should not be prescribed for patients with neutropenia (<1/nL), active tuberculosis or severe infections, or severe liver impairment and during pregnancy.

A combination with other bDMARDs or strong immunodepressants as azathioprine, cyclosporine, or tacrolimus should be avoided due to the elevated risk of infection and the lack of experience.

Use during Pregnancy: drugs from the JAK-inhibitor family have not been tested in pregnant women yet. As both drugs are small molecules, it seems likely that cross the placenta. Women taking JAK-inhibitors should be instructed to use safe contraceptive methods throughout the treatment and up to one week (Baricitinib) to four weeks (Tofacitinib) after a discontinuation of the drug.

Perioperative management: Although there are not enough long-term data for a conclusive recommendation on perioperative management, JAK-inhibitors in general have a short elimination-time. It therefore seems possible to continue treatment until shortly before surgery. The therapy should then be paused until proper wound-healing is achieved.

Ongoing development: Two selective JAK-1-Inhibitors are currently undergoing Phase 3 trials for clinical approval: Upadacitinib and Filgotinib. Both drugs show promising results in terms of efficacy and safety. Another JAK-inhibitor currently being evaluated in Phase 3 trials is the selective JAK-3-Inhibitor Peficitinib, with limited data available so far [80,81].

7. Conclusions

The therapeutic strategies of RA have improved dramatically over the past 3 decades. At that time, only a few drugs were available and therapy started late in disease course. With the T2T strategy and the possibility to choose from different mode of actions, the aim of stable remission can be reached and joint destruction can be prevented. Equally important to the choice of drugs is to diagnose the disease early and to start therapy within 6–12 weeks after disease onset. Early arthritis clinics headed by experienced rheumatologists are a good tool to achieve this. T2T further improves the prognosis of RA.

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References

1. Hoes, J.N.; Jacobs, J.W.G.; Boers, M.; Boumpas, D.; Buttgerit, F.; Caeyers, N.; Choy, E.H.; Cutolo, M.; Silva, J.A.P.D.; Esselens, G.; et al. EULAR evidence-based recommendations on the management of systemic glucocorticoid therapy in rheumatic diseases. *Ann. Rheum. Dis.* **2007**, *66*, 1560–1567. [[CrossRef](#)] [[PubMed](#)]
2. Smolen, J.S.; Landewe, R.; Breedveld, F.C.; Buch, M.; Burmester, G.; Dougados, M.; Emery, P.; Gaujoux-Viala, C.; Gossec, L.; Nam, J.; 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. [[CrossRef](#)] [[PubMed](#)]
3. Smolen, J.S.; Breedveld, F.C.; Burmester, G.R.; Bykerk, V.; Dougados, M.; Emery, P.; Kvien, T.K.; Navarro-Compan, M.V.; Oliver, S.; Schoels, M.; et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann. Rheum. Dis.* **2016**, *75*, 3–15. [[CrossRef](#)] [[PubMed](#)]
4. Aletaha, D.; Smolen, J.S. Diagnosis and management of rheumatoid arthritis: A review. *JAMA* **2018**, *320*, 1360–1372. [[CrossRef](#)] [[PubMed](#)]
5. Fiehn, C.; Holle, J.; Iking-Konert, C.; Leipe, J.; Weseloh, C.; Frerix, M.; Alten, R.; Behrens, F.; Baerwald, C.; Braun, J.; et al. S2e guideline: Treatment of rheumatoid arthritis with disease-modifying drugs. *Z. Rheumatol.* **2018**, *77*, 3553.
6. Verschueren, P.; De Cock, D.; Corluy, L.; Joos, R.; Langenaken, C.; Taelman, V.; Raeman, F.; Ravelingien, I.; Vandevyvere, K.; Lenaerts, J.; et al. Effectiveness of methotrexate with step-down glucocorticoid remission induction (COBRA Slim) versus other intensive treatment strategies for early rheumatoid arthritis in a treat-to-target approach: 1-year results of CareRA, a randomised pragmatic open-label superiority trial. *Ann. Rheum. Dis.* **2017**, *76*, 511–520. [[PubMed](#)]

7. Smolen, J.S.; Kalden, J.R.; Scott, D.L.; Rozman, B.; Kvien, T.K.; Larsen, A.; Loew-Friedrich, I.; Oed, C.; Rosenburg, R. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: A double-blind, randomised, multicentre trial. *Lancet* **1999**, *353*, 259–266. [CrossRef]
8. Brown, P.M.; Pratt, A.G.; Isaacs, J.D. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. *Nat. Rev. Rheumatol.* **2016**, *12*, 731–742. [CrossRef]
9. Kremer, J.M. Major Side Effects of Low-Dose Methotrexate. Available online: www.UpToDate.com (accessed on 2 April 2019).
10. Shea, B.; Swinden, M.V.; Ghogomu, E.T.; Ortiz, Z.; Katchamart, W.; Rader, T.; Bombardier, C.; A Wells, G.; Tugwell, P. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *Cochrane Database Syst. Rev.* **2013**, CD000951. [CrossRef]
11. Kruger, K.; Albrecht, K.; Rehart, S.; Scholz, R. Recommendations of the German society for rheumatology on the perioperative approach under therapy with DMARDs and biologicals in inflammatory rheumatic diseases. *Z. Rheumatol.* **2014**, *73*, 77–84. [CrossRef]
12. Østensen, M.; Khamashta, M.; Lockshin, M.; Parke, A.; Brucato, A.; Carp, H.; Doria, A.; Rai, R.; Meroni, P.L.; Cetin, I.; et al. Anti-inflammatory and immunosuppressive drugs and reproduction. *Arthritis Res. Ther.* **2006**, *8*, 209. [CrossRef] [PubMed]
13. Breedveld, F.C.; Dayer, J. Leflunomide: Mode of action in the treatment of rheumatoid arthritis. *Ann. Rheum. Dis.* **2000**, *59*, 841–849. [CrossRef] [PubMed]
14. Kruger, K.; Bolten, W. Treatment with leflunomide in rheumatoid arthritis. *Z. Rheumatol.* **2005**, *64*, 96–101. [CrossRef] [PubMed]
15. Fox, R.; Helfgott, S.M. Pharmacology, Dosing, and Adverse Effects of Leflunomide in the Treatment of Rheumatoid Arthritis. Available online: www.UpToDate.com (accessed on 2 April 2019).
16. Smedegård, G.; Björk, J. Sulphasalazine: Mechanism of Action in Rheumatoid Arthritis. *Rheumatology* **1995**, *34*, 7–15. [CrossRef]
17. Michael, H.; Weisman, R.Z.R. Sulfasalazine: Pharmacology, Administration, and Adverse Effects in the Treatment of Rheumatoid Arthritis. Available online: www.UpToDate.com (accessed on 2 April 2019).
18. Pullar, T.; A Box, S. Sulphasalazine in the treatment of rheumatoid arthritis. *Rheumatology* **1997**, *36*, 382–386.
19. Fachinformation Sulfasalazin HEXAL. Available online: https://s3.eu-central-1.amazonaws.com/prod-cerebro-ifap/media_all/72466.pdf (accessed on 2 April 2019).
20. Keffer, J.; Probert, L.; Cazlaris, H.; Georgopoulos, S.; Kaslaris, E.; Kioussis, D.; Kollias, G. Transgenic mice expressing human tumour necrosis factor: A predictive genetic model of arthritis. *EMBO J.* **1991**, *10*, 4025–4031. [CrossRef] [PubMed]
21. Emery, P. Infliximab: A new treatment for rheumatoid arthritis. *Hosp. Med.* **2001**, *62*, 150–152. [CrossRef]
22. Moreland, L.W. Recent advances in anti-tumour necrosis factor (TNF) therapy in rheumatoid arthritis: Focus on the soluble TNF receptor p75 fusion protein, etanercept. *BioDrugs* **1999**, *11*, 201–210. [CrossRef]
23. Mikuls, T.R.; Moreland, L.W. TNF blockade in the treatment of rheumatoid arthritis: Infliximab versus etanercept. *Expert Opin. Pharmacother.* **2001**, *2*, 75–84. [CrossRef]
24. Rau, R. Adalimumab (a fully human anti-tumour necrosis factor alpha monoclonal antibody) in the treatment of active rheumatoid arthritis: The initial results of five trials. *Ann. Rheum. Dis.* **2002**, *61*, 70–73. [CrossRef]
25. Kay, J.; Matteson, E.L.; Dasgupta, B.; Nash, P.; Durez, P.; Hall, S.; Hsia, E.C.; Han, J.; Wagner, C.; Xu, Z.; et al. Golimumab in patients with active rheumatoid arthritis despite treatment with methotrexate: A randomized, double-blind, placebo-controlled, dose-ranging study. *Arthritis Rheum.* **2008**, *58*, 964–975. [CrossRef] [PubMed]
26. Smolen, J.; Landewe, R.B.; Mease, P.; Brzezicki, J.; Mason, D.; Luijckens, K.; van Vollenhoven, R.F.; Kavanaugh, A.; Schiff, M.; Burmester, G.R.; et al. Efficacy and safety of certolizumab pegol plus methotrexate in active rheumatoid arthritis: The RAPID 2 study. A randomised controlled trial. *Ann. Rheum. Dis.* **2009**, *68*, 797–804. [CrossRef] [PubMed]
27. Fleischmann, R.; Vencovsky, J.; van Vollenhoven, R.F.; Borenstein, D.; Box, J.; Coteur, G.; Goel, N.; Brezinschek, H.P.; Innes, A.; Strand, V. Efficacy and safety of certolizumab pegol monotherapy every 4 weeks in patients with rheumatoid arthritis failing previous disease-modifying antirheumatic therapy: The FAST4WARD study. *Ann. Rheum. Dis.* **2009**, *68*, 805–811. [CrossRef]

28. Fleischmann, R.; van Vollenhoven, R.F.; Vencovsky, J.; Alten, R.; Davies, O.; Mountian, I.; de Longueville, M.; Carter, D.; Choy, E. Long-Term Maintenance of Certolizumab Pegol Safety and Efficacy, in Combination with Methotrexate and as Monotherapy, in Rheumatoid Arthritis Patients. *Rheumatol. Ther.* **2017**, *4*, 57–69. [[CrossRef](#)] [[PubMed](#)]
29. Keystone, E.C.; Breedveld, F.C.; Kupper, H.; Li, Y.; Florentinus, S.; Sainsbury, I. Long-term use of adalimumab as monotherapy after attainment of low disease activity with adalimumab plus methotrexate in patients with rheumatoid arthritis. *RMD Open* **2018**, *4*, e000637. [[CrossRef](#)] [[PubMed](#)]
30. Narayanan, S.; Lu, Y.; Hutchings, R.; Baynton, E.; Hautamaki, E. Comparison of Disease Status And Outcomes of Patients With Rheumatoid Arthritis (Ra) Receiving Adalimumab or Etanercept Monotherapy In Europe. *Value Heal.* **2014**, *17*, A374. [[CrossRef](#)] [[PubMed](#)]
31. Gaubitz, M.; Göttl, K.-H.; Behmer, O.; Lippe, R.; Meng, T.; Löschmann, P.-A. Etanercept is effective as monotherapy or in combination with methotrexate in rheumatoid arthritis: Subanalysis of an observational study. *Clin. Rheumatol.* **2017**, *36*, 1989–1996. [[CrossRef](#)] [[PubMed](#)]
32. Cheifetz, A.; Mayer, L. Monoclonal antibodies, immunogenicity, and associated infusion reactions. *Mount Sinai J. Med.* **2005**, *72*, 250–256.
33. Weinblatt, M.E.; Schiff, M.; Valente, R.; van der Heijde, D.; Citera, G.; Zhao, C.; Maldonado, M.; Fleischmann, R. Head-to-head comparison of subcutaneous abatacept versus adalimumab for rheumatoid arthritis: Findings of a phase IIIb, multinational, prospective, randomized study. *Arthritis Rheum.* **2013**, *65*, 28–38. [[CrossRef](#)]
34. Cheifetz, A.; Smedley, M.; Martín, S.; Reiter, M.; Leone, G.; Mayer, L.; Plevy, S. The incidence and management of infusion reactions to infliximab: A large center experience. *Am. J. Gastroenterol.* **2003**, *98*, 1315–1324. [[CrossRef](#)]
35. A Wells, G.; Christensen, R.; Ghogomu, E.T.; Maxwell, L.; Macdonald, J.K.; Filippini, G.; Skoetz, N.; Francis, D.K.; Lopes, L.C.; Guyatt, G.H.; et al. Adverse effects of biologics: A network meta-analysis and Cochrane overview. *Cochrane Database Syst. Rev.* **2011**.
36. Calabrese, L.H.; Calabrese, C.; Kirchner, E. The 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis Should Include New Standards for Hepatitis B Screening: Comment on the Article by Singh et al. *Arthritis Rheum.* **2016**, *68*, 723–724. [[CrossRef](#)] [[PubMed](#)]
37. Hastings, R.; Ding, T.; Butt, S.; Gadsby, K.; Zhang, W.; Moots, R.J.; Deighton, C. Neutropenia in patients receiving anti-tumor necrosis factor therapy. *Arthritis Rheum.* **2010**, *62*, 764–769. [[CrossRef](#)] [[PubMed](#)]
38. van Oosten, B.W.; Barkhof, F.; Truyen, L.; Boringa, J.B.; Bertelsmann, F.W.; von Blomberg, B.M.; Woody, J.N.; Hartung, H.P.; Polman, C.H. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. *Neurology* **1996**, *47*, 1531–1534. [[CrossRef](#)] [[PubMed](#)]
39. Kwon, H.J.; Cote, T.R.; Cuffe, M.S.; Kramer, J.M.; Braun, M.M. Case reports of heart failure after therapy with a tumor necrosis factor antagonist. *Ann. Intern. Med.* **2003**, *138*, 807–811. [[CrossRef](#)] [[PubMed](#)]
40. Gabriel, S.E. Tumor necrosis factor inhibition: A part of the solution or a part of the problem of heart failure in rheumatoid arthritis? *Arthritis Rheum.* **2008**, *58*, 637–640. [[CrossRef](#)] [[PubMed](#)]
41. Marchioni, R.M.; Lichtenstein, G.R. Tumor necrosis factor-alpha inhibitor therapy and fetal risk: A systematic literature review. *World J. Gastroenterol.* **2013**, *19*, 2591–2602. [[CrossRef](#)]
42. Malek, A.; Sager, R.; Schneider, H. Maternal-fetal transport of immunoglobulin-G and its subclasses during the 3rd trimester of human-pregnancy. *Am. J. Reprod. Immunol.* **1994**, *32*, 8–14. [[CrossRef](#)]
43. Mahadevan, U.; Wolf, D.C.; Dubinsky, M.; Cortot, A.; Lee, S.D.; Siegel, C.A.; Ullman, T.; Glover, S.; Valentine, J.F.; Rubin, D.T.; et al. Placental transfer of anti-tumor necrosis factor agents in pregnant patients with inflammatory bowel disease. *Clin. Gastroenterol. Hepatol.* **2013**, *11*, 286–292. [[CrossRef](#)]
44. Clowse, M.E.B.; Scheuerle, A.E.; Chambers, C.; Afzali, A.; Kimball, A.B.; Cush, J.J.; Cooney, M.; Shaughnessy, L.; Vanderkelen, M.; Forger, F. Pregnancy Outcomes After Exposure to Certolizumab Pegol: Updated Results From a Pharmacovigilance Safety Database. *Arthritis Rheumatol.* **2018**, *70*, 1399–1407. [[CrossRef](#)]
45. Kontinen, L.; Kankaanpää, E.; Luosujärvi, R.; Blåfield, H.; Vuori, K.; Hakala, M.; Rantalaiho, V.; Savolainen, E.; Uutela, T.; Nordström, D.; et al. Effectiveness of anakinra in rheumatic disease in patients naive to biological drugs or previously on TNF blocking drugs: An observational study. *Clin. Rheumatol.* **2006**, *25*, 882–884. [[CrossRef](#)] [[PubMed](#)]
46. Mertens, M.; Singh, J.A. Anakinra for Rheumatoid Arthritis: A Systematic Review. *J. Rheumatol.* **2009**, *36*, 1118–1125. [[CrossRef](#)] [[PubMed](#)]

47. Eungdamrong, J.; Boyd, K.P.; A Meehan, S.; Latkowski, J.-A. Muckle-Wells treatment with anakinra. *Dermatol. Online J.* **2013**, *19*.
48. Kohler, B.M.; Lorenz, H.-M.; Blank, N. IL1-blocking therapy in colchicine-resistant familial Mediterranean fever. *Eur. J. Rheumatol.* **2018**, *5*, 230–234. [[CrossRef](#)] [[PubMed](#)]
49. Vounotrypidis, P.; Sakellariou, G.T.; Zisopoulos, D.; Berberidis, C. Refractory relapsing polychondritis: Rapid and sustained response in the treatment with an IL-1 receptor antagonist (anakinra). *Rheumatology* **2006**, *45*, 491–492. [[CrossRef](#)] [[PubMed](#)]
50. Gratton, S.B.; Scalapino, K.J.; Fye, K.H. Case of anakinra as a steroid-sparing agent for gout inflammation. *Arthritis Rheum.* **2009**, *61*, 1268–1270. [[CrossRef](#)] [[PubMed](#)]
51. Fleischmann, R.M.; Tesser, J.; Schiff, M.H.; Schechtman, J.; Burmester, G.-R.; Bennett, R.; Modafferi, D.; Zhou, L.; Bell, D.; Appleton, B. Safety of extended treatment with anakinra in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2006**, *65*, 1006–1012. [[CrossRef](#)] [[PubMed](#)]
52. Chang, Z.; Spong, C.Y.; Jesus, A.A.; Davis, M.A.; Plass, N.; Stone, D.L.; Chapelle, D.; Hoffmann, P.; Kastner, D.L.; Barron, K.; et al. Anakinra Use During Pregnancy in Patients with Cryopyrin-Associated Periodic Syndromes (CAPS). *Arthritis Rheumatol.* **2014**, *66*, 3227–3232. [[CrossRef](#)] [[PubMed](#)]
53. Gabay, C.; Emery, P.; van Vollenhoven, R.; Dikranian, A.; Alten, R.; Pavelka, K.; Kleerman, M.; Musselman, D.; Agarwal, S.; Green, J.; et al. Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): A randomised, double-blind, controlled phase 4 trial. *Lancet* **2013**, *381*, 1541–1550. [[CrossRef](#)]
54. Zhang, X.; Chen, Y.-C.; Fettner, S.; Rowell, L.; Gott, T.; Grimsey, P.; Unsworth, A. Pharmacokinetics and pharmacodynamics of tocilizumab after subcutaneous administration in patients with rheumatoid arthritis. *Int. J. Clin. Pharmacol. Ther.* **2013**, *51*, 620–630. [[CrossRef](#)]
55. Burmester, G.R.; Lin, Y.; Patel, R.; van Adelsberg, J.; Mangan, E.K.; Graham, N.M.; van Hoogstraten, H.; Bauer, D.; Ignacio Vargas, J.; Lee, E.B. Efficacy and safety of sarilumab monotherapy versus adalimumab monotherapy for the treatment of patients with active rheumatoid arthritis (MONARCH): A randomised, double-blind, parallel-group phase III trial. *Ann. Rheum. Dis.* **2017**, *76*, 840–847. [[CrossRef](#)] [[PubMed](#)]
56. Genovese, M.C.; Rubbert-Roth, A.; Smolen, J.S.; Kremer, J.; Khraishi, M.; Gómez-Reino, J.; Sebba, A.; Pilsen, R.; Williams, S.; Van Vollenhoven, R. Longterm Safety and Efficacy of Tocilizumab in Patients with Rheumatoid Arthritis: A Cumulative Analysis of Up to 4.6 Years of Exposure. *J. Rheumatol.* **2013**, *40*, 768–780. [[CrossRef](#)] [[PubMed](#)]
57. Saito, J.; Yakuwa, N.; Kaneko, K.; Takai, C.; Goto, M.; Nakajima, K.; Yamatani, A.; Murashima, A. Tocilizumab during pregnancy and lactation: Drug levels in maternal serum, cord blood, breast milk and infant serum. *Rheumatology* **2019**. [[CrossRef](#)] [[PubMed](#)]
58. Nakajima, K.; Watanabe, O.; Mochizuki, M.; Nakasone, A.; Ishizuka, N.; Murashima, A. Pregnancy outcomes after exposure to tocilizumab: A retrospective analysis of 61 patients in Japan. *Mod. Rheumatol.* **2016**, *26*, 667–671. [[CrossRef](#)] [[PubMed](#)]
59. Maxwell, L.; Singh, J.A. Abatacept for rheumatoid arthritis. *Cochrane Database Syst. Rev.* **2009**, CD007277. [[CrossRef](#)] [[PubMed](#)]
60. Herrero-Beaumont, G.; Calatrava, M.J.M.; Castañeda, S. Abatacept Mechanism of Action: Concordance With Its Clinical Profile. *Reumatol. Clínica (Engl. Ed.)* **2012**, *8*, 78–83. [[CrossRef](#)]
61. Atzeni, F.; Sarzi-Puttini, P.; Mutti, A.; Bugatti, S.; Cavagna, L.; Caporali, R. Long-term safety of abatacept in patients with rheumatoid arthritis. *Autoimmun. Rev.* **2013**, *12*, 1115–1117. [[CrossRef](#)]
62. Smolen, J.S.; Keystone, E.C.; Emery, P.; Breedveld, F.C.; Betteridge, N.; Burmester, G.R.; Dougados, M.; Ferraccioli, G.; Jaeger, U.; Klareskog, L.; et al. Consensus statement on the use of rituximab in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2007**, *66*, 143–150. [[CrossRef](#)]
63. Harrold, L.R.; John, A.; Best, J.; Zlotnick, S.; Karki, C.; Li, Y.; Greenberg, J.D.; Kremer, J.M. Impact of rituximab on patient-reported outcomes in patients with rheumatoid arthritis from the US Corrona Registry. *Clin. Rheumatol.* **2017**, *36*, 2135–2140. [[CrossRef](#)]
64. Chatzidionysiou, K.; Lie, E.; Nasonov, E.; Lukina, G.; Hetland, M.L.; Tarp, U.; Gabay, C.; van Riel, P.L.; Nordstrom, D.C.; Gomez-Reino, J.; et al. Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonist has failed: Pooled data from 10 European registries. *Ann. Rheum. Dis.* **2011**, *70*, 1575–1580. [[CrossRef](#)]

65. Lee, Y.H.; Bae, S.C.; Song, G.G. The efficacy and safety of rituximab for the treatment of active rheumatoid arthritis: A systematic review and meta-analysis of randomized controlled trials. *Rheumatol. Int.* **2011**, *31*, 1493–1499. [[CrossRef](#)] [[PubMed](#)]
66. Chakravarty, E.F.; Murray, E.R.; Kelman, A.; Farmer, P. Pregnancy outcomes after maternal exposure to rituximab. *Blood* **2011**, *117*, 1499–1506. [[CrossRef](#)] [[PubMed](#)]
67. Iwata, S.; Tanaka, Y. Progress in understanding the safety and efficacy of Janus kinase inhibitors for treatment of rheumatoid arthritis. *Expert Rev. Clin. Immunol.* **2016**, *12*, 1047–1057. [[CrossRef](#)] [[PubMed](#)]
68. Migita, K.; Izumi, Y.; Torigoshi, T.; Satomura, K.; Izumi, M.; Nishino, Y.; Jiuchi, Y.; Nakamura, M.; Kozuru, H.; Nonaka, F.; et al. Inhibition of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway in rheumatoid synovial fibroblasts using small molecule compounds. *Clin. Exp. Immunol.* **2013**, *174*, 356–363. [[CrossRef](#)] [[PubMed](#)]
69. Migita, K.; Koga, T.; Komori, A.; Torigoshi, T.; Maeda, Y.; Izumi, Y.; Sato, J.; Jiuchi, Y.; Miyashita, T.; Yamasaki, S.; et al. Influence of Janus Kinase Inhibition on Interleukin 6-mediated Induction of Acute-phase Serum Amyloid A in Rheumatoid Synovium. *J. Rheumatol.* **2011**, *38*, 2309–2317. [[CrossRef](#)] [[PubMed](#)]
70. Arkin, M.R.; Wells, J.A. Small-molecule inhibitors of protein–protein interactions: Progressing towards the dream. *Nat. Rev. Drug Discov.* **2004**, *3*, 301–317. [[CrossRef](#)] [[PubMed](#)]
71. Dhillon, S. Tofacitinib: A Review in Rheumatoid Arthritis. *Drugs* **2017**, *77*, 1987–2001. [[CrossRef](#)]
72. Kremer, J.; Li, Z.G.; Hall, S.; Fleischmann, R.; Genovese, M.; Martin-Mola, E.; Isaacs, J.D.; Gruben, D.; Wallenstein, G.; Krishnaswami, S.; et al. Tofacitinib in combination with nonbiologic disease-modifying antirheumatic drugs in patients with active rheumatoid arthritis: A randomized trial. *Ann. Intern. Med.* **2013**, *159*, 253–261. [[CrossRef](#)]
73. Taylor, P.C.; Keystone, E.C.; van der Heijde, D.; Weinblatt, M.E.; Del Carmen Morales, L.; Reyes Gonzaga, J.; Yakushin, S.; Ishii, T.; Emoto, K.; Beattie, S.; et al. Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis. *N. Engl. J. Med.* **2017**, *376*, 652–662. [[CrossRef](#)]
74. Fleischmann, R.; Schiff, M.; van der Heijde, D.; Ramos-Remus, C.; Spindler, A.; Stanislav, M.; Zerbini, C.A.; Gurbuz, S.; Dickson, C.; de Bono, S.; et al. Baricitinib, Methotrexate, or Combination in Patients With Rheumatoid Arthritis and No or Limited Prior Disease-Modifying Antirheumatic Drug Treatment. *Arthritis Rheumatol.* **2017**, *69*, 506–517. [[CrossRef](#)]
75. Dougados, M.; van der Heijde, D.; Chen, Y.C.; Greenwald, M.; Drescher, E.; Liu, J.; Beattie, S.; Witt, S.; de la Torre, I.; Gaich, C.; et al. Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: Results from the RA-BUILD study. *Ann. Rheum. Dis.* **2017**, *76*, 88–95. [[CrossRef](#)] [[PubMed](#)]
76. Yamaoka, K. Benefit and Risk of Tofacitinib in the Treatment of Rheumatoid Arthritis: A Focus on Herpes Zoster. *Drug Saf.* **2016**, *39*, 823–840. [[CrossRef](#)] [[PubMed](#)]
77. Cohen, S.B.; Koenig, A.; Wang, L.; Kwok, K.; Mebus, C.A.; Riese, R.; Fleischmann, R. Efficacy and safety of tofacitinib in US and non-US rheumatoid arthritis patients: Pooled analyses of phase II and III. *Clin. Exp. Rheumatol.* **2016**, *34*, 32–36. [[PubMed](#)]
78. Keystone, E.C.; Taylor, P.C.; Drescher, E.; Schlichting, D.E.; Beattie, S.D.; Berclaz, P.Y.; Lee, C.H.; Fidelus-Gort, R.K.; Luchi, M.E.; Rooney, T.P.; et al. Safety and efficacy of baricitinib at 24 weeks in patients with rheumatoid arthritis who have had an inadequate response to methotrexate. *Ann. Rheum. Dis.* **2015**, *74*, 333–340. [[CrossRef](#)] [[PubMed](#)]
79. Smolen, J.S.; Genovese, M.C.; Takeuchi, T.; Hyslop, D.L.; Macias, W.L.; Rooney, T.; Chen, L.; Dickson, C.L.; Riddle Camp, J.; Cardillo, T.E.; et al. Safety Profile of Baricitinib in Patients with Active Rheumatoid Arthritis with over 2 Years Median Time in Treatment. *J. Rheumatol.* **2019**, *46*, 7–18. [[CrossRef](#)] [[PubMed](#)]
80. Westhovens, R. Clinical efficacy of new JAK inhibitors under development. Just more of the same? *Rheumatology* **2019**, *58* (Suppl. 1), i27–i33. [[CrossRef](#)]
81. Serhal, L.; Edwards, C.J. Upadacitinib for the treatment of rheumatoid arthritis. *Expert Rev. Clin. Immunol.* **2018**, *15*, 1–13. [[CrossRef](#)]





Review

The Potential Role of Genomic Medicine in the Therapeutic Management of Rheumatoid Arthritis

Marialbert Acosta-Herrera *, David González-Serna and Javier Martín

Institute of Parasitology and Biomedicine López-Neyra, CSIC, Av. del Conocimiento 17. Armilla, 18016 Granada, Spain; sna.david@ipb.csic.es (D.G.-S.); javiermartin@ipb.csic.es (J.M.)

* Correspondence: m.acostaherrera@ipb.csic.es; Tel.: +34-958-181-621; Fax: +34-958-181-632

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Abstract: During the last decade, important advances have occurred regarding understanding of the pathogenesis and treatment of rheumatoid arthritis (RA). Nevertheless, response to treatment is not universal, and choosing among different therapies is currently based on a trial and error approach. The specific patient's genetic background influences the response to therapy for many drugs: In this sense, genomic studies on RA have produced promising insights that could help us find an effective therapy for each patient. On the other hand, despite the great knowledge generated regarding the genetics of RA, most of the investigations performed to date have focused on identifying common variants associated with RA, which cannot explain the complete heritability of the disease. In this regard, rare variants could also contribute to this missing heritability as well as act as biomarkers that help in choosing the right therapy. In the present article, different aspects of genetics in the pathogenesis and treatment of RA are reviewed, from large-scale genomic studies to specific rare variant analyses. We also discuss the shared genetic architecture existing among autoimmune diseases and its implications for RA therapy, such as drug repositioning.

Keywords: rheumatoid arthritis; pharmacogenomics; methotrexate; anti-TNF; personalized medicine

1. Introduction

Human diversity includes, among other things, differential responses to environmental stimuli and stress and differential drug metabolism and responses to treatments [1]. This diversity has been in part revealed since the completion of the Human Genome Project, which has improved our knowledge on the underlying biology of disease and its impact on healthcare. Since the completion of this project, a developing field called “personalized medicine” has aimed to adapt medical care to the genetic background of individuals, including diagnosis, therapeutic decision-making, health outcomes, and policy implications of clinical use (National Human Genome Research Institute (NHGRI), <https://www.genome.gov/>).

The most common variations in human DNA are single-nucleotide polymorphisms (SNPs), which are simple substitutions of one nucleotide for another at a given position [2]. These variants can map into gene coding or noncoding sequences of the genome, and the most studied to date have been biallelic SNPs [3]. These genome variations have been the objects of study of personalized medicine, which has revealed how subtle variations on the genome are responsible for large differences in health outcomes [1]. The main goal of these studies has been to detect associations between genetic variants and a trait or disease [4]. Under the common disease/common variant hypothesis, where common diseases are likely influenced by common genetic variants in the population, traditionally these studies have been focused on candidate gene association studies by comparing the allele frequency of the SNPs in affected and unaffected individuals. If the observed differences were not due to random chance, then they were considered to be associated with the disease or trait. Gene selection was based on

the plausible mechanisms involved in disease pathogenesis [1]. At present and thanks to advances in genotyping technology and information on the haplotype structure of the genome, these studies are now directed toward the entire genome in so-called genome-wide association studies (GWAS). These studies are hypothesis-free (where no prior knowledge of the biological pathways is needed) and hypothesis-generating, as the new associations may pinpoint new molecular mechanisms never anticipated before. However, larger sample sizes are needed to fulfill the stringent threshold for statistical significance due to multiple testing adjustments (p -value $< 5 \times 10^{-8}$).

Since the completion of the first GWAS in 2005 [5], human genetic research has been a key player in the discovery of new biological pathways underlying complex diseases. Furthermore, genomic information may allow for the identification of patients with differential abilities to metabolize drugs, assess drug reactions, and eventually develop individualized treatments [6,7], which is the aim of the “treat-to-target” approach, where the therapeutic goal is to reach a state of disease remission or at least lower disease activity [8]. Many challenges continue to exist in the interpretation of GWAS findings. However, it has become fairly clear that drug development with genetic support from GWAS data is twice as likely to reach approval for its use as without this support (from phase I to approval in the different phases of drug development) [9]. In a recent paper by Nelson et al., the authors assessed publicly available GWAS results and combined them with the commercial Informa Pharmaprojects database. They found that those genes associated with a broad spectrum of human diseases were enriched in target genes for drugs approved in the United States or the European Union, highlighting the importance of the provided genetic knowledge in different drug mechanisms. The authors commented that this correlation may be explained by genes with prominent phenotypic changes that might be the most responsive to drug-induced alterations [9].

Interestingly, there have been several cases of genes associated with diseases that were effective drug targets. The most recognized examples have come from cholesterol metabolism: For instance, the gene *HMGCR*, which is associated with serum cholesterol levels [10], is a known target for statins. Additionally, loss of function mutations on the gene *PCSK9* have been described [11], and drugs targeting this gene have been developed to lower cholesterol levels [12,13].

2. Genetics and Therapy Development in Rheumatoid Arthritis

In the largest genetic study of rheumatoid arthritis (RA) conducted to date [14], the authors performed a three-stage transethnic meta-analysis in a total of 100,000 subjects of European and Asian ancestry by evaluating ~10,000,000 SNPs. Stage 1 revealed 57 associated loci, including 17 that had never been associated with the disease before. Afterward, the authors conducted a two-stage replication study for the suggestive loci (p -value $< 5 \times 10^{-6}$), and in a combined analysis of the three stages, they were able to identify 42 novel loci in the transethnic meta-analysis, increasing the total number of RA risk loci to 101. These loci represented a total of 377 associated genes, which were then prioritized. Interestingly, the authors assessed if the associated loci were useful for drug target validation and how approved drugs for other diseases could be linked to RA risk genes, evaluating not only the protein products of the RA-associated genes but also the protein that directly interacts with them in a protein–protein interaction network. From this assessment, the authors found a significant overlap among approved drugs for the treatment of RA that targeted genes that were considered RA risk genes in genetic studies. Additionally, the authors assessed if approved drugs for other diseases might be linked to RA risk genes for drug repurposing and found, for instance, the cases of *CDK4* and *CDK6*, which are targets for different types of cancer treatment [15] and have been shown to weaken disease activity in animal models of RA [15]. All of the above evidence has shown how human genetic data have the potential to be integrated with other biological information to enable personalized treatments.

3. Pharmacogenomic Studies in Rheumatoid Arthritis

Personalized medicine offers fully customized drugs for each person, attending to different conditions (such as genetics), and pharmacogenomics may identify the individual patient’s signature,

which could help guide treatment selection mostly based on an assessment of genomic variants associated with drug response [16]. In the case of RA, even though routine compounds are suitable for most patients, some of them may not have the desired effect when taking these drugs [17], and patients may remain with high disease activity and irreversible joint damage as a possible consequence [18]. Thus, biomarkers are needed that help us differentiate between good and bad responses to a specific treatment. Although great progress has been made in this field, the objective of finding predictive genetic biomarkers that clearly define the grade of response to specific treatments is still far away. The use of genetic variants as biomarkers that could predict the response to a specific treatment has several advantages, as these variants are stable and would remain unaltered, unlike changes in gene expression and epigenetics, which are highly dependent on the environment.

3.1. Genomic Predictors of Methotrexate

Methotrexate (MTX) is a disease-modifying antirheumatic drug (DMARD) used as first-line therapy in RA [19], and great efforts have been directed toward finding predictive biomarkers of MTX response. These investigations have been centered on analyzing genes involved in the key molecular pathways affecting MTX absorption and metabolism, such as cytokine production, drug transport, or nucleotide synthesis. Most of these studies have been carried out in common variants, and the strongest and most replicated association discovered was for the solute carrier family 19 member 1 (*SLC19A1*) gene, a transport carrier that allows MTX to enter cells. In this sense, several studies have reported the association of rs1051266 with intracellular MTX levels. Indeed, a recent meta-analysis that included 12 studies confirmed the association of this polymorphism with MTX treatment response [20]. The methylene tetrahydrofolate reductase (*MTHFR*) gene has also been one of the most studied genes, as the encoded enzyme is key in the MTX pathway (C677T and A1298C are the most commonly studied SNPs associated with MTX response and toxicity [21,22]): However, there were conflicting results in two meta-analyses [23,24]. Another commonly studied polymorphism is the 347 C/G in the 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (*ATIC*) gene, which was recently meta-analyzed in six studies that confirmed its association with responses to and the toxicity of MTX [23]. Although many other SNPs have been associated with MTX response and toxicity in RA patients, most of them could not be replicated, and the studies had a low sample size.

To date, two GWAS have been performed involving MTX activity: Senapati et al. [25] suggested multiple novel risk loci involved in thymidylate synthase (*TYMS*) regulation, and Taylor et al. [26] conducted the largest GWAS on MTX response, including 1424 early RA patients and finding a suggestive association in neuregulin 3 (*NRG3*). However, the authors were not able to replicate their findings. Thus, the existence of predictive models, including several SNPs from different genes associated with MTX response, could help in the creation of specific treatments associated with pharmacogenomics. In this regard, the clinical pharmacogenetics model of response to MTX (CP-MTX) combined clinical data and genotypes from four SNPs in MTX-relevant genes (*MTHF1D* rs2236225, *ATIC* rs2372536, *AMPD1* rs17602729, and *ITPA* rs1127354). This model has been validated in independent samples, and its benefits and costs have been addressed in informing MTX prescription [27–30]. However, it is expected that many more genes and many more variants with modest effects contribute to response to treatment, and therefore the model could be further improved by including other clinical variables and updating the list of associated genetic variants.

The major RA susceptibility region corresponds to the human leukocyte antigen (HLA) locus, concretely the *HLA-DRB1* shared epitope, which is associated with a more severe disease [31]. In this regard, there have been conflicting results regarding the association of the shared epitope with a lower MTX efficacy in monotherapy [32,33]. An additional *HLA-DR* locus, *HLA-DRB4*, is present in nearly all haplotypes containing the strongest associated *HLA-DRB1* alleles [34]. In this sense, a recent study compared MTX responders and nonresponders after stratification for *HLA-DRB4* expression, highlighting that response to MTX is characterized by preponderant innate and adaptive immune activation, respectively [35].

3.2. Genomic Predictors of Tumor Necrosis Factor (TNF) Inhibitors

Biomarkers able to predict responses to biological drugs have received lots of attention. In this line, tumor necrosis factor inhibitors (TNFi) remain the most commonly prescribed first-line biologics, even when these drugs are ineffective in up to 30% of patients [36]. Thus, more than 40 candidate gene studies and 6 GWAS regarding the response to TNFi have been performed to date [37,38]. One of the most commonly studied SNPs is G308A in the tumor necrosis factor (*TNFA*) gene, which has been associated with increased efficacy of adalimumab, infliximab, and etanercept [39,40]. In addition, Krintel et al. [41] suggested an association with *PDE3A-SLCO1C1*, and their results were replicated in a Spanish independent sample, reaching genome-wide significance [42]. Other SNPs linked to clinical responses in anti-TNF therapy are located on protein tyrosine phosphatase receptor type C (*PTPRC*): This has been consistently replicated in independent study samples [43–45]. Other studies have assessed the involvement of the fragment C gamma receptor (*FCGR*) [46], the TNF receptor superfamily 1B (*TNFRSF1B*) [47], and mitogen-activated protein kinase 14 (*MAPK14*) [40] in associations with TNFi response.

There have been many research efforts regarding the HLA region and its implication for TNFi therapies, as it happens with MTX response. One of the first associations observed involved two *HLA-DRB1* alleles encoding the shared epitope, including *0101 and *0404, in response to etanercept [48]. Subsequent studies confirmed the association of this locus with anti-TNF treatments, specifically with amino acid positions 11, 71, and 74 [31]. Furthermore, another study identified polymorphisms within the nonclassical *HLA-E* gene associated with clinical outcomes of anti-TNF therapy in female RA patients [49]. Unfortunately, the majority of studies that have been performed to date regarding pharmacogenetics of anti-TNF therapies have revealed inconsistent results, and very few of them have been robustly replicated [50,51]. This lack of replicability might be due to a lack of consensus on the criteria to differentiate the good versus bad responders [51].

Interestingly, a recent study by Sieberts et al. [52] showed that common SNP information did not improve significantly predictive models in contrast to other clinical information. They performed a community-based open assessment and tested a wide range of state-of-the-art modeling methodologies. However, the authors acknowledged some limitations when the number of risk loci was in the order of hundreds or when heritability was better explained by rare variations or copy number variants, which could be the case for TNFi response.

3.3. Other Genomic Predictors

DMARDs such as MTX and biologic agents are the drugs mainly used to treat RA. Nevertheless, there are other concomitant therapies used to reduce inflammation and relieve pain, including steroids and nonsteroidal anti-inflammatory drugs (NSAIDs). In this regard, two studies observed a better response to the combination therapy of MTX and glucocorticoids in RA patients carrying the mutant allele of the C3435T SNP of the multidrug-resistance 1 (*MDR1*) gene [53,54]. In addition, a subsequent study observed that carrying the SNP G2677A/T of the *MDR1* gene was significantly associated with response to glucocorticoid treatment [55].

On the other hand, like other DMARDs, one-third of patients fail to respond to MTX treatment, either because of inefficiency or adverse events. In those cases, leflunomide represents a potential drug to replace MTX as a treatment [19]. Pharmacogenetic studies have indicated an impact of the CYP1A2*1F mutation of the cytochrome P450 family 1 subfamily A member 2 (*CYP1A2*) gene in leflunomide toxicity [56]. Another study observed that rs3213422 of the dihydroorotate dehydrogenase (*DHODH*) gene was associated with leflunomide toxicity and therapeutic effects [57]. Finally, estrogen receptor gene SNPs could influence the response to leflunomide therapy in females [58].

4. Genetic Studies and Rare Variants

International collaborative efforts have enabled the recruitment of unprecedented sizes of study participants: However, despite the increase in statistical power, today many challenges still exist in the interpretation of findings from GWAS, which cannot account for much of the heritability of diseases (the “missing heritability” paradigm) [59]. The possible contribution of rare variants (minor allele frequency (MAF) < 0.5%) with larger effects on this missing heritability has been much discussed, and this may require composite association tests of overall “mutational load” in cases and controls. These genetic variations are not well captured by genotyping arrays. Therefore, whole-exome (WES) and whole-genome sequencing (WGS) is the technology of choice: It generates millions of sequence reads in parallel, increasing the speed and generated volume of data [59]. This technology will provide a deeper characterization of genetic variants within the entire frequency spectrum and their relationship to disease susceptibility.

The impact of rare human variations on complex diseases is still limited, but for most traits, there is an inverse relationship between allele frequency and effect size, with the rarer alleles being those with a higher odds ratio, resembling those in Mendelian disorders [6]. A limited number of studies have assessed these rare genetic variations with success in RA. Li et al. [60] performed a WES study on 124 subjects from an Asian population. The authors identified genes enriched in deleterious variants using a gene burden test that were involved in innate immunity pathways and contributed to the risk of RA in a Han Chinese population. In the case of Bowes et al. [61], the authors utilized an interesting approach by exploiting low-frequency GWAS data by partitioning the data into gene-centric bins and collapsing their genotypes into a single count. The authors were able to prioritize signals mapping to *TNFAIP3*, a known RA risk gene, with replicable results in independent study samples. This study highlighted a previously known hypothesis where genes harboring common variants also harbored rarer variants with larger effects that had not been captured in previous GWAS [59]. To further confirm this hypothesis, Diogo et al. [62] assessed the contribution of rare and common variants in candidate genes from GWAS on RA by deep-sequencing their protein-coding regions. The authors exome-sequenced 25 RA risk genes from GWAS and found an aggregation of rare variants in *IL2RA* and *IL2RB*. They additionally assessed the aggregate contribution of low-frequency and common coding variants and observed an enrichment of coding variants with a nominal signal of association. The authors finally acknowledged the need for increased sample sizes to comprehensively identify variants distributed across the allele frequency spectrum associated with RA.

Interestingly, Eyre et al. [63] evaluated if previously associated linkage peaks were enriched with rare variants. They found that the distributions of rare variants differed significantly among regions showing linkage evidence, but that this effect depended on associations in the HLA region. Along the same line, Bang et al. [64] performed targeted exome sequencing in Korean RA patients. They comprehensively analyzed 10,588 variants of 398 genes and identified 13 nonsynonymous variants with nominal associations and 17 genes with nominal burden signals. However, the authors did not find a significant enrichment of coding variants associated with RA. As mentioned before, there has been little success in confidently identifying rare variants associated with RA, and in the majority of cases, studies have been performed on biological candidate genes. Only completely resequencing the whole exome or genome will eventually pinpoint novel genes harboring rare variants and significantly contribute to the proper assessment of RA heritability.

Interestingly, rare variants have also been evaluated in responses to treatment, as in the case of TNFi. Cui et al. [65] sequenced the coding region of 750 genes in 1,094 RA patients treated with anti-TNF. The authors applied single-variant association, gene-based associations, and gene set analyses in TNF pathway genes: However, they were not able to identify rare and low-frequency protein-coding variants that significantly contributed to anti-TNF treatment response.

5. Shared Genetics in Autoimmunity and Drug Repurposing

Autoimmune disorders, such as RA, are very heterogeneous and share symptoms, risk genes, comorbidities, and familial aggregation, suggesting a common genetic architecture that is extensively recognized in autoimmunity [66,67]. Several studies have revealed these shared genetics through simple comparisons of the associated genes [68], as was the case in a study conducted by our group, where González-Serna et al. [69] considered the association of a rare variant in the *TNFSF13B* gene with RA and replicated this association in systemic lupus erythematosus (SLE) patients. *TNFSF13B* encodes the (B-cell activating factor) BAFF cytokine, which is essential for B-cell homeostasis and the regulation of B-cell maturation, differentiation, and survival [70]. The assessed risk variant is functional and results in a shorter transcript that escapes microRNA inhibition, leading to an increase in the production of the BAFF cytokine. Additionally, it has been observed that this variant is strongly associated with high levels of total IgG and IgM and with reduced monocyte counts [71]. Our reported association with RA highlights the BAFF variant as a common genetic risk factor in autoimmunity. Interestingly, belimumab is a monoclonal antibody targeting human BAFF and was the first targeted therapy approved for SLE [72–74], highlighting the potential for drug repurposing for genetically related conditions.

Recently, a combination of large-scale studies including different phenotypes has proven to be a very useful tool in the identification of shared genetic risk variants and shared pathways involved in these diseases in a systematic fashion [75–77]. The first example is a meta-GWAS that combined 10 pediatric autoimmune diseases and revealed new shared loci with immunoregulatory functions [78]. Another big study by Ellinghaus et al. [79] combined immunochip data from five chronic inflammatory diseases and also found shared genetic loci in seronegative conditions, such as ankylosing spondylitis, psoriasis, primary sclerosing cholangitis, Crohn's disease, and ulcerative colitis. Furthermore, two recent studies by Marquez et al. [80] and Acosta-Herrera et al. [81] assessed the genetic overlap in autoimmune diseases by combining Immunochip and GWAS data, respectively. The authors identified shared risk variants in autoimmunity and common biological mechanisms and suggested novel genes as drug targets as well as promising candidates for drug repurposing. These studies highlighted how a combination of different related phenotypes can contribute to the determination of causal variants in disease and might help in the establishment of personalized treatments.

6. Future Perspectives

Most RA treatments are nonspecific and are based on a trial-and-error approach. Ineffective treatments affect the quality of life of patients, increasing the probability of adverse events and the eventual development of greater disabilities. It is therefore crucial to develop mechanisms to deliver the right drug to the right patients, which is the ultimate goal of precision medicine. In this sense, a small number of associated loci have been consistently replicated regarding treatment response: One of the reasons might be the lack of statistical power of these assessments. RA is a complex and heterogeneous disease, and therefore the response to treatment in RA patients might be influenced by multiple effects of many genetic variants with moderate effect sizes. Thus, larger studies with proper statistical power and more homogeneous classifications of phenotypes are needed to validate previous findings.

Moreover, response to treatment is normally measured with several composite scores, including the disease activity score (DAS-28), American College of Rheumatology, or EULAR response criteria, hindering the possibility of validating the results. Well-described and homogenous measures of responses are critical in pharmacogenetic studies. Additionally, most studies have investigated common genetic variations in treatment response, and a broader assessment of the frequency spectrum might be necessary.

Establishing the association of genes should continue with discoveries of the functional implications of such associations. As commented before, genetic variants alone do not fully explain the risk of suffering the disease, making complementary strategies necessary, including transcriptomic and

epigenomics approaches: For example, Plant et al. [82] identified differentially methylated positions on DNA as biomarkers of response to TNFi therapy. Additionally, Spiliopoulou et al. [83] showed that the CD39 and CD40 pathways could be relevant to targeted drug therapy by evaluating the relations of TNFi responses with locus-specific scores, constructed from GWAS data.

Finally, the integration of all of this information with clinical and environmental data will eventually help us to identify the biological mechanisms underlying the development of the disease, to establish accurate biomarkers for patient stratification, and to tailor treatment to genetic architecture, increasing the probability of obtaining an adequate response to a particular drug and eventually achieving disease remission.

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References

1. Subramanian, G.; Adams, M.D.; Venter, J.C.; Broder, S. Implications of the human genome for understanding human biology and medicine. *JAMA* **2001**, *286*, 2296–2307. [[CrossRef](#)] [[PubMed](#)]
2. Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; et al. Initial sequencing and analysis of the human genome. *Nature* **2001**, *409*, 860–921. [[PubMed](#)]
3. 1000 Genomes Project Consortium; Abecasis, G.R.; Auton, A.; Brooks, L.D.; DePristo, M.A.; Durbin, R.M.; Handsaker, R.E.; Kang, H.M.; Marth, G.T.; McVean, G.A. An integrated map of genetic variation from 1092 human genomes. *Nature* **2012**, *491*, 56–65. [[PubMed](#)]
4. Timpson, N.J.; Greenwood, C.M.T.; Soranzo, N.; Lawson, D.J.; Richards, J.B. Genetic architecture: the shape of the genetic contribution to human traits and disease. *Nat. Rev. Genet.* **2018**, *19*, 110–124. [[CrossRef](#)] [[PubMed](#)]
5. Klein, R.J.; Zeiss, C.; Chew, E.Y.; Tsai, J.Y.; Sackler, R.S.; Haynes, C.; Henning, A.K.; SanGiovanni, J.P.; Mane, S.M.; Mayne, S.T.; et al. Complement factor H polymorphism in age-related macular degeneration. *Science* **2005**, *308*, 385–389. [[CrossRef](#)] [[PubMed](#)]
6. Bomba, L.; Walter, K.; Soranzo, N. The impact of rare and low-frequency genetic variants in common disease. *Genome Biol.* **2017**, *18*, 77. [[CrossRef](#)] [[PubMed](#)]
7. Lauschke, V.M.; Milani, L.; Ingelman-Sundberg, M. Pharmacogenomic biomarkers for improved drug therapy—recent progress and future developments. *AAPS J.* **2017**, *20*, 4. [[CrossRef](#)] [[PubMed](#)]
8. Burmester, G.R.; Pope, J.E. Novel treatment strategies in rheumatoid arthritis. *Lancet* **2017**, *389*, 2338–2348. [[CrossRef](#)]
9. Nelson, M.R.; Tipney, H.; Painter, J.L.; Shen, J.; Nicoletti, P.; Shen, Y.; Floratos, A.; Sham, P.C.; Li, M.J.; Wang, J.; et al. The support of human genetic evidence for approved drug indications. *Nat. Genet.* **2015**, *47*, 856–860. [[CrossRef](#)]
10. Kathiresan, S. Lp(a) lipoprotein redux—from curious molecule to causal risk factor. *N. Engl. J. Med.* **2009**, *361*, 2573–2574. [[CrossRef](#)]
11. Youngblom, E.; Pariani, M.; Knowles, J.W. Familial Hypercholesterolemia. In *GeneReviews*®; Adam, M.P., Ardinger, H.H., Pagon, R.A., Wallace, S.E., Bean, L.J.H., Stephens, K., Amemiya, A., Eds.; University of Washington: Seattle, WA, USA, 1993.
12. Lopez, D. Inhibition of PCSK9 as a novel strategy for the treatment of hypercholesterolemia. *Drug News Perspect.* **2008**, *21*, 323–330. [[CrossRef](#)] [[PubMed](#)]
13. Steinberg, D.; Witztum, J.L. Inhibition of PCSK9: A powerful weapon for achieving ideal LDL cholesterol levels. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 9546–9547. [[CrossRef](#)] [[PubMed](#)]

14. Okada, Y.; Wu, D.; Trynka, G.; Raj, T.; Terao, C.; Ikari, K.; Kochi, Y.; Ohmura, K.; Suzuki, A.; Yoshida, S.; et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **2014**, *506*, 376–381. [[CrossRef](#)] [[PubMed](#)]
15. Sekine, C.; Sugihara, T.; Miyake, S.; Hirai, H.; Yoshida, M.; Miyasaka, N.; Kohsaka, H. Successful treatment of animal models of rheumatoid arthritis with small-molecule cyclin-dependent kinase inhibitors. *J. Immunol.* **2008**, *180*, 1954–1961. [[CrossRef](#)] [[PubMed](#)]
16. Kurko, J.; Besenyei, T.; Laki, J.; Glant, T.T.; Mikecz, K.; Szekanecz, Z. Genetics of rheumatoid arthritis—A comprehensive review. *Clin. Rev. Allergy Immunol.* **2013**, *45*, 170–179. [[CrossRef](#)] [[PubMed](#)]
17. Cronstein, B.N. Pharmacogenetics in the rheumatic diseases, from prêt-à-porter to haute couture. *Nat. Clin. Pract. Rheumatol.* **2006**, *2*, 2–3. [[CrossRef](#)] [[PubMed](#)]
18. Finckh, A.; Liang, M.H.; van Herckenrode, C.M.; de Pablo, P. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. *Arthritis Rheum.* **2006**, *55*, 864–872. [[CrossRef](#)]
19. Smolen, J.S.; Landewe, R.; Breedveld, F.C.; Buch, M.; Burmester, G.; Dougados, M.; Emery, P.; Gaujoux-Viala, C.; Gossec, L.; Nam, J.; 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. [[CrossRef](#)]
20. Li, X.; Hu, M.; Li, W.; Gu, L.; Chen, M.; Ding, H.; Vanarsa, K.; Du, Y. The association between reduced folate carrier-1 gene 80G/A polymorphism and methotrexate efficacy or methotrexate related-toxicity in rheumatoid arthritis: A meta-analysis. *Int. Immunopharmacol.* **2016**, *38*, 8–15. [[CrossRef](#)]
21. Van Ede, A.E.; Laan, R.F.; Blom, H.J.; Huizinga, T.W.; Haagsma, C.J.; Giesendorf, B.A.; de Boo, T.M.; van de Putte, L. The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. *Arthritis Rheum.* **2001**, *44*, 2525–2530. [[CrossRef](#)]
22. Berkun, Y.; Levartovsky, D.; Rubinow, A.; Orbach, H.; Aamar, S.; Grenader, T.; Abou Atta, I.; Mevorach, D.; Friedman, G.; Ben-Yehuda, A. Methotrexate related adverse effects in patients with rheumatoid arthritis are associated with the A1298C polymorphism of the MTHFR gene. *Ann. Rheum. Dis.* **2004**, *63*, 1227–1231. [[CrossRef](#)] [[PubMed](#)]
23. Lee, Y.H.; Bae, S.C. Association of the ATIC 347 C/G polymorphism with responsiveness to and toxicity of methotrexate in rheumatoid arthritis: a meta-analysis. *Rheumatol. Int.* **2016**, *36*, 1591–1599. [[CrossRef](#)] [[PubMed](#)]
24. Owen, S.A.; Lunt, M.; Bowes, J.; Hider, S.L.; Bruce, I.N.; Thomson, W.; Barton, A. MTHFR gene polymorphisms and outcome of methotrexate treatment in patients with rheumatoid arthritis: analysis of key polymorphisms and meta-analysis of C677T and A1298C polymorphisms. *Pharmacogenom. J.* **2013**, *13*, 137–147. [[CrossRef](#)] [[PubMed](#)]
25. Senapati, S.; Singh, S.; Das, M.; Kumar, A.; Gupta, R.; Kumar, U.; Jain, S.; Juyal, R.C.; Thelma, B.K. Genome-wide analysis of methotrexate pharmacogenomics in rheumatoid arthritis shows multiple novel risk variants and leads for TYMS regulation. *Pharmacogenet. Genomics* **2014**, *24*, 211–219. [[CrossRef](#)] [[PubMed](#)]
26. Taylor, J.C.; Bongartz, T.; Massey, J.; Mifsud, B.; Spiliopoulou, A.; Scott, I.C.; Wang, J.; Morgan, M.; Plant, D.; Colombo, M.; et al. Genome-wide association study of response to methotrexate in early rheumatoid arthritis patients. *Pharmacogenom. J.* **2018**, *18*, 528–538. [[CrossRef](#)]
27. Wessels, J.A.; van der Kooij, S.M.; le Cessie, S.; Kievit, W.; Barrera, P.; Allaart, C.F.; Huizinga, T.W.; Guchelaar, H.J.; Pharmacogenetics Collaborative Research Group. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. *Arthritis Rheum.* **2007**, *56*, 1765–1775. [[CrossRef](#)]
28. Kooloos, W.M.; Wessels, J.A.; van der Kooij, S.M.; Allaart, C.F.; Huizinga, T.W.; Guchelaar, H.J. Optimization of the clinical pharmacogenetic model to predict methotrexate treatment response: the influence of the number of haplotypes of MTHFR 1298A-677C alleles on probability to respond. *Ann. Rheum. Dis.* **2009**, *68*, 1371. [[CrossRef](#)]
29. Franssen, J.; Kooloos, W.M.; Wessels, J.A.; Huizinga, T.W.; Guchelaar, H.J.; van Riel, P.L.; Barrera, P. Clinical pharmacogenetic model to predict response of MTX monotherapy in patients with established rheumatoid arthritis after DMARD failure. *Pharmacogenomics* **2012**, *13*, 1087–1094. [[CrossRef](#)]

30. Lopez-Rodriguez, R.; Ferreiro-Iglesias, A.; Lima, A.; Bernardes, M.; Pawlik, A.; Paradowska-Gorycka, A.; Swierkot, J.; Slezak, R.; Gonzalez-Alvaro, I.; Narvaez, J.; et al. Evaluation of a clinical pharmacogenetics model to predict methotrexate response in patients with rheumatoid arthritis. *Pharmacogenom. J.* **2018**, *18*, 539–545. [[CrossRef](#)]
31. Viatte, S.; Plant, D.; Han, B.; Fu, B.; Yarwood, A.; Thomson, W.; Symmons, D.P.; Worthington, J.; Young, A.; Hyrich, K.L.; et al. Association of HLA-DRB1 haplotypes with rheumatoid arthritis severity, mortality, and treatment response. *JAMA* **2015**, *313*, 1645–1656. [[CrossRef](#)]
32. Saruhan-Direskeneli, G.; Inanc, M.; Fresko, I.; Akkoc, N.; Dalkilic, E.; Erken, E.; Karaaslan, Y.; Kinikli, G.; Oksel, F.; Pay, S.; et al. The role of HLA-DRB1 shared epitope alleles in predicting short-term response to leflunomide in rheumatoid arthritis. *Rheumatology (Oxford)* **2007**, *46*, 1842–1844. [[CrossRef](#)] [[PubMed](#)]
33. Hider, S.L.; Silman, A.J.; Thomson, W.; Lunt, M.; Bunn, D.; Symmons, D.P. Can clinical factors at presentation be used to predict outcome of treatment with methotrexate in patients with early inflammatory polyarthritis? *Ann. Rheum. Dis.* **2009**, *68*, 57–62. [[CrossRef](#)] [[PubMed](#)]
34. Heldt, C.; Listing, J.; Sozeri, O.; Blasing, F.; Frischbutter, S.; Muller, B. Differential expression of HLA class II genes associated with disease susceptibility and progression in rheumatoid arthritis. *Arthritis Rheum.* **2003**, *48*, 2779–2787. [[CrossRef](#)]
35. Stuhlmüller, B.; Mans, K.; Tandon, N.; Bonin, M.O.; Smiljanovic, B.; Sorensen, T.A.; Schendel, P.; Martus, P.; Listing, J.; Detert, J.; et al. Genomic stratification by expression of HLA-DRB4 alleles identifies differential innate and adaptive immune transcriptional patterns - A strategy to detect predictors of methotrexate response in early rheumatoid arthritis. *Clin. Immunol.* **2016**, *171*, 50–61. [[CrossRef](#)] [[PubMed](#)]
36. Hetland, M.L.; Christensen, I.J.; Tarp, U.; Dreyer, L.; Hansen, A.; Hansen, I.T.; Kollerup, G.; Linde, L.; Lindegaard, H.M.; Poulsen, U.E.; et al. Direct comparison of treatment responses, remission rates, and drug adherence in patients with rheumatoid arthritis treated with adalimumab, etanercept, or infliximab: results from eight years of surveillance of clinical practice in the nationwide Danish DANBIO registry. *Arthritis Rheum.* **2010**, *62*, 22–32. [[PubMed](#)]
37. Bek, S.; Bojesen, A.B.; Nielsen, J.V.; Sode, J.; Bank, S.; Vogel, U.; Andersen, V. Systematic review and meta-analysis: pharmacogenetics of anti-TNF treatment response in rheumatoid arthritis. *Pharmacogenom. J.* **2017**, *17*, 403–411. [[CrossRef](#)] [[PubMed](#)]
38. Massey, J.; Plant, D.; Hyrich, K.; Morgan, A.W.; Wilson, A.G.; Spiliopoulou, A.; Colombo, M.; McKeigue, P.; Isaacs, J.; Cordell, H.; et al. Genome-wide association study of response to tumour necrosis factor inhibitor therapy in rheumatoid arthritis. *Pharmacogenom. J.* **2018**, *18*, 657–664. [[CrossRef](#)] [[PubMed](#)]
39. Seitz, M.; Wirthmüller, U.; Moller, B.; Villiger, P.M. The -308 tumour necrosis factor- α gene polymorphism predicts therapeutic response to TNF α -blockers in rheumatoid arthritis and spondyloarthritis patients. *Rheumatology (Oxford)* **2007**, *46*, 93–96. [[CrossRef](#)] [[PubMed](#)]
40. Coulthard, L.R.; Taylor, J.C.; Eyre, S.; Biologics in Rheumatoid Arthritis Genetics and Genomics; Robinson, J.L.; Wilson, A.G.; Isaacs, J.D.; Hyrich, K.; Emery, P.; Barton, A.; et al. Genetic variants within the MAP kinase signalling network and anti-TNF treatment response in rheumatoid arthritis patients. *Ann. Rheum. Dis.* **2011**, *70*, 98–103. [[CrossRef](#)]
41. Krintel, S.B.; Palermo, G.; Johansen, J.S.; Germer, S.; Essioux, L.; Benayed, R.; Badi, L.; Ostergaard, M.; Hetland, M.L. Investigation of single nucleotide polymorphisms and biological pathways associated with response to TNF α inhibitors in patients with rheumatoid arthritis. *Pharmacogenet. Genomics* **2012**, *22*, 577–589. [[CrossRef](#)]
42. Acosta-Colman, I.; Palau, N.; Tornero, J.; Fernandez-Nebro, A.; Blanco, F.; Gonzalez-Alvaro, I.; Canete, J.D.; Maymo, J.; Ballina, J.; Fernandez-Gutierrez, B.; et al. GWAS replication study confirms the association of PDE3A-SLCO1C1 with anti-TNF therapy response in rheumatoid arthritis. *Pharmacogenomics* **2013**, *14*, 727–734. [[CrossRef](#)] [[PubMed](#)]
43. Cui, J.; Saevarsdottir, S.; Thomson, B.; Padyukov, L.; van der Helm-van Mil, A.H.; Nititham, J.; Hughes, L.B.; de Vries, N.; Raychaudhuri, S.; Alfredsson, L.; et al. Rheumatoid arthritis risk allele PTPRC is also associated with response to anti-tumor necrosis factor alpha therapy. *Arthritis Rheum.* **2010**, *62*, 1849–1861. [[PubMed](#)]
44. Plant, D.; Prajapati, R.; Hyrich, K.L.; Morgan, A.W.; Wilson, A.G.; Isaacs, J.D.; Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate; Barton, A. Replication of association of the PTPRC gene with response to anti-tumor necrosis factor therapy in a large UK cohort. *Arthritis Rheum.* **2012**, *64*, 665–670. [[CrossRef](#)] [[PubMed](#)]

45. Ferreiro-Iglesias, A.; Montes, A.; Perez-Pampin, E.; Canete, J.D.; Raya, E.; Magro-Checa, C.; Vasilopoulos, Y.; Sarafidou, T.; Caliz, R.; Ferrer, M.A.; et al. Replication of PTPRC as genetic biomarker of response to TNF inhibitors in patients with rheumatoid arthritis. *Pharmacogenom. J.* **2016**, *16*, 137–140. [[CrossRef](#)] [[PubMed](#)]
46. Iotchkova, V.; Huang, J.; Morris, J.A.; Jain, D.; Barbieri, C.; Walter, K.; Min, J.L.; Chen, L.; Astle, W.; Cocca, M.; et al. Discovery and refinement of genetic loci associated with cardiometabolic risk using dense imputation maps. *Nat. Genet.* **2016**, *48*, 1303–1312. [[CrossRef](#)] [[PubMed](#)]
47. Canet, L.M.; Filipescu, I.; Caliz, R.; Lupianez, C.B.; Canhao, H.; Escudero, A.; Segura-Catena, J.; Soto-Pino, M.J.; Ferrer, M.A.; Garcia, A.; et al. Genetic variants within the TNFRSF1B gene and susceptibility to rheumatoid arthritis and response to anti-TNF drugs: a multicenter study. *Pharmacogenet. Genom.* **2015**, *25*, 323–333. [[CrossRef](#)]
48. Criswell, L.A.; Lum, R.F.; Turner, K.N.; Woehl, B.; Zhu, Y.; Wang, J.; Tiwari, H.K.; Edberg, J.C.; Kimberly, R.P.; Moreland, L.W.; et al. The influence of genetic variation in the HLA-DRB1 and LTA-TNF regions on the response to treatment of early rheumatoid arthritis with methotrexate or etanercept. *Arthritis Rheum.* **2004**, *50*, 2750–2756. [[CrossRef](#)]
49. Iwaszko, M.; Swierkot, J.; Kolossa, K.; Jeka, S.; Wiland, P.; Bogunia-Kubik, K. Polymorphisms within the human leucocyte antigen-E gene and their associations with susceptibility to rheumatoid arthritis as well as clinical outcome of anti-tumour necrosis factor therapy. *Clin. Exp. Immunol.* **2015**, *182*, 270–277. [[CrossRef](#)]
50. Ferreiro-Iglesias, A.; Montes, A.; Perez-Pampin, E.; Canete, J.D.; Raya, E.; Magro-Checa, C.; Vasilopoulos, Y.; Caliz, R.; Ferrer, M.A.; Joven, B.; et al. Evaluation of 12 GWAS-drawn SNPs as biomarkers of rheumatoid arthritis response to TNF inhibitors. A potential SNP association with response to etanercept. *PLoS ONE* **2019**, *14*, e0213073. [[CrossRef](#)]
51. Bluett, J.; Barton, A. Precision Medicine in Rheumatoid Arthritis. *Rheum. Dis. Clin. North. Am.* **2017**, *43*, 377–387. [[CrossRef](#)]
52. Sieberts, S.K.; Zhu, F.; Garcia-Garcia, J.; Stahl, E.; Pratap, A.; Pandey, G.; Pappas, D.; Aguilar, D.; Anton, B.; Bonet, J.; et al. Crowdsourced assessment of common genetic contribution to predicting anti-TNF treatment response in rheumatoid arthritis. *Nat. Commun.* **2016**, *7*, 12460. [[CrossRef](#)] [[PubMed](#)]
53. Pawlik, A.; Wrzesniewska, J.; Fiedorowicz-Fabrycy, I.; Gawronska-Szklarz, B. The MDR1 3435 polymorphism in patients with rheumatoid arthritis. *Int. J. Clin. Pharmacol. Ther.* **2004**, *42*, 496–503. [[CrossRef](#)] [[PubMed](#)]
54. Drozdziak, M.; Rudas, T.; Pawlik, A.; Kurzawski, M.; Czerny, B.; Gornik, W.; Herczynska, M. The effect of 3435C>T MDR1 gene polymorphism on rheumatoid arthritis treatment with disease-modifying antirheumatic drugs. *Eur. J. Clin. Pharmacol.* **2006**, *62*, 933–937. [[CrossRef](#)] [[PubMed](#)]
55. Cuppen, B.V.; Pardali, K.; Kraan, M.C.; Marijnissen, A.C.; Yrliid, L.; Olsson, M.; Bijlsma, J.W.; Lafeber, F.P.; Fritsch-Stork, R.D. Polymorphisms in the multidrug-resistance 1 gene related to glucocorticoid response in rheumatoid arthritis treatment. *Rheumatol. Int.* **2017**, *37*, 531–536. [[CrossRef](#)] [[PubMed](#)]
56. Bohanec Grabar, P.; Rozman, B.; Tomsic, M.; Suput, D.; Logar, D.; Dolzan, V. Genetic polymorphism of CYP1A2 and the toxicity of leflunomide treatment in rheumatoid arthritis patients. *Eur. J. Clin. Pharmacol.* **2008**, *64*, 871–876. [[CrossRef](#)] [[PubMed](#)]
57. Pawlik, A.; Herczynska, M.; Kurzawski, M.; Safranow, K.; Dziedziejko, V.; Drozdziak, M. The effect of exon (19C>A) dihydroorotate dehydrogenase gene polymorphism on rheumatoid arthritis treatment with leflunomide. *Pharmacogenomics* **2009**, *10*, 303–309. [[CrossRef](#)]
58. Dziedziejko, V.; Kurzawski, M.; Safranow, K.; Chlubek, D.; Pawlik, A. The effect of ESR1 and ESR2 gene polymorphisms on the outcome of rheumatoid arthritis treatment with leflunomide. *Pharmacogenomics* **2011**, *12*, 41–47. [[CrossRef](#)] [[PubMed](#)]
59. Manolio, T.A.; Collins, F.S.; Cox, N.J.; Goldstein, D.B.; Hindorf, L.A.; Hunter, D.J.; McCarthy, M.I.; Ramos, E.M.; Cardon, L.R.; Chakravarti, A.; et al. Finding the missing heritability of complex diseases. *Nature* **2009**, *461*, 747–753. [[CrossRef](#)]
60. Araki, Y.; Mimura, T. The Histone Modification Code in the Pathogenesis of Autoimmune Diseases. *Mediators Inflamm.* **2017**, *2017*, 2608605. [[CrossRef](#)]
61. Stahl, E.A.; Raychaudhuri, S.; Remmers, E.F.; Xie, G.; Eyre, S.; Thomson, B.P.; Li, Y.; Kurreeman, F.A.; Zhenakova, A.; Hinks, A.; et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat. Genet.* **2010**, *42*, 508–514. [[CrossRef](#)]

62. Diogo, D.; Kurreeman, F.; Stahl, E.A.; Liao, K.P.; Gupta, N.; Greenberg, J.D.; Rivas, M.A.; Hickey, B.; Flannick, J.; Thomson, B.; et al. Rare, low-frequency, and common variants in the protein-coding sequence of biological candidate genes from GWASs contribute to risk of rheumatoid arthritis. *Am. J. Hum. Genet.* **2013**, *92*, 15–27. [[CrossRef](#)] [[PubMed](#)]
63. Eyre, S.; Ke, X.; Lawrence, R.; Bowes, J.; Panoutsopoulou, K.; Barton, A.; Thomson, W.; Worthington, J.; Zeggini, E. Examining the overlap between genome-wide rare variant association signals and linkage peaks in rheumatoid arthritis. *Arthritis Rheum.* **2011**, *63*, 1522–1526. [[CrossRef](#)] [[PubMed](#)]
64. Bang, S.Y.; Na, Y.J.; Kim, K.; Joo, Y.B.; Park, Y.; Lee, J.; Lee, S.Y.; Ansari, A.A.; Jung, J.; Rhee, H.; et al. Targeted exon sequencing fails to identify rare coding variants with large effect in rheumatoid arthritis. *Arthritis Res. Ther.* **2014**, *16*, 447. [[CrossRef](#)] [[PubMed](#)]
65. Cui, J.; Diogo, D.; Stahl, E.A.; Canhao, H.; Mariette, X.; Greenberg, J.D.; Okada, Y.; Pappas, D.A. The role of rare protein-coding variants in anti-tumor necrosis factor treatment response in rheumatoid arthritis. *Arthritis Rheum.* **2017**, *69*, 735–741. [[CrossRef](#)] [[PubMed](#)]
66. Zhernakova, A.; van Diemen, C.C.; Wijmenga, C. Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat. Rev. Genet.* **2009**, *10*, 43–55. [[CrossRef](#)] [[PubMed](#)]
67. Zhernakova, A.; Withoff, S.; Wijmenga, C. Clinical implications of shared genetics and pathogenesis in autoimmune diseases. *Nat. Rev. Endocrinol.* **2013**, *9*, 646–659. [[CrossRef](#)] [[PubMed](#)]
68. Wang, N.; Shen, N.; Vyse, T.J.; Anand, V.; Gunnarson, I.; Sturfelt, G.; Rantapaa-Dahlqvist, S.; Elvin, K.; Truedsson, L.; Andersson, B.A.; et al. Selective IgA deficiency in autoimmune diseases. *Mol. Med.* **2011**, *17*, 1383–1396. [[CrossRef](#)]
69. Gonzalez-Serna, D.; Ortiz-Fernandez, L.; Vargas, S.; Garcia, A.; Raya, E.; Fernandez-Gutierrez, B.; Lopez-Longo, F.J.; Balsa, A.; Gonzalez-Alvaro, I.; Narvaez, J.; et al. Association of a rare variant of the TNFSF13B gene with susceptibility to rheumatoid arthritis and systemic lupus erythematosus. *Sci. Rep.* **2018**, *8*, 8195. [[CrossRef](#)]
70. Woodland, R.T.; Schmidt, M.R.; Thompson, C.B. BlyS and B cell homeostasis. *Semin. Immunol.* **2006**, *18*, 318–326. [[CrossRef](#)]
71. Steri, M.; Orru, V.; Idda, M.L.; Pitzalis, M.; Pala, M.; Zara, I.; Sidore, C.; Faa, V.; Floris, M.; Deiana, M.; et al. Overexpression of the Cytokine BAFF and Autoimmunity Risk. *N. Engl. J. Med.* **2017**, *376*, 1615–1626. [[CrossRef](#)]
72. Vincent, F.B.; Morand, E.F.; Schneider, P.; Mackay, F. The BAFF/APRIL system in SLE pathogenesis. *Nat. Rev. Rheumatol.* **2014**, *10*, 365–373. [[CrossRef](#)] [[PubMed](#)]
73. Furie, R.; Petri, M.; Zamani, O.; Cervera, R.; Wallace, D.J.; Tegzova, D.; Sanchez-Guerrero, J.; Schwarting, A.; Merrill, J.T.; Chatham, W.W.; et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum.* **2011**, *63*, 3918–3930. [[CrossRef](#)] [[PubMed](#)]
74. Navarra, S.V.; Guzman, R.M.; Gallacher, A.E.; Hall, S.; Levy, R.A.; Jimenez, R.E.; Li, E.K.; Thomas, M.; Kim, H.Y.; Leon, M.G.; et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: A randomised, placebo-controlled, phase 3 trial. *Lancet* **2011**, *377*, 721–731. [[CrossRef](#)]
75. Lopez-Isac, E.; Martin, J.E.; Assassi, S.; Simeon, C.P.; Carreira, P.; Ortego-Centeno, N.; Freire, M.; Beltran, E.; Narvaez, J.; Alegre-Sancho, J.J.; et al. Cross-disease meta-analysis of genome-wide association studies for systemic sclerosis and rheumatoid arthritis reveals irf4 as a new common susceptibility locus. *Arthritis Rheumatol.* **2016**, *68*, 2338–2344. [[CrossRef](#)] [[PubMed](#)]
76. Marquez, A.; Vidal-Bralo, L.; Rodriguez-Rodriguez, L.; Gonzalez-Gay, M.A.; Balsa, A.; Gonzalez-Alvaro, I.; Carreira, P.; Ortego-Centeno, N.; Ayala-Gutierrez, M.M.; Garcia-Hernandez, F.J.; et al. A combined large-scale meta-analysis identifies COG6 as a novel shared risk locus for rheumatoid arthritis and systemic lupus erythematosus. *Ann. Rheum. Dis.* **2017**, *76*, 286–294. [[CrossRef](#)] [[PubMed](#)]
77. Coenen, M.J.; Trynka, G.; Heskamp, S.; Franke, B.; van Diemen, C.C.; Smolonska, J.; van Leeuwen, M.; Brouwer, E.; Boezen, M.H.; Postma, D.S.; et al. Common and different genetic background for rheumatoid arthritis and coeliac disease. *Hum. Mol. Genet.* **2009**, *18*, 4195–4203. [[CrossRef](#)] [[PubMed](#)]
78. Li, Y.R.; Li, J.; Zhao, S.D.; Bradford, J.P.; Mentch, F.D.; Maggadottir, S.M.; Hou, C.; Abrams, D.J.; Chang, D.; Gao, F.; et al. Meta-analysis of shared genetic architecture across ten pediatric autoimmune diseases. *Nat. Med.* **2015**, *21*, 1018–1027. [[CrossRef](#)] [[PubMed](#)]

79. Ellinghaus, D.; Jostins, L.; Spain, S.L.; Cortes, A.; Bethune, J.; Han, B.; Park, Y.R.; Raychaudhuri, S.; Pouget, J.G.; Hubenthal, M.; et al. Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nat. Genet.* **2016**, *48*, 510–518. [[CrossRef](#)]
80. Marquez, A.; Kerick, M.; Zhernakova, A.; Gutierrez-Achury, J.; Chen, W.M.; Onengut-Gumuscu, S.; Gonzalez-Alvaro, I.; Rodriguez-Rodriguez, L.; Rios-Fernandez, R.; Gonzalez-Gay, M.A.; et al. Meta-analysis of ImmunoChip data of four autoimmune diseases reveals novel single-disease and cross-phenotype associations. *Genome Med.* **2018**, *10*, 97. [[CrossRef](#)]
81. Acosta-Herrera, M.; Kerick, M.; Gonzalez-Serna, D.; Myositis Genetics, C.; Scleroderma Genetics, C.; Wijmenga, C.; Franke, A.; Gregersen, P.K.; Padyukov, L.; Worthington, J.; et al. Genome-wide meta-analysis reveals shared new loci in systemic seropositive rheumatic diseases. *Ann. Rheum. Dis.* **2019**, *78*, 311–319. [[CrossRef](#)]
82. Plant, D.; Webster, A.; Nair, N.; Oliver, J.; Smith, S.L.; Eyre, S.; Hyrich, K.L.; Wilson, A.G.; Morgan, A.W.; Isaacs, J.D.; et al. Differential methylation as a biomarker of response to etanercept in patients with rheumatoid arthritis. *Arthritis Rheumatol.* **2016**, *68*, 1353–1360. [[CrossRef](#)] [[PubMed](#)]
83. Spiliopoulou, A.; Colombo, M.; Plant, D.; Nair, N.; Cui, J.; Coenen, M.J.; Ikari, K.; Yamanaka, H.; Saevarsdottir, S.; Padyukov, L.; et al. Association of response to TNF inhibitors in rheumatoid arthritis with quantitative trait loci for CD40 and CD39. *Ann. Rheum. Dis.* **2019**. (E-pub ahead of print). [[CrossRef](#)] [[PubMed](#)]



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Review

Golimumab for Rheumatoid Arthritis

Eleftherios Pelechas, Paraskevi V. Voulgari and Alexandros A. Drosos *

Rheumatology Clinic, Department of Internal Medicine, Medical School, University of Ioannina, 45110 Ioannina, Greece; pelechas@doctors.org.uk (E.P.); pvoulgar@cc.uoi.gr (P.V.V.)

* Correspondence: adrosos@cc.uoi.gr; Tel.: +30-2651007503; Fax: +30-2651007054

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Abstract: Since the advent of infliximab for the treatment of rheumatoid arthritis (RA), new genetically-engineered molecules have appeared. This review aims to present the current data and body of evidence for golimumab (GLM). Safety, efficacy, tolerability and immunogenicity are all being investigated, not only through phase III trials (GO-BEFORE, GO-FORWARD, GO-AFTER, GO-MORE, GO-FURTHER, GO-NICE), but also through studies of real-world data. It seems that GLM in the subcutaneous form is an efficacious molecule with a good safety profile at the standard dosage scheme, but a 100 mg subcutaneous dose is associated with a higher risk of opportunistic infections, lymphoma and demyelination. Furthermore, when compared to other tumor necrosis factor- α molecules, it is non-inferior, and, at some points, such as when it comes to immunogenicity and persistence of the drug, it has a better profile. In summary, GLM is an effective, well-tolerated option for the treatment of RA, for both the clinician and patients who are seeking a convenient dosage scheme.

Keywords: rheumatoid arthritis; TNF α ; golimumab; efficacy; tolerability; immunogenicity

1. Introduction

Nowadays, rheumatology has been transformed into one of the most impactful specialties in the field of medicine, mainly due to a better understanding of the way our immune system responds to different internal and external stimuli [1]. The idea of neutralizing tumor necrosis factor (TNF) α via a specific antibody emerged in the mid-1980s. The hypothesis was that reducing TNF α levels would restore the balance in the cytokine system. Thus infliximab (INF), a chimeric human-murine monoclonal antibody that binds with high affinity to both soluble and transmembrane forms of TNF α , but not to lymphotoxin α (TNF β), was developed with the employment of genetic engineering techniques. Since the advent of INF, four more genetically engineered molecules have been marketed: etanercept (ETN), adalimumab (ADA), certolizumab (CTZ) and golimumab (GLM), each employing a slightly different compositional and pharmacodynamic approach. In addition, anti-TNF α biosimilars have come of age and are already on the market [2,3]. Nevertheless, even with the appearance of different molecules targeting rheumatoid arthritis (RA), the unmet needs for the treatment of the disease remain high [4].

2. Golimumab

GLM is a human IgG1 κ monoclonal antibody produced by a murine hybridoma cell line with recombinant DNA technology [5], which has been shown to improve the signs and symptoms of RA in adults in large, randomized, placebo-controlled phase III trials [6–10]. It is the latest anti-TNF α approved by the Food and Drug Administration (FDA), in 2009, under the brand name Simponi. In Europe, a once-monthly 50-mg subcutaneous (s.c.) formulation of the TNF α GLM is approved as monotherapy and/or in combination with methotrexate (MTX). Other approved indications of GLM are for the treatment of psoriatic arthritis (PsA) and axial spondyloarthritis (AxSpA)—comprising

ankylosing spondylitis (AS) and non-radiographic axial spondyloarthritis (nr-AxSpA) in adults, and polyarticular juvenile idiopathic arthritis (pJIA) in children (50 mg/month if body weight > 40 kg). In patients with body weight greater than 100 kg and for all the above indications who do not achieve an adequate clinical response after 3–4 doses, increasing the dose to 100 mg once a month may be considered, taking into account the increased risk of certain serious adverse drug reactions. Finally, GLM has been also approved for ulcerative colitis. The initial dose should be 200 mg, followed by 100 mg at week two. Patients who have an adequate response should receive 50 mg at week six and every four weeks thereafter, whereas for those with an inadequate response or with body weight greater than 80 kg, 100 mg at week six and every four weeks thereafter.

3. Pharmacological Properties of GLM

GLM acts principally by targeting and neutralizing TNF α with the ultimate goal to prevent inflammation as well as cartilage degradation and bone destruction [11]. In pivotal phase III trials (but also in different sub-studies in patients with RA and other inflammatory arthritides), when administered alone or in combination with MTX, it showed that there is a significant reduction in serum acute phase reactants and other inflammatory biomarkers [12–15].

GLM exhibits dose-proportional pharmacokinetics and this is why patients with different body weights should receive different dosage schemes. The median time to maximum plasma concentration is 2–7 days following a single s.c. injection. Steady-state plasma concentrations can be achieved at 12 weeks of repeated injections and the mean absolute bioavailability is approximately 50% [16]. The mean elimination half-life is estimated to be approximately 12 days. In patients receiving MTX with GLM, the mean steady-state trough concentrations were 30% higher than those receiving GLM alone. The concomitant use of MTX reduces the apparent clearance of GLM by approximately 35% [17].

4. Clinical Efficacy

The clinical efficacy of GLM in inflammatory arthritides has been shown in a series of phase III trials but also in several sub-studies (Table 1) [8–10,18–23]. More specifically, in RA there is sufficient data supporting the therapeutic efficacy of the drug.

In the GO-BEFORE study (NCT00264537), a total of 637 MTX-naïve patients with active RA were randomized (1:1:1:1) to placebo + MTX (group 1), GLM 100 mg + placebo (group 2), GLM 50 mg + MTX (group 3), or GLM 100 mg + MTX (group 4). This study did not detect significant differences in ACR50 response (primary endpoint) between the combination therapy groups (3 and 4) of GLM (50 mg/100 mg) every four weeks plus MTX and MTX as monotherapy. A difference would have been seen if the ACR 20 response had been considered. Thus, the modified intend-to-treat (ITT) analysis of the primary endpoint and other prespecified efficacy measures demonstrated that the efficacy of GLM + MTX is better than, and the efficacy of GLM alone is similar to, the efficacy of MTX alone in reducing RA symptoms in MTX naïve patients, with no unexpected safety concerns [8,18].

In the GO-FORWARD study (NCT00264550), a total of 444 patients with active RA despite MTX therapy were randomly assigned (3:3:2:2) to placebo injections + MTX capsules (group 1), GLM 100 mg injections + placebo capsules (group 2) GLM 50 mg injections + MTX capsules (group 3) and GLM 100 mg injections + MTX capsules (group 4). The co-primary endpoints were the proportion of patients with >ACR20% improvement at week 14 and change from baseline in the health assessment questionnaire-disability index (HAQ-DI) score at week 24. In the aforementioned groups ACR20 response at week 14 was achieved by 33.1%/44.4%/55.1%/56.2%, respectively, whereas at week 24, median improvements from baseline in HAQ-DI score (0.13) were: 0.13 ($p = 0.240$); 0.38 ($p < 0.001$); 0.50 ($p < 0.001$), respectively [9,19]. The conclusion of this study was that the addition of GLM to MTX in patients with active RA despite MTX therapy, significantly reduced the signs and symptoms of RA and improvement of physical function.

The GO-AFTER study (NCT00299546) evaluated the efficacy and safety of GLM in subjects who have active RA and have been treated previously with >1 dose of a biologic anti-TNF α agent (ETN,

ADA, INF). A total of 461 patients from 10 countries were randomly allocated to receive s.c. injections of placebo (group 1), GLM 50 mg s.c. (group 2) or GLM 100 mg s.c. (group 3) every four weeks. MTX, sulfasalazine (SSZ), hydroxychloroquine (HCQ), oral corticosteroids (CS) and non-steroidal anti-inflammatory drugs (NSAIDs) were carried on at stable doses. As primary endpoint, an ACR20 improvement at week 14 should be achieved by patients who discontinued previous anti-TNF α treatment due to lack of effectiveness or reasons unrelated to effectiveness, such as intolerance and accessibility issues. In groups 1–3, 18%/35%/38% respectively achieved ACR 20 at week 14. The conclusion of this study was that GLM reduces the signs and symptoms of RA in patients with active disease who had previously received >1 anti-TNF α [10,20].

Table 1. Summary of GLM trials.

| Trial (Clinical Trial Identifier Number) | Official Title | Study Type (Phase) | Indication | Number of Participants |
|--|--|----------------------------|-------------|------------------------|
| GO-BEFORE (NCT00264537) | A multicentre, randomized, double-blind, placebo-controlled trial of golimumab, a fully human anti-TNF α monoclonal antibody, administered subcutaneously, in methotrexate-naïve subjects with active rheumatoid arthritis | Clinical Trial (Phase III) | RA | 637 |
| GO-FORWARD (NCT00264550) | A multicentre, randomized, double-blind, placebo-controlled trial of golimumab, a fully human anti-TNF α monoclonal antibody, administered subcutaneously, in subjects with active Rheumatoid arthritis despite methotrexate therapy | Clinical trial (Phase III) | RA | 444 |
| GO-AFTER (NCT00299546) | A multicentre, randomized, double-blind, placebo-controlled trial of golimumab, a fully human anti-TNF α monoclonal antibody, administered subcutaneously in subjects with active rheumatoid arthritis and previously treated with biologic anti-TNF α Agent(s) | Clinical trial (Phase III) | RA | 461 |
| GO-MORE (NCT00975130) | An open-label study assessing the addition of subcutaneous golimumab (GLM) to conventional disease-modifying antirheumatic drug (DMARD) therapy in biologic-naïve subjects with rheumatoid arthritis (Part 1), followed by a randomized study assessing the value of combined intravenous and subcutaneous GLM administration aimed at inducing and maintaining remission (Part 2) | Clinical trial (Phase III) | RA | 3366 |
| GO-FURTHER (NCT00973479) | A multicentre, randomized, double-blind, placebo-controlled trial of golimumab, an anti-TNF α monoclonal antibody, administered intravenously, in patients with active rheumatoid arthritis despite methotrexate therapy | Clinical trial (Phase III) | RA | 592 |
| GO-NICE (NCT01313858) | Non-interventional study investigating the use of golimumab in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis | Observational | RA, PsA, AS | 1613 |

GLM: golimumab; TNF: tumor necrosis factor; RA: rheumatoid arthritis; PsA: psoriatic arthritis; AS: ankylosing spondylitis; nr-AxSpA: non-radiographic axial spondyloarthritis.

In the *GO-MORE* study (NCT00975130) a total number of 3366 patients were enrolled in order to evaluate the efficacy and safety of s.c. GLM as add-on therapy in patients with active RA in typical clinical practice settings (use of csDMARDs and Cs). A four-weeks add-on of 50 mg s.c. GLM for a period of six months were given in part one of the study whereas in part two, patients not on remission were randomly assigned to receive intravenous (i.v.) + s.c. (group 1) or s.c. GLM to month 12. Neither in part one nor part two of the study a statistically significant difference was observed apart from the efficacy and safety of GLM as an add-on therapy for csDMARD-refractory RA in a

typical clinical practice population. This study concluded that there is no additional efficacy of the i.v. + s.c. scheme of GLM over the s.c. regimen [21].

The *GO-FURTHER* study (NCT00973479) evaluated not only the safety and efficacy but also the radiographic progression through two years of treatment with i.v. GLM + MTX in an open-label extension of a phase III trial of patients with active RA despite MTX therapy. A total number of 592 patients with active RA were randomized (2:1) to i.v. GLM 2 mg/kg + MTX (group one), or placebo + MTX (group 2) at weeks 0 and 4, and every eight weeks thereafter. ACR 20/50/70 response criteria were measured as well as the 28-joint count disease activity score using the C-reactive protein (DAS-28-CRP), physical function and quality of life, and changes in the modified Sharp/van der Heijde scores (SHS). The ACR responses at week 100 were 68.1%/43.8%/23.5% respectively. Physical function, quality of life and clinical response were maintained throughout the study period (two years). The SHS score was 0.74 in group 1 and 2.10 in group 2 ($p = 0.005$). As far as it concerns the AE, 79.1% had at least one and 18.2% had a serious AE. This study demonstrated that in patients with active RA, despite MTX, i.v. GLM + MTX showed significant inhibition of structural damage at weeks 24 and 52 and substantial clinical improvement with no safety signs up to one year [22].

The *GO-NICE* study (NCT01313858), aimed to document patient and treatment characteristics as well as clinical effectiveness and safety in adult patients newly treated with the 50 mg s.c. GLM every four weeks under real-life conditions. Of the 1613 patients, 1458 were eligible for final analysis and of those 474 patients were suffering from RA. The mean age of those patients was 54.9 ± 13.4 years, 72.8% were females and 64.7% biologic-naïve. The DAS-28-erythrocyte sedimentation rate (ESR) decreased from 5.0 to 2.9 after 24 months ($p < 0.0001$). As reported, most AE were of mild or moderate nature, and no new safety signals were detected [23].

Finally, there are several other studies regarding the persistence of GLM treatment in patients with RA. Thomas et al. [24] in a retrospective, observational study of all patients treated with GLM in four Academic centers in Greece during a four-year period examined the long-term survival on drug (SOD) of patients not only with RA (166 patients) but also PsA (82 patients) and AS (80 patients). The estimated SOD at two and three years was 68% and 62% respectively (69% and 60% for RA patients) concluding that GLM showed a high three-year SOD with a low rate of discontinuation due to AEs. Furthermore, Rotar et al. [25] analyzed prospectively the collected data of all patients treated with GLM and other TNFs for seven years and were suffering from RA, AS, and PsA. The authors concluded that the persistence of GLM in RA-treated patients is lower compared with the AS and PsA patients but it is higher among those patients treated with other anti-TNFs. Svedbom et al. [26] in a systematic review of real-world evidence in immune-mediated rheumatic diseases including RA, examined the persistence to treatment with s.c. GLM but also to other anti-TNF α molecules. Of 376 available references identified, 12 studies with a total of 4910 patients met the inclusion criteria. In four studies that included comparisons to other biologics, GLM was either statistically noninferior or statistically superior to other treatments. Serrano et al. [27] in a prospective monocentric cohort of RA patients treated with GLM and a total number of 61 patients (mean age 55.1 ± 14.1 years; 85.2% females; RF + 70%; anti-CCP + 78%) showed that GLM survival time was better when used as first or second biological and with concomitant use of csDMARDs. Aaltonen et al., based on Kaplan-Meier survival analysis in a systematic review regarding the anti-TNF α , showed that the probability of discontinuing the treatment within 6, 12, 24, and 36 months was 16%, 27%, 37%, and 43%, respectively in patients with RA. SOD was better among the patients with no prior bDMARD therapy than among those using anti-TNF α as their second or third bDMARD. CTZ (41%) and INF (38%) were associated with higher probability of treatment discontinuation within 12 months compared to ADA (25%), ETN (25%), and GLM (25%) [28].

5. Tolerability and Immunogenicity

Data from the pivotal phase III trials in adults with RA but also the open-label extension studies, support that s.c. GLM is generally a well-tolerated therapeutic option [8–10]. Overall, in these

trials, upper-respiratory infections (32.0% vs. 8.8% with placebo), nasopharyngitis (17.4% vs. 6.4%), followed by elevated aminotransferase levels (11.9% vs. 5.2%) and hypertension (9.8% vs. 2.7%) were the most common AE in the 50 mg s.c. dose. Injection-site reactions (ISRs) were reported by 11.0% vs. 2.8% of GLM and placebo recipients, the most common being injection-site erythema (5.8% vs. 1.1%) [29]. Tuberculosis, opportunistic infections, lymphoma, and demyelination incidence appeared to be higher among patients receiving GLM 100 mg s.c. dose.

As far as it concerns the immunogenicity, Thomas et al. documented in a systematic review for the immunogenicity of TNF inhibitors that GLM and ETN were the least immunogenic (3.8% and 1.2% respectively) whereas the most immunogenic were INF (25.3%), followed by ADA (14.1%) and CTZ (6.9%). The clinical significance of the anti-drug antibodies (ADAbs) in the sera of patients with RA is associated with decreased clinical response [30].

6. Conclusions

GLM is one of five anti-TNF α inhibitors approved for the treatment of RA, but also other inflammatory arthritides [31]. It is a newer, second-generation anti-TNF α and for this reason the clinical experience is less in comparison with the older ones such as INF, ETA and ADA (first generation TNF α inhibitors). On the other hand, the growing body of evidence through the open-label extension trials of pivotal studies and those from several medical centers in patients with RA, confirm the efficacy and safety of the drug. Furthermore, with clinical and radiological benefits being sustained and no new safety signals being identified, GLM seems an attractive choice for the treatment of RA. Other, important elements that make this choice attractive, are the low levels of immunogenicity, the low rate of drug discontinuation in comparison with the other anti-TNFs and the dosage scheme (every four weeks) which seems to be a point of major significance when a physician-patient sharing decision occurs. One concern is the tendency of higher incidence of opportunistic infections, lymphoma and demyelination in the 100 mg s.c. injection, and it should be used with caution in patients with higher body weight or poor response to treatment with the 50 mg dosage scheme.

As there are no head-to-head trials comparing it with the other anti-TNF α inhibitors, the indirect comparison of all five agents suggests that possibly GLM is better tolerated than ADA, CTZ and INF in terms of the risks of serious infection and of discontinuing treatment due to AEs. In summary, GLM is an effective, well-tolerated option for the treatment of RA for both the clinician but also for the patients seeking a convenient dosage scheme.

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References

1. McInnes, I.B.; Schett, G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet* **2017**, *389*, 2328–2337. [CrossRef]
2. Pelechas, E.; Voulgari, P.V.; Drosos, A.A. ABP501 for the treatment of rheumatoid arthritis. *Expert Opin. Biol. Ther.* **2018**, *18*, 317–322. [CrossRef] [PubMed]
3. Pelechas, E.; Drosos, A.A. Etanercept biosimilars SB-4. *Expert Opin. Biol. Ther.* **2019**, *19*, 173–179. [CrossRef]
4. Kaltounoudis, E.; Pelechas, E.; Voulgari, P.V.; Drosos, A.A. Unmet needs in the treatment of rheumatoid arthritis. An observational study and a real-life experience from a single university center. *Semin. Arthritis Rheum.* **2019**, *48*, 597–602. [CrossRef] [PubMed]
5. Summary of Product Characteristics. Available online: https://www.ema.europa.eu/en/documents/product-information/simponi-epar-product-information_en.pdf (accessed on 19 March 2019).
6. Singh, J.A.; Noorbaloochi, S.; Singh, G. Golimumab for rheumatoid arthritis: A systematic review. *J. Rheumatol.* **2010**, *37*, 1096–1104. [CrossRef]
7. Papagoras, C.; Voulgari, P.V.; Drosos, A.A. Golimumab, the newest TNF- α blocker, comes of age. *Clin. Exp. Rheumatol.* **2015**, *33*, 570–577. [PubMed]

8. Emery, P.; Fleischmann, R.M.; Moreland, L.W.; Hsia, E.C.; Strusberg, I.; Durez, P.; Nash, P.; Amante, E.J.; Churchill, M.; Park, W.; et al. Golimumab, a human anti-tumor necrosis factor α monoclonal antibody, injected subcutaneously every four weeks in methotrexate-naïve patients with active rheumatoid arthritis: Twenty-four-week results of a phase III, multicenter, randomized, double-blind, placebo-controlled study of Golimumab before methotrexate as first-line therapy for early-onset rheumatoid arthritis. *Arthritis Rheum.* **2009**, *60*, 2272–2283. [[PubMed](#)]
9. Keystone, E.C.; Genovese, M.C.; Klareskog, L.; Hsia, E.C.; Hall, S.T.; Miranda, P.C.; Pazdur, J.; Bae, S.C.; Palmer, W.; Zrubek, J.; et al. Golimumab, a human antibody to tumour necrosis factor α given by monthly subcutaneous injections, in active rheumatoid arthritis despite methotrexate therapy: The GO-FORWARD Study. *Ann. Rheum. Dis.* **2009**, *68*, 789–796. [[CrossRef](#)]
10. Smolen, J.S.; Kay, J.; Doyle, M.K.; Landewé, R.; Matteson, E.L.; Wollenhaupt, J.; Gaylis, N.; Murphy, F.T.; Neal, J.S.; Zhou, Y.; et al. Golimumab in patients with active rheumatoid arthritis after treatment with tumour necrosis factor α inhibitors (GO-AFTER study): A multicenter, randomised, double-blind, placebo-controlled, phase III trial. *Lancet* **2009**, *374*, 210–221. [[CrossRef](#)]
11. Oldfield, V.; Plosker, G.L. Golimumab: In the treatment of rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *BioDrugs* **2009**, *23*, 125–135. [[CrossRef](#)] [[PubMed](#)]
12. Visvanathan, S.; Rahman, M.U.; Keystone, E.; Genovese, M.; Klareskog, L.; Hsia, E.; Mack, M.; Buchanan, J.; Elashoff, M.; Wagner, C. Association of serum markers with improvement in clinical response measures after treatment with Golimumab in patients with active rheumatoid arthritis despite receiving methotrexate: Results from the GO-FORWARD study. *Arthritis Res. Ther.* **2010**, *12*, R211. [[CrossRef](#)] [[PubMed](#)]
13. Inman, R.D.; Baraliakos, X.; Hermann, K.A.; Braun, J.; Deodhar, A.; van der Heijde, D.; Xu, S.; Hsu, B. Serum biomarkers and changes in clinical/MRI evidence of Golimumab-treated patients with ankylosing spondylitis: Results of the randomized, placebo-controlled GO-RAISE study. *Arthritis Res. Ther.* **2016**, *18*, 304. [[CrossRef](#)]
14. Wagner, C.L.; Visvanathan, S.; Elashoff, M.; McInnes, I.B.; Mease, P.J.; Krueger, G.G.; Murphy, F.T.; Papp, K.; Gomez-Reino, J.J.; Mack, M.; et al. Markers of inflammation and bone remodeling associated with improvement in clinical response measures in psoriatic arthritis patients treated with Golimumab. *Ann. Rheum. Dis.* **2013**, *72*, 83–88. [[CrossRef](#)] [[PubMed](#)]
15. Wagner, C.L.; Visvanathan, S.; Braun, J.; van der Heijde, D.; Deodhar, A.; Hsu, B.; Mack, M.; Elashoff, M.; Inman, R.D. Serum markers associated with clinical improvement in patients with ankylosing spondylitis treated with Golimumab. *Ann. Rheum. Dis.* **2012**, *71*, 674–680. [[CrossRef](#)]
16. Zhou, H.; Jang, H.; Fleischmann, R.M.; Bouman-Thio, E.; Xu, Z.; Marini, J.C.; Pendley, C.; Jiao, Q.; Shankar, G.; Marciniak, S.J.; et al. Pharmacokinetics and safety of Golimumab, a fully human anti-TNF α monoclonal antibody, in subjects with rheumatoid arthritis. *J. Clin. Pharmacol.* **2007**, *47*, 383–396. [[CrossRef](#)] [[PubMed](#)]
17. Xu, Z.; Vu, T.; Lee, H.; Hu, C.; Ling, J.; Yan, H.; Baker, D.; Beutler, A.; Pendley, C.; Wagner, C.; et al. Population pharmacokinetics of Golimumab, an anti-tumor necrosis factor-alpha human monoclonal antibody, in patients with psoriatic arthritis. *J. Clin. Pharmacol.* **2009**, *49*, 1056–1070. [[CrossRef](#)] [[PubMed](#)]
18. Emery, P.; Fleischmann, R.M.; Strusberg, I.; Durez, P.; Nash, P.; Amante, E.J.; Churchill, M.; Park, W.; Pons-Estel, B.; Han, C.; et al. Efficacy and safety of subcutaneous golimumab in methotrexate-naïve patients with rheumatoid arthritis: Five-year results of a randomized clinical trial. *Arthritis Care Res.* **2016**, *68*, 744–752. [[CrossRef](#)]
19. Keystone, E.C.; Genovese, M.C.; Hall, S.; Bae, S.C.; Han, C.; Gathany, T.A.; Xu, S.; Zhou, Y.; Leu, J.H.; Hsia, E.C. Safety and efficacy of subcutaneous golimumab in patients with active rheumatoid arthritis despite methotrexate therapy: Final 5-year results of the GO-FORWARD trial. *J. Rheumatol.* **2016**, *43*, 298–306. [[CrossRef](#)] [[PubMed](#)]
20. Smolen, J.S.; Kay, J.; Doyle, M.; Landewé, R.; Matteson, E.L.; Gaylis, N.; Wollenhaupt, J.; Murphy, F.T.; Xu, S.; Zhou, Y.; et al. Golimumab in patients with active rheumatoid arthritis after treatment with tumor necrosis factor alpha inhibitors: Findings with up to five years of treatment in the multicenter, randomized, double-blind, placebo-controlled, phase 3 GO-AFTER study. *Arthritis Res. Ther.* **2015**, *17*, 14. [[CrossRef](#)]
21. Combe, B.; Dasgupta, B.; Louw, I.; Pal, S.; Wollenhaupt, J.; Zerbini, C.A.; Beaulieu, A.D.; Schulze-Koops, H.; Durez, P.; Yao, R.; et al. GO-MORE investigators. Efficacy and safety of golimumab as add-on therapy to disease-modifying antirheumatic drugs: Results of the GO-MORE study. *Ann. Rheum. Dis.* **2014**, *73*, 1477–1486. [[CrossRef](#)]

22. Weinblatt, M.E.; Westhovens, R.; Mendelsohn, A.M.; Kim, L.; Lo, K.H.; Sheng, S.; Noonan, L.; Lu, J.; Xu, Z.; Leu, J.; et al. GO-FURTHER investigators. Radiographic benefit and maintenance of clinical benefit with intravenous golimumab therapy in patients with active rheumatoid arthritis despite methotrexate therapy: Results up to 1 year of the phase 3, randomised, multicenter, double-blind, placebo-controlled GO-FURTHER trial. *Ann. Rheum. Dis.* **2014**, *73*, 2152–2159. [PubMed]
23. Kruger, K.; Burmester, G.R.; Wassenberg, S.; Bohl-Bühler, M.; Thomas, M.H. Effectiveness and safety of golimumab in patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis under real-life clinical conditions: Non-interventional GO-NICE study in Germany. *BMJ Open* **2018**, *8*, e021082. [PubMed]
24. Thomas, K.; Flouri, I.; Repa, A.; Fragiadaki, K.; Sfrikakis, P.P.; Koutsianas, C.; Kaltsonoudis, E.; Voulgari, P.V.; Drosos, A.A.; Petrikou, E.; et al. High 3-year golimumab survival in patients with rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis: Real world data from 328 patients. *Clin. Exp. Rheumatol.* **2018**, *36*, 254–262. [PubMed]
25. Rotar, Z.; Tomsic, M.; Praprotnik, S. The persistence of golimumab compared to other tumour necrosis factor- α inhibitors in daily clinical practice for the treatment of rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis: Observations from the Slovenian nation-wide longitudinal registry of patients treated with biologic disease-modifying antirheumatic drugs. *Clin. Rheumatol.* **2019**, *38*, 297–305. [CrossRef]
26. Svedbom, A.; Storck, C.; Kachroo, S.; Govoni, M.; Khalifa, A. Persistence with golimumab in immune-mediated rheumatic diseases: A systematic review of real-world evidence in rheumatoid arthritis, axial spondyloarthritis, and psoriatic arthritis. *Patient Prefer. Adherence* **2017**, *11*, 719–729. [CrossRef]
27. Serrano, B.; Gonzalez, C.M.; Gonzalez, R.; Martinez-Barrio, J.; Gabriel Ovalles-Bonilla, J.; Carlos Nieto, J.; Janta, I.; Valor, L.; Longo, F.J.L.; Monteagudo, I.; et al. Golimumab retention rate in patients with rheumatoid arthritis. Predictors of long-term retention [abstract]. *Arthritis Rheumatol.* **2017**, *69* (Suppl. 10). Available online: <https://acrabstracts.org/abstract/golimumab-retention-rate-in-patients-with-rheumatoid-arthritis-predictors-of-long-term-retention/> (accessed on 27 December 2018).
28. Aaltonen, K.J.; Joensuu, J.T.; Pirila, L.; Kauppi, M.; Uutela, T.; Varjolahti-Lehtinen, T.; Yli-Kerttula, T.; Isomäki, P.; Nordström, D.; Sokka, T. Drug survival on tumour necrosis factor inhibitors in patients with rheumatoid arthritis in Finland. *Scand. J. Rheumatol.* **2017**, *46*, 359–363. [CrossRef]
29. Kay, J.; Fleischmann, R.; Keystone, E.; Hsia, E.C.; Hsu, B.; Zhou, Y.; Goldstein, N.; Braun, J. Five-year safety data from 5 clinical trials of subcutaneous golimumab in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *J. Rheumatol.* **2016**, *43*, 2120–2130. [CrossRef]
30. Thomas, S.S.; Borazan, N.; Barroso, N.; Duan, L.; Taroumian, S.; Kretzmann, B.; Bardales, R.; Elashoff, D.; Vangala, S.; Furst, D.E. Comparative immunogenicity of TNF inhibitors: Impact on clinical efficacy and tolerability in the management of autoimmune diseases. A systematic review and Meta-analysis. *BioDrugs* **2015**, *29*, 241–258. [CrossRef]
31. Frampton, J.E. Golimumab: A Review in Inflammatory Arthritis. *BioDrugs* **2017**, *31*, 263–274. [CrossRef]



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Perspective

How to Get the Most from Methotrexate (MTX) Treatment for Your Rheumatoid Arthritis Patient?—MTX in the Treat-to-Target Strategy

Peter. C. Taylor ^{1,*}, Alejandro Balsa Criado ², Anne-Barbara Mongey ³, Jerome Avouac ⁴, Hubert Marotte ⁵ and Rudiger B. Mueller ⁶

¹ Botnar Research Centre, NDORMS, University of Oxford, Windmill Road, Oxford OX3 7LD, UK

² Rheumatology unit, University Hospital La Paz, Institute for Health Research—IdiPAZ, Universidad Autonoma de Madrid, 28046 Madrid, Spain; alejandro.balsa@salud.madrid.org

³ St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland; anne.b.mongey@ucd.ie

⁴ Paris Descartes University, Sorbonne Paris Cité, Rheumatology Department, Cochin Hospital, Assistance Publique-Hôpitaux de Paris, 75014 Paris, France; javouac@me.com

⁵ SAINBIOSE, INSERM U1059, Université de Lyon, Saint-Etienne, France Service de Rhumatologie, CHU Saint-Etienne, 42055 Saint-Etienne, France; Hubert.marotte@chu-st-etienne.fr

⁶ Division of Rheumatology, Medical University Department, Kantonsspital Aarau, 5001 Aarau, Switzerland; ruediger.mueller@ksa.ch

* Correspondence: peter.taylor@kennedy.ox.ac.uk; Tel.: +44-1865737830

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Abstract: Methotrexate (MTX) is a remarkable drug with a key role in the management of rheumatoid arthritis (RA) at every stage of its evolution. Its attributes include good overall efficacy for signs and symptoms, inhibition of structural damage and preservation of function with acceptable and manageable safety, a large dose-titratable range, options for either an oral or parenteral route of administration, and currently unrivalled cost-effectiveness. It has a place as a monotherapy and also as an anchor drug that can be safely used in combination with other conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) or used concomitantly with biological DMARDs or targeted synthetic DMARDs. MTX is not without potential issues regarding toxicity, notably hepatotoxicity and bone marrow toxicity, as well as tolerability problems for some, but not all, patients. But many of these issues can be mitigated or managed. In the face of a welcome expansion in available targeted therapies for the treatment of RA, MTX looks set to remain at the foundation of pharmacotherapy for the majority of people living with RA and other inflammatory rheumatic diseases. In this article, we provide an evidence-based discussion as to how to achieve the best outcomes with this versatile drug in the context of a treat-to-target strategy for the management of RA.

Keywords: methotrexate; rheumatoid arthritis; tolerability; efficacy; posology; titration; oral route; subcutaneous route; bioavailability; effectiveness

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation, pain, stiffness, and progressive joint destruction with a detrimental impact on joint function, work ability, and health-related quality of life (HrQoL) [1]. The primary goal of treating patients with RA is to maximize long-term HrQoL through control of symptoms, prevention of structural damage, normalization of function, and participation in social and work-related activities. Abrogation of inflammation is considered the most important way to achieve optimal outcomes [2], and its achievement is facilitated by Treat-to-Target (T2T) recommendations [2].

The T2T paradigm has emerged during the last decade and was incorporated in recommendations issued primarily in 2010 and recently updated [2]. The T2T paradigm relies on five principles: the definition of a treatment target; the close and regular assessment of disease activity using composite measures that include joint counts; the regular adaptation of therapy, if the target is not achieved within a particular timeframe; the consideration of individual patients' aspects; and shared decision-making with the patient. A T2T strategy is more effective in terms of reducing disease activity compared to routine care [3], and has a beneficial effect on working ability. T2T principles underpin the current recommendations of European League Against Rheumatism (EULAR) Task Force [4], American College of Rheumatology (ACR) guidelines [5], and the Canadian Rheumatology Association (CRA) recommendations [6] and the Asian APLAR guidelines [7]. T2T overarching principles affirm the necessity of a shared decision between patient and the whole team involved in the care of RA [2].

The ideal treatment target is remission [8], or, if remission cannot be achieved, low disease activity (LDA). Remission is associated with the best achievable outcomes including limitation or prevention of joint damage [9], preservation of physical function, and work capacity as well as optimizing overall quality of life [10,11]. Furthermore, remission reduces comorbidity risks [12]. Treatment targets should however be adapted according to the presence of comorbidities, individual patient factors and drug-associated risks [2] to ensure the most favorable benefit: risk ratio for any given patient. Treatment should be titrated to the therapeutic response assessed by composite measures of disease activity, ideally with therapy adjustment at least every three months [2] if the patient is not in remission, a recommendation based on evidence provided by clinical trials [13–15], until the desired treatment target is reached.

Early treatment [16] and rapid attainment of the targeted endpoint are critical. Disease-modifying anti-rheumatic drugs (DMARDs) are the main therapy of RA. The most commonly prescribed conventional synthetic (cs) DMARDs are methotrexate (MTX), leflunomide, sulfasalazine, and hydroxychloroquine [6]. Based on its efficacy, safety, large dose-titratable range, options for either an oral or parenteral route of administration, and cost-effectiveness, MTX holds a unique place in the management of RA, with a role at every stage of the evolution of this chronic condition. MTX monotherapy is recommended as an initial pharmacological strategy [4–6], but it can also be used as an “anchor drug” in combination with another conventional synthetic disease-modifying antirheumatic drugs (csDMARD), any biological DMARD (bDMARD), or targeted synthetic DMARD (tsDMARD) [4]. MTX is currently the most commonly used first-line therapy for RA in the world [17].

While recommendations advocate the use of methotrexate at various positions in the contemporary treatment paradigm, the heterogeneity of presentation of RA and versatility of approaches to effective MTX use are such that there is need for evidence- and experience-based supplementary information to enable clinicians to get the most out of MTX for their patients in the T2T era. Notably, this process includes allaying any misconceptions that patients may have concerning this therapy, adapting dose and administration routes for optimal efficacy, mitigating toxicity and tolerability issues, and taking into account the relatively slow kinetic of response, which can be managed by the use of bridging steroids as appropriate.

In this position paper, we address these and other issues regarding the optimal use of MTX in a target population comprising adult (age ≥ 18 years) patients meeting current classification criteria for RA and patients with early inflammatory arthritis suspected of having RA but not yet fulfilling classification criteria.

2. Pharmacology

2.1. Pharmacokinetics

MTX (4-amino-10-methylfolic acid), an antifolate agent that was licensed for a RA indication in the 1980s [18], is a prodrug that becomes active when glutamated within cells, exhibiting a high binding activity for dihydrofolate reductase (DHFR) [19].

Oral MTX is absorbed from the small gut by an active transport mechanism involving the proton-coupled folate transporter. Bioavailability ranges from 30% to 70% [20] and plateaus for single oral dose >15 mg [21], suggesting an absorption limitation [22]. Intracellular MTX is gradually polyglutamated (MTXGlu_n). There is a competitive process between glutamation and deglutamation and steady-state levels of intracellular MTX are reached in a median time of 28 weeks with variability between patients due to different polyglutamation rates [23,24]. The major determinants of MTXGlu_n concentrations are age, renal function, and MTX dose [25]. Serum concentration of MTX falls rapidly following an intravenous administration [26]. The plasma half-life is 4.5 h to 10 h [27] but MTX is retained within cells long after its serum clearance. Excretion of MTX is mainly renal, occurring through glomerular filtration and active tubular excretion. Seventy-five percent of MTX is excreted unchanged in the urine with large interpatient variability [28].

The active transport mechanism limits oral MTX absorption resulting in lower bioavailability of higher MTX doses. Interestingly, bioavailability may increase by splitting the oral dose [22] or switching to a parenteral route [22]. Polyglutamation is progressive, explaining the slow onset of the action of MTX [19] and the delay before maximal benefit. In some patients with low glutamation rates, a relatively low dosing strategy will lengthen the time required for adequate drug concentration to be achieved [23]. This may be detrimental, as higher intracellular MTXGlu_n levels have been associated with better clinical response [29]. The subcutaneous (SC) administration route is associated with a significant increase in long-chain MTXGlu_n when compared to the oral route [30]. Renal function impacts the generation of MTXGlu_n. The anti-inflammatory effects of MTX in patients with RA are much more prolonged than its plasma half-life might suggest due to the accumulation of polyglutamated metabolites in tissues. Since the MTX excretion mainly involves the kidney, renal failure, or competitive excretion with other drugs in patients receiving multiple treatments will prolong MTX serum half-life [31].

2.2. Mode of Action

The mechanisms by which low-dose MTX exerts its therapeutic effect in RA remain incompletely understood [19]. MTX has several important anti-inflammatory actions mediated through a variety of pathways that do not involve folate antagonism. The main putative mechanisms are the promotion of adenosine release, inhibition of purine and pyrimidine synthesis, inhibition of methyl donors, generation of reactive oxygen species, downregulation of adhesion-molecule expression, modification of cytokine profiles, and downregulation of eicosanoids and matrix metalloproteinases [19]. Adenosine exerts an anti-inflammatory activity by down-regulating the activation and proliferation of T-Lymphocytes. Adenosine receptors are overexpressed on immune cells and synoviocytes of patients with RA, probably because of the high tumor necrosis factor (TNF) levels. Inhibition of DHFR prevents the regeneration from dihydrofolate to tetrahydrofolate, an essential factor for the production of folate cofactors required for purine and pyrimidine synthesis [32]. Inhibition of DHFR also inhibits the production of methyl donors and attenuates the formation of polyamines.

Since the anti-inflammatory properties of MTX are not mediated by folate antagonism, some toxic effects may be prevented or minimized by folic acid supplementation (not taken on the same day as MTX) without loss of therapeutic benefit.

In the future, pharmacogenetic advances may help to predict non-responses to MTX, thus directing patients to combination therapy as initial treatment. Promising results have recently been published [33,34].

3. MTX: A Forefront Position among csDMARDs

3.1. Flexible

More intensive treatment interventions provide the best chance of achieving remission in RA patients [35]. However, potential benefits need to be balanced against potential risks and every

effort must be made to ensure acceptable tolerability of any given therapeutic regime. The most frequent strategy for management of early RA is a “step-up” approach, where MTX is initiated at a dose estimated to be well tolerated and then titrated up according to therapeutic response. Current recommendations for management of RA [5,36] advocate the initial use of MTX as a monotherapy with a bridging steroid to give more rapid symptomatic relief before the clinic benefits of MTX become apparent. Some authors have advocated a step-down approach to treatment, initiating MTX at a high dose and arguing that this takes advantage of a “window of opportunity” for best outcomes early in the disease [37]. However, this process has the potential disadvantage of making tolerability issues more challenging to manage. Whatever the strategy, MTX has a central role to play, both as a monotherapy and as an “anchor drug”, for many successful DMARD combinations whether conventional, targeted synthetic, or biologic [38]. Some unique attributes of MTX contributing to this versatility include the wide dose titratable range, choice of administration routes, the favorable efficacy:toxicity ratio, utility in DMARD combinations, potential ancillary benefits, and outstanding cost-effectiveness. Unfortunately, despite treatment guidelines, up to 50% of patients [39] do not continue MTX when treatment with a biologic is initiated. Over the last few years there has been a discussion about tsDMARD or bDMARD monotherapy. Some authors suggest that MTX administered in a tolerated dose should be continued as a combination partner until remission is achieved [40]. This process is recommended because the highest benefits for all targeted therapies are generally observed when they are used with concomitant MTX.

MTX is marketed in the form of tablets with increments of 2.5, 5, or 10 mg/tablet, and as prefilled syringes or auto-injectors with various doses per injection, allowing one to finely tune the weekly dose. Oral and (SC) are the most widely routes used in RA patients. SC injections should be considered for weekly doses of 15 mg or higher due to the better bioavailability of MTX when administered parenterally.

3.2. Clinically Efficient

The TEAR study has demonstrated that low disease activity illustrated with the Disease Activity Score-28 with the erythrocyte sedimentation rate (DAS28-ESR) can be achieved by 28% of patients with early RA treated with MTX monotherapy [41]. It was a 2 × 2 factorial design comparing four treatment arms (immediate combination of MTX + etanercept (ETN); immediate combination of MTX + hydroxychloroquine (HCQ) + sulfasalazine (SSZ)); initial MTX with step-up treatment adding ETN; initial MTX with step-up treatment adding HCQ and SSZ. The study aimed to assess if it was better to intensively treat all early RA cases with drug combinations or to reserve this procedure for those who do not achieve low disease activity (DAS28-ESR < 3.2) after 24 weeks. At week 24, 28% of the patients from step-up groups achieved the treatment target. Similar results were achieved in the BeST study [42]. An even higher rate was demonstrated by Mueller et al in real-life [43]. Moreover, no difference was found between groups with respect to disease activity after 48 weeks and 102 weeks of treatment. Favorable results obtained with initial MTX monotherapy in the step-up groups were maintained until week 102. Moreover, there was no difference in radiologic progression when comparing step-up with combination therapy in a post-hoc analysis [38]. Since differences in outcomes were negligible between patient groups that were started at a baseline on an aggressive combination therapy regimen, or were delayed before stepping up (by 6 months), the authors concluded that there is no penalty for delaying step-up therapy until it is clinically indicated [38]. In comparison with those patients exhibiting a sustained, good response to MTX monotherapy, aggressive combined treatment would only result in a higher risk of side effects and higher costs. Importantly, these results were obtained employing a strict MTX dosage escalation regime (10 mg/w, escalated to 15 mg/w at week 6 and 20 mg/w at week 12, unless the patients had zero tender joints and zero swollen joints at that visit). LDA, but not remission, was the key factor that determined whether to step-up to combination therapy at week 24. The SWEFOT study reported similar results with 29.8% of patients meeting EULAR criteria for response after 3 months of MTX monotherapy [44].

3.3. Ancillary Benefits

Besides its efficacy in ameliorating arthritis, MTX may have ancillary benefits. Patients with RA have a higher risk of cardiovascular disease, infection, and cancer [45–47], resulting in reduced survival. Low-dose MTX is reported to reduce all-cause mortality in RA by 60% and cardiovascular mortality by 70%, while other DMARDs have no effect on mortality. Protective effects appear to be independent of MTX dose [48]. Another study reported that all-cause mortality was reduced by 70% in a vast cohort of RA patients treated with MTX when compared to those receiving other treatments [49]. Protective effects were evident only after one year of MTX use and were independent of the effect on RA activity [49]. The biological mechanisms underpinning this protection are not fully understood. Effects of MTX on the survival of RA patients are likely to be mediated by its anti-inflammatory properties, since MTX failed to demonstrate any protective effects on cardiovascular events in patients with previous myocardial infarction or multivessel coronary disease who additionally had type 2 diabetes or metabolic syndrome but no major inflammation [50].

Interstitial lung disease (ILD) is a common extra-articular manifestation of RA [51], affecting around 10% of patients [52]. Chronic lung disease (CLD) and ILD increase the risk of death in RA patients [53,54]. Methotrexate has been associated with severe lung toxicity, including ILD [55]. However, there is recent data that questions the results of case studies or observational studies linking methotrexate to pulmonary damage. Two meta-analyses also failed to associate methotrexate with ILD; in the first one [56], a meta-analysis of clinical trials (22 studies with 8584 patients with RA), the use of MTX in patients with RA was associated with an increased risk of total respiratory complications (RR 1.10), mainly due to an increased risk of respiratory infections (RR 1.11) and acute pneumonitis (RR: 7.81) but it was not observed that treatment with MTX increased the risk of death due to lung disease (RR 1.53, 0.46–5.01). In the second one [57], a meta-analysis of the clinical trials of psoriasis, psoriatic arthritis, and inflammatory bowel disease, all clinical conditions without pulmonary manifestations, did not find an increase in lung disease in patients treated with MTX. A recent analysis of a large prospective cohort of predominantly male US Veterans showed no increase in mortality risk in RA-CLD patients treated with MTX or biologics [54]. Moreover, in a recent study, MTX was a strong predictor of survival in RA patients with ILD [53].

The role for MTX in the prevention of dementia remains controversial. Inflammation is a common feature of both RA and dementia [58]. Despite the blood brain barrier, systemic inflammation is associated with cerebral inflammation [58]. A retrospective population-based study involving more than 11,000 RA patients, including 70.6% of csDMARD users, revealed that csDMARDs users had a 40% risk reduction of dementia. The strongest effect was with the use of MTX [59].

Felty's syndrome comprises a triad of RA, neutropenia, and splenomegaly, occurring in less than 1% of RA patients. Pseudo Felty's syndrome is characterized by RA, monoclonal expansion of lymphocytes, and neutropenia. The main complication is infection, whose risk increases with the depth and duration of neutropenia [60]. MTX is the first-line treatment of Felty's syndrome and is indicated, despite neutropenia [61,62].

3.4. Cost-Effectiveness

Therapeutic decisions should also be guided by cost-effectiveness considerations. Based on a decision analysis model extended to lifetime duration, early MTX monotherapy was more cost-effective than the early combination of MTX and TNF Inhibitor (TNFi)-based biologics [63]. The cost-effectiveness ratio of the early MTX strategy was less than \$5,000/quality-adjusted life year (QALY) and more than \$150,000/QALY for TNFis. In patients who failed to respond or to tolerate oral MTX; switching to SC MTX was described as more cost-effective than adding bDMARDs [64], since a large proportion of these patients achieved adequate response with SC MTX and, therefore, did not require more expensive therapy [65]. A cost-minimization analysis based on UK costs indicated that a routine use of SC MTX following oral MTX failure had the potential to save an estimated £7,197 (5536 US\$) per patient in the first year of therapy [64]. Prefilled syringes and auto-injectors facilitate self-administration of SC

MTX by the patients, thus increasing costs savings. In addition, in some countries, the prescription of bDMARDs can be reimbursed only if the patient did not adequately respond to at least 2 csDMARDs, if not contra-indicated [66,67].

3.5. *Combinable with Other Treatments*

MTX is a great combination partner with other csDMARDs and bDMARDs. The most popular csDMARDs combinations are MTX and HCQ and the triple combination of MTX, HCQ, and SSZ. The combination of MTX and HCQ is synergistic and gave better results than MTX alone in a head-to-head comparison after 6 months of treatment, but the difference was no longer significant after 12 months [68]. Combining MTX and HCQ may be useful in patients who have a good response to MTX, attaining LDA but not remission [69]. The efficacy of this combination may be explained by pharmacokinetic interactions. Concomitant administration of HCQ increases the mean area under the curve (AUC) of MTX blood concentration, decreases the maximum MTX concentration (C_{max}), and extends the time to reach C_{max} [67]. A reduced C_{max} may also explain the diminution of acute liver adverse effects. However, extra vigilance for MTX adverse events is recommended, especially in patients with impaired renal function [67]. Triple therapy combining MTX, HCQ, and SSZ may be a valuable alternative to the addition of bDMARDs to MTX, in cases of inadequate response to MTX and in the absence of poor prognosis factors. In a randomized trial comparing a triple combination of csDMARDs versus a regimen of MTX and etanercept (ETN), patients receiving triple therapy adhered for a longer time to their regimen than patients receiving MTX + ETN, despite similar outcomes [70], thus reflecting better acceptability. Infections were more frequent with the MTX+ETN regimen, whereas gastrointestinal side effects were more frequent with the triple combination [71]. Conversely, in a network meta-analysis of 33 studies, the odds of remission were lower with triple therapy than with the combination of MTX and TNFi [72]. However, the analysis considered both patients with inadequate response to MTX and MTX-naïve patients. These results were contested by a recent meta-analysis showing that triple association of csDMARDs and the combination of MTX and bDMARD as a second-line treatment in patients with insufficient response to MTX gave similar results [69]. Nonetheless, this result provides additional evidence supporting conventional combinations over bDMARDs after an inadequate response to MTX.

MTX is also a valuable partner in combined therapy with tsDMARD and bDMARDs. Such combinations have been shown to significantly improve clinical manifestations of RA [73–76]. Around two thirds of patients will have to step-up from MTX monotherapy to combined strategies [41]. In addition, RA patients with severe presentation and poor prognostic factors may require combined therapy upfront to prevent irreversible joint damage and functional impairment. The efficacy of targeted therapies is enhanced when the bDMARD is administered in combination with MTX. This may be due to complementary mechanisms of action, pharmacokinetic interactions, and reduction of the immunogenicity of the biologic agent.

The CONCERTO study was the first blinded, controlled trial to address the relationship between MTX dose and serum drug concentrations in RA patients treated with adalimumab. Steady-state trough serum concentrations of adalimumab were almost two-fold higher in patients concomitantly receiving MTX [77]. Patients were randomized into four groups with an increasing MTX dose (from 2.5/week to 20 mg/week, orally). There was no difference between the two highest MTX doses on clinical, radiographic, and functional response, whereas both doses were associated with better outcomes when comparing to the two lowest doses [77]. In parallel, adalimumab serum concentrations were higher with 10 or 20 mg MTX than with 2.5 or 5 mg MTX, mimicking the clinical findings. These results suggested that MTX at a dose of 10 mg/w reduced the clearance of adalimumab and increased the adalimumab serum level, thereby enhancing its efficacy. The MUSICA study [78] evaluated the effects of low and high MTX doses in combination with the initiation of adalimumab in patients who did not adequately respond to MTX. Patients with a stable MTX dose of at least 15 mg/week prior to screening were randomized to receive blinded MTX, at either 7.5 or 20 mg/week in combination with open-label

40 mg of adalimumab every other week. Since low-doses of MTX associated to adalimumab failed to show non-inferiority as compared to high doses for most clinical, functional, and ultrasound outcomes, the MUSICA study results do not support routine MTX reduction at the moment of adalimumab initiation. However, in a proportion of patients who may need to reduce MTX for toxicity reasons, much of the clinical efficacy in association with adalimumab may be retained, particularly when MTX dose reductions are modest.

Secondary failure to bDMARDs after an initial response can occur in as many as 30% of patients [79]. Development of anti-drug antibodies (ADAb) is one of the main drivers of loss of treatment efficacy [80–84]. ADAb have been described with infliximab, adalimumab, but not with etanercept. In a recent cross-sectional study on Spanish RA patients experiencing secondary failure while receiving TNFi, the prevalence of adalimumab ADAb was 29.3%, the one of infliximab ADA was 27.3%, and none of the patients treated with ETN developed ADAb [84]. Randomized-controlled trials reported ADAb in a small proportion on patients on golimumab and certolizumab [80]. ADAb either neutralize the active drug or decrease the serum drug concentration, resulting in a loss of clinical response [85]. Around 80% of patients who tested positive to ADAb had no detectable drug in the serum [84]. In a retrospective cohort of RA patients who were biologic-naïve at the time of enrolment, and were then treated with infliximab for a median time of 5.75 years, ADAb against infliximab were found in 33% of patients, in 24% of responders, and in all non-responder patients [83].

MTX may prevent [84,86] or reverse [87] the formation of ADAb and so maintain efficacy to TNFi, prolong drug survival, and prevent immune complex-mediated adverse events [80,83]. The pooled risk ratio for the formation of ADAb in patients receiving combined therapy with immunomodulators versus that of patients receiving anti-TNF monotherapy was 0.49 in a meta-analysis of 35 studies involving 6790 patients with inflammatory bowel disease [88]. The development of ADAb is reduced by the concomitant use of MTX in a dose-dependent manner [89]. It is, therefore, desirable to prescribe bDMARDs in combination with concomitant MTX when it is satisfactorily tolerated with toxicity. However, in the event of tolerability issues with MTX, the dose can be lowered while still providing efficacy benefits in combination, as illustrated in the CONCERTO [77] and MUSICA [90] studies.

3.6. Challenges Associated with MTX Use

When prescribing MTX as first-line therapy in RA patients, the physician has to face several challenges, such as explaining the time-course of the clinical benefits to the patient, improving patient compliance and adherence to therapy and monitoring, and avoiding unnecessary premature discontinuation of MTX are at the forefront of physician concerns.

The collective findings of combination therapy studies with MTX monotherapy arms, such as SWEFOT [44], CAMERA [14], TEAR [41], OPTIMA [91], and PREMIER [74], illustrate that, in general, it takes 6 months to see a full response to MTX. In patients with early RA and no poor prognostic factors, the relatively slow onset of action of MTX does not jeopardize clinical or functional outcomes. During this period, and especially during the first months, patients do not experience the full clinical benefit of MTX but are maybe exposed to adverse events. This is a crucial period in which too many patients discontinue MTX. The risk of premature stopping can be mitigated by the provision of clear and complete information to the patient. The slow kinetic of onset of symptomatic improvement with MTX can be mitigated by co-prescription of corticosteroids as a bridging therapy.

The best outcomes with the use of MTX are achieved when a shared decision-making approach to treatment is used in which the patient is an active participant. Patients need to understand and take responsibility for attendance for blood tests to ensure close monitoring of laboratory parameters, as well as following recommendations for folic acid supplementation in order to reduce the likelihood of certain adverse events. Reassuring tolerability and safety of low-dose MTX have recently been studied confirmed in the large placebo-controlled CIRT study aimed at determining if low-dose MTX could reduce the incidence of atherosclerotic events in patients with metabolic disorders and a history of myocardial infarction or multivessel coronary disease [50]. The study included 2391 patients who

were allocated to the MTX arm. The median follow-up was 2.3 years before the study was stopped because of its futility. Most frequent AEs were infections, mouth sores and oral pain, unintended weight loss, leukopenia, and elevation of liver enzymes more than three times the normal range. Severe AEs are far less frequent. No case of marrow depletion was reported, even if leukopenia was frequent [50]. However, the report of more non-basal skin cancers in the MTX group was unexpected and deserves further exploration.

Elevations of liver enzymes must alert the physician to potential hepatotoxicity, in which case it may be prudent to temporarily reduce the MTX dose or even to discontinue the drug. Acute elevations of liver enzymes occur relatively frequently but are usually transient and generally resolve spontaneously. Rarely, persistent abnormal liver function tests reveal hepatotoxic effects of MTX, justifying treatment cessation. Among 41 patients receiving low-dose MTX for rheumatic diseases, experiencing elevation of liver enzymes, and underwent liver biopsy, only 2 patients had histological signs of direct hepatic MTX toxicity. Most frequently, liver biopsies revealed autoimmune hepatitis-like lesions, especially in patients with RA [92]. However, it is important to avoid the unnecessarily premature discontinuation of MTX for reasons of small elevations in transaminases. It is important to bear in mind other causes of transaminitis, such as alcohol consumption or over-the-counter use of nonsteroidal anti-inflammatory drugs (NSAIDs). The limited data available on the effects of alcohol consumption on the risk of liver toxicity in patients with RA who are receiving MTX are insufficient to draw firm conclusions on the amount of alcohol that patients receiving MTX can safely consume. International guidelines vary. The American College of Rheumatology recommends that alcohol should be avoided whilst on MTX [93], while EULAR advises avoiding MTX in patients with a history of alcohol abuse, without specifying a restriction of alcohol consumption otherwise [94]. A systematic literature review found little evidence to provide specific guidelines regarding alcohol consumption [95]. Following their analysis, they estimated a 3% risk for the development of severe liver disease in patients who drink over 100 g of alcohol (12.5 units) per week in the absence of additional risk factors such as obesity, diabetes, or hepatitis. The authors advised caution in using MTX in patients with risk factors. A more recent study suggested that a weekly consumption of <14 units of alcohol per week does not appear to be associated with an increased risk of hepatotoxicity as defined by transaminitis based on aspartate transaminase (AST) or alanine transaminase (ALT), >3 times the upper limit of normal. Consuming between 15 and 21 units is associated with a “possible risk”, and over 21 units with a significantly increased risk of transaminitis [96]. However, this definition of hepatotoxicity is somewhat arbitrary as it is not supported by the use of hepatic scans or histology. Furthermore, allowing alcohol consumption of up to 14 units/week may place patients at risk of hepatotoxicity, such as hepatic fibrosis and cirrhosis [97]. In practice, many rheumatologists allow patients without additional risk factors for hepatotoxicity to consume small to moderate amounts of alcohol while taking MTX, preferably avoiding alcohol consumption on the days that they take MTX. Decisions regarding alcohol consumption should be made on an individual patient basis after taking into consideration the presence of other risk factors.

The next section of this position-paper will detail how to deal with these challenges in real-life conditions in order to get the most out of MTX as a first-line treatment.

4. How to Get the Most out of MTX as a First-Line Treatment?

Despite the many advantages of MTX, it may still be used suboptimally. Studies indicate that half of patients discontinue oral MTX within 2 years and that MTX is still frequently stopped rather than combined with bDMARDs or tsDMARDs when these target therapies are initiated. The risk of discontinuation is higher in older patients [98–100]. Patients should be treated early since a longer duration of RA decreases the probability of response [101]. Baseline high disease activity increases the probability of non-response in most studies [102,103].

When initiating MTX, the choice of dose, route of administration, and approach to subsequent dose escalation is not an exact science. Choices may depend on several factors, including the age,

gender, ethnicity, weight, and renal function of the patient. These choices will be made with the intent to minimize any tolerability issues and drug-related toxicity, as well as to optimize efficacy and maximize adherence to MTX.

4.1. Dealing with Modifiable Predictors of Response to MTX

Some factors have been shown to impact the response to MTX. Of these, many such as age [104], gender [104], and pharmacogenetic profile [34] cannot be modified. However, others such as smoking may be modifiable, and physicians should endeavor to address this.

Smoking may affect the pharmacokinetic and pharmacodynamics properties of MTX [25]. Smoking habits are predictors of poor response [102,104,105] in a dose-dependent manner [103], with heavy smokers being at higher risk of non-response. In addition, given the higher cardiovascular risk attached to RA patients, smoking cessation is highly desirable [106,107]. Even if it is a demanding objective [107], smoking cessation should be a priority for physicians.

Alcohol consumption, particularly when excessive, has been implicated in the risk of inadequate response to MTX [103]. For most patients, a modest alcohol intake is acceptable, provided that hepatic monitoring remains within safe limits.

Setting realistic but positive expectations about the likely magnitude of benefit, speed of onset, and mitigation strategies to limit toxicity and tolerability issues for MTX may favorably influence the drug's efficacy via improved compliance. High quality education plays a crucial role in achieving this goal. The impact of patient anxiety has also been underscored in limiting the potential benefits of MTX and can be accentuated by the ready availability of misinformation through the internet and other sources. In a multivariate model predicting non-response to MTX in a real-life setting, the risk of non-response was higher in patients who were negative for rheumatoid factor, had a higher Health Assessment Questionnaire (HAQ) score, a higher tender joint count, a lower DAS28 score, and a higher anxiety score [108]. Nonetheless, anxiety is a modifiable risk factor. This issue should be addressed during the shared decision-making process. This process should include exploring the patient's expectations regarding the disease and its treatment, providing honest and positive messages about achievable outcomes, and allaying unfounded fears about adverse effects of therapy. Educating the patient may lower the level of anxiety and improve clinical outcomes.

4.2. Choosing the Route of Administration

When starting MTX, the physician has the choice between oral or parenteral administration. Although MTX can be given by intramuscular (IM) injection, the subcutaneous route is the preferred parenteral administration mode.

Depending on the MTX dose, the bioavailability differs between oral and subcutaneous routes. Oral MTX at a dose of 7.5 mg/week has a 100 percent bioavailability. However, bioavailability is reduced by 30% starting at just 15 mg/week due to gut absorption limitations [22,109]. This threshold is also the point where the bioavailability of oral MTX reaches a plateau in RA patients [21,110]. Consequently, for a patient receiving a weekly dose of 20 mg, intestinal absorption may be reduced by 30% and the effective dose actually received by the patients may be only 14 mg/week. Despite this fact, orally administered doses greater than 15 mg/week are frequently used to control disease activity.

By contrast, the bioavailability of SC MTX is greater than that of oral MTX and does not depend on the MTX dose with a linear increase in systemic exposure for increasing doses [21]. The ratio of dose-normalised $AUC_{0-24\text{ h}}$ of the SC MTX compared to oral MTX was 127.61 (90% CI 122.30 to 133.15) in a cross-over study comparing the two administration routes of the same MTX dose [21]. The AUC ratio between SC and oral MTX increases with higher doses [110]. Therefore, upon switching from an oral to SC MTX formulation, a patient may experience a bioequivalent increase of more than 6 mg MTX per week. The practical implication of this is that for many patients, there may be no advantage to increasing oral MTX dose above 15 mg/week [21]. Rather, RA patients with an inadequate clinical response to orally administered MTX may benefit from the higher drug exposure offered when

switched to the SC formulation [21]. Initiating the MTX regimen with SC administration has been associated with better clinical outcomes at the same dosages [111]. Initial treatment with SC MTX has been associated with lower rates of treatment change, no difference in toxicity, and some improvements in disease control compared to oral MTX over the first year, in a large cohort of early RA [112].

4.3. Starting at the Right Dose

As monotherapy, the dose of MTX can range from 7.5 to 25 mg/week, depending on national guidelines and physician's preference. Such a range allows a wide variety of practices. Indeed, considerable heterogeneity exists in rheumatologist prescribing behaviours [113].

The recommended MTX dose at initiation is generally 10–15 mg/week [94] but should be personalized, depending on age, ethnicity, body weight and prior history of intolerance to other medications. In the interventional C-OPERA study involving Japanese patients, the initial MTX dose was 8 mg/week [114]. Considering the average patient body weight in the Japanese population, a MTX dose of 8 mg/week gave a similar MTX dose, per pound of body weight, to a 10 mg MTX dose in the USA or Europe [114]. In the French ESPOIR cohort involving 813 patients recruited in 14 regional centres the median MTX dose at initiation was 12.5 mg/week [113] with two peaks in the distribution at 10 mg and 15 mg/week. An optimal treatment defined by a starting dose of at least 10 mg/week during the first 3 months, with escalation to at least 20 mg/week at 6 months, has been associated with better clinical outcomes at two years in this cohort [113]. Of note, only 26.3% of patients were considered to have had an optimal dose.

Clinical response is associated with longer-chain polyglutamates, which take 3–8 weeks to become detectable in erythrocytes [23]. These data raise the question as to whether a more rapid attainment of a steady state could be achieved with alternative dosing strategies, such as more rapid dose escalation or starting therapy with higher doses. Starting with a high oral dose does not make pharmacologic sense due to the reduced bioavailability of oral MTX above 15 mg/week. Recent trials have used higher initial MTX dosage (20–30 mg/week) [115]. However, combining the results of 31 studies involving 5589 patients, a meta-regression analysis did not support higher effectiveness of increasing MTX dose in monotherapy [116]. In the CONCERTO study which investigated the effects of starting with various dosages of oral MTX in combination with adalimumab 40 mg every other week, there was no difference in clinical or radiographic outcomes of patients starting with a 10 mg/week MTX dose, compared to those starting with a 20 mg/week dose [77]. Starting with higher oral doses may also result in discontinuation due to adverse events, such as nausea.

In patients who reintroduce MTX after previous discontinuation for tolerability problems, the MTX dose selection should be determined by the last tolerated dose.

4.4. Escalating and Adapting the MTX Dose

In the context of a treat-to-target strategy, the large dose-titratable range of MTX offers numerous possibilities of dose adjustment before combining with bDMARDs and tsDMARDs. Around one third of DMARD-naïve patients will respond to MTX monotherapy and maintain favourable outcomes for several years as reported in the SWEFOT study [117]. The MTX dose should be increased until remission or at least LDA. In clinical trials comparing bDMARDs to MTX monotherapy, 25% of the patients allocated to the MTX arm reached ACR70 criterion within 6 months, bringing them in the range of LDA [118]. With the subcutaneous route [111], the proportion of patients reaching LDA was even higher.

Titration is highly influenced by the starting dose and initial response to treatment. An optimal treatment defined by a starting dose of at least 10 mg/week during the first 3 months with escalation to at least 20 mg/week at 6 months has been associated with better clinical outcomes at two years in the ESPOIR cohort [113]. In the C-EARLY study comparing certolizumab + MTX and placebo + MTX in DMARD-RA patients with poor prognostic factors, MTX was started at 10 mg/week and escalated by 5 mg every 2 weeks, if tolerated, to a maximum of 25 mg/week by week 8. In the MTX

monotherapy group, 39.4% of patients reached LDA at week 52. Withdrawals due to AEs occurred for 9.2% of patients [119]. These results suggest that the MTX dose should not be less than 10 mg/week and that dose escalation should be as rapid as tolerated. Maximum dose varies according to the region. The usual maximum MTX dose is 25 mg/week in USA and Europe although some patients may benefit from higher doses. The maximum recommended dose in China is 20 mg/week [120] and in Japan, 16 mg/week [114]. In a randomized controlled trial in patients with active RA despite oral MTX, intramuscular (IM) MTX, starting at a dose of 15 mg/week then increasing to 45 mg/week, did not improve disease control; however, higher doses were generally well tolerated [121]. The CRA 2012 Working Group recommends individualized dosing (oral or parenteral) titrated, to a usual maximum dose of 25 mg/week, by rapid escalation [6]. The French Society for Rheumatology recommends rapid dose escalation (e.g., 5 mg increments every 1–4 weeks) reaching an optimal dose of MTX (15 to 25 mg/week) within 4–8 weeks [122], depending on effectiveness and safety, as well as individual characteristics. The use of split oral dosing (2 half-doses at 8 h interval on one day of the week) has been reported to improve bioavailability, tolerance, adherence, and efficacy [123]. Given the higher bioavailability of SC MTX beyond 15 mg/week, as compared to oral MTX, switching to SC MTX before increasing the MTX dose beyond 15 mg/week could be good practice. In addition, switching to SC MTX may also prevent dose-dependent gastrointestinal side effects [124].

When remission without structural progression is achieved in a patient who is not taking glucocorticoid therapy, and sustained for many months or even years, therapy de-escalation according to tight control principles could be considered [122]. However, withdrawing MTX bears the risk of losing the state of remission and difficulties in regaining a good outcome after a post-withdrawal flare [118].

4.5. Switching to SC Route

In patients with an inadequate response or intolerance to oral MTX, parenteral administration should be considered [6]. In Germany, the 2012 adapted EULAR Task Force recommendations and treatment algorithm in RA support optimization of MTX monotherapy with parenteral administration [125]. Similarly, the British Society for Rheumatology 2008 guidelines for DMARD use recommends switching to IM or SC MTX if oral MTX is ineffective or not tolerated [126].

Parenteral administration has been reported to reduce disease activity in patients who have an inadequate response to oral MTX [127]. Conversely, a change from parenteral to oral MTX has been associated with disease flare [128], increased disease activity, and greater frequency of gastrointestinal side effects. Switching back to intramuscular MTX improved disease manifestations and reduced side effects [129].

Switching patients treated with oral MTX to an SC formulation should be considered when the maximum tolerated dose has been reached without achieving LDA. The SC route may also be proposed upfront to the patient at the time of MTX initiation, although this has relatively greater economic implications. However, data from a randomized controlled trial of 375 MTX-naïve RA patients favoured initiation of 15 mg once weekly MTX therapy by the SC route, which resulted in higher ACR20 and 70 response rates than oral MTX with a similar safety profile at 24 weeks [111]. Furthermore, in a large retrospective cohort study, Harris et al. reported that patients taking SC MTX received higher starting and maximum doses than those on oral MTX (>15 mg starting dose and >20 mg maximum dose) [130], perhaps reflecting the better tolerability of the parenterally delivered drug.

There have been advocates in Canada, Germany, and the United Kingdom for the use of SC MTX in order to optimize achievable efficacy of MTX prior to commencing biologic treatment or even to prevent or delay the need for biologic therapy with attendant health economic benefits [6,125,126]. Indeed, multiple lines of evidence suggest that SC MTX, which bypasses first-pass metabolism in the gastrointestinal tract, appears to be an effective option in patients who have had an inadequate response or are intolerant to oral MTX. Switching such patients to SC MTX has been shown to result in higher and more constant bioavailability [22], to be more effective at the same dosage [111], and to have less

intense gastrointestinal side effects compared to oral MTX [124]. Higher bioavailability and increased long-chain MTXGlu_n may translate into efficacy [30,65,131,132]. A retrospective population-based study in 156 patients who were intolerant or unresponsive to oral MTX found that switching to SC MTX ($n = 78$) resulted in a decrease in RA disease severity, with good tolerability reported in these patients [65]. In the retrospective analysis of the US Veterans Affairs database of patients treated with injectable MTX after failing prior oral MTX, higher doses of MTX (>20 mg/wk) were achieved more readily with SC administration, and were associated with a significantly longer duration of MTX monotherapy before therapeutic change or the addition of other DMARDs/biologic agents, as compared to the oral formulation [133]. In the previously mentioned clinical trial comparing SC and oral MTX in 375 RA patients, after 16 weeks, patients from the oral group not fulfilling ACR20 criteria were switched from 15 mg of oral MTX to 15 mg of SC MTX following which 30% went on to achieve ACR20 responses [111]. In keeping with this observations, but in a real-world setting, retrospective analysis of 103 RA patients who switched from oral to SC MTX showed significant improvements in DAS-28 scores in patients who switched due to inefficacy or intolerability to prior oral MTX [132].

A prospective survey (i.e., office questionnaires) study evaluating patients ($n = 70$; each serving as their own control) with long-lasting RA who were switched from oral MTX to SC MTX due to side effects reported that when receiving the SC formulation, patients experienced less intense GI side effects, with no patients reporting vomiting or diarrhoea AEs [124].

SC MTX should also be considered in patients with poor compliance to oral MTX. There is currently no clear biomarker available to measure adherence to MTX in daily practice, despite some data suggesting a strong correlation between MTXGlu_n and compliance assessed by the electronic monitoring of treatment intakes with the Medication Event Monitoring System [134].

4.6. Giving Time for MTX to Achieve its Maximum Clinical Benefit and Using Bridge Therapies

Whether MTX is initiated at a higher dose, or a lower dose with upward titration, it will still take up to six months to achieve a maximum clinical benefit. Nevertheless, therapeutic response to MTX should be assessed after 3 months of treatment with the objective of a clinical improvement at least 50% [4]. If this short-term target is not reached, treatment adjustment should be considered.

Intra-articular glucocorticoids or short term systemic glucocorticoids may be used as part of the initial treatment strategy while waiting for MTX to take effect [6]. When MTX and intra-articular glucocorticoids were used with a treat-to-target approach in patients with early RA in the context of a clinical trial, this strategy effectively decreased synovitis, osteitis, and tenosynovitis and halted structural damage progression, as judged by MRI [135]. The EULAR Task Force recommends using glucocorticoids in combination with csDMARDs primarily as bridging therapy until the csDMARD reaches its maximum effect but corticosteroids should be tapered as rapidly as clinically feasible [4,6]. Intra-articular glucocorticoid application may be considered in residually inflamed or reactivated joint [4].

4.7. Preventing or Dealing with Adverse Events by Folic Acid Supplementation

In clinical practice, MTX-related toxicity may limit optimum treatment. Mild toxicity occurs in about 60% of patients and roughly seven to 38% of patients discontinue MTX within the first year of treatment due to toxicity [18,136,137]. Predisposing factors include existing folate deficiency, advanced age, cumulative MTX dose, renal insufficiency, and concomitant use of other folate inhibitors. A folate deficiency may cause side effects, such as mouth sores, abdominal pain, elevation of liver enzymes, or bone marrow depletion. Folate deficiency is frequent in RA patients and even more in those treated with MTX [138]. In a meta-analysis of six clinical studies, RA patients treated with low-dose MTX and “dummy” folic or folinic acid frequently experienced side effects: nausea and vomiting were reported by 35% of patients, abnormal liver blood tests were reported by 21% of patients, 22% of patients experienced mouth sores [139]. By contrast, genuine folic acid and folinic acid supplementation greatly reduced discontinuation of MTX compared to placebo supplementation in a placebo-controlled

double-blind clinical trial. This was mainly due to the MTX stopping rules based on elevations of liver enzymes [137]. By delaying or preventing a premature switch from MTX to a far more expensive bDMARD or tsDMARD, folate supplementation may contribute to health costs savings [140].

There is a large variability of folate supplementation practices amongst rheumatologists. In a study of 2467 incident users of MTX in the Veterans Health Administration database, 27% of patients were not prescribed folic acid within 30 days of MTX initiation. After 20 months, only 50% of patients continued to receive folic acid [141].

In a Cochrane review of patients on MTX therapy for RA, folic acid or folinic acid supplementation reduced the risk of gastrointestinal side effects (nausea, vomiting, and abdominal pain) by 26% (not statistically significant); abnormal serum transaminase elevations were decreased by 77%; the risk of premature discontinuations from MTX for any reason was reduced by 61%. A trend towards a reduction in stomatitis was demonstrated but did not reach statistical significance [139]. Folate supplementation did not negatively impact the efficacy of MTX. These results were confirmed by a recent meta-analysis of seven studies involving 709 patients [142]. A third meta-analysis of 68 studies (not limited to RA) of patients taking MTX revealed that MTX increased the risk of nausea and vomiting, elevated transaminase levels, mucosal ulcerations, leucopenia, thrombocytopenia and infectious events. The concomitant prescription of folic acid or folinic acid was associated with a significantly lower risk of any adverse events [143].

No evidence of a significant difference between folic or folinic acid has been reported, but given its low-cost, folic acid may be the most cost-effective therapy [139]. Prescription of at least 5 mg folic acid per week with MTX therapy is strongly recommended [94] to avoid or prevent side effects. Taking folic acid two days before MTX intake may improve its tolerability. Folic acid supplementation can be increased to 5 mg/day, other than the methotrexate dosing day, if needed. However, a pilot study recently compared two folate supplementation regimens with folic acid (5 mg/week and 0.8 mg/week) with similar results [144]. Folinic acid should be administered on a weekly basis the day after MTX. Although daily use of folic acid other than the MTX day does not appear to affect MTX efficacy, dosing of folinic acid close to MTX administration or dose of folinic acid over 7.5 mg/week may hinder MTX efficacy.

4.8. Potential Toxicities

Most frequent adverse events of MTX are gastrointestinal and there is a correlation between the MTX dose and the intensity of side effects [124]. Other possible side effects include anaemia, neutropenia, increased risk of bruising and dermatitis. Gastric side effects can be reduced both by switching patients to the SC route [124] and by folate supplementation [139]. Elevation of transaminases is frequent, but MTX treated patients are not at increased risk of symptomatic or severe liver related adverse events [55]. The incidence of clinically important cytopenia in patients treated with low dose MTX is estimated to be less than 1%. A complete blood count, liver and renal biochemistry, and a chest radiograph should be ordered prior to initiating MTX therapy [6]. Screening for hepatitis B and C should be considered and HIV testing is recommended in high-risk patients [6].

MTX is excreted via the kidneys and is contraindicated in patients with an estimated glomerular filtration rate of less than 30 mL/min. Patients with impaired renal function should be closely monitored particularly for hematologic toxicity.

Concomitant intake of NSAIDs may reduce renal function, thereby increasing MTX bioavailability. In a registry-based analysis, concomitant use of NSAIDs and MTX increased the risk of serious adverse events with a higher risk of renal failure and cytopenia [145]. However, it should be remembered that NSAIDs, per se, may increase liver enzymes. Therefore, if raised transaminases are observed in patients taking concomitant MTX and NSAIDs, it is worth considering a decrease (or cessation) in the dose of NSAIDs rather than diminishing the MTX dose. The physician should pay attention to self-medication with NSAIDs. MTX should not be used with trimethoprim-sulfamethoxazole when used in a twice-daily regimen for treatment of an infection since the combination may result in

significant bone marrow toxicity. However, it can be used for patients taking prophylactic doses of trimethoprim-sulfamethoxazole (dosed three times per week) for prevention of *Pneumocystis Carinii*. The main drug interactions are summarized in Table 1.

Table 1. Drug interactions. MTX, methotrexate.

| Interactions | Source of Interactions |
|--|---|
| Increase MTX levels | <ul style="list-style-type: none"> • Allopurinol, triamterene • Decrease renal MTX clearance: ciprofloxacin, cephalothin, penicillin, probenecid, sulfonamides • Decrease MTX excretion: diuretics, proton pump inhibitors • Increase MTX reabsorption by the kidney tubule: probenecid |
| Decrease MTX levels | <ul style="list-style-type: none"> • Decrease intestinal absorption of MTX: chloramphenicol, tetracyclines |
| Increase the risk of bone marrow suppression | <ul style="list-style-type: none"> • Chloramphenicol, co-trimoxazole, pyrimethamine, sulfonamides, trimethoprine-sulfamethoxazole |
| Increase liver toxicity | <ul style="list-style-type: none"> • Alcohol, leflunomide |

MTX-induced pneumonitis is a potentially life-threatening adverse effect [146]. It is an idiosyncratic hypersensitivity reaction due to activated T-cell-mediated (CD4 and CD8) stimulation of type 2 alveolar cells to release cytokines which lead to the recruitment of inflammatory cells, resulting in alveolitis. Acute or subacute pneumonitis related to methotrexate usually occurs during the first year of treatment [55,147]. It appears rapidly with low grade fever or high fever, nonproductive cough, and dyspnea that usually progresses to respiratory failure. The presence of eosinophilia in peripheral blood but not in bronchoalveolar lavage is common (50%). A diffuse interstitial pattern or a mixed interstitial-alveolar pattern (nodular or patchy infiltrates) is observed in the chest radiograph and high-resolution CT usually reveals the pattern of a usual interstitial pneumonia or a nonspecific interstitial pneumonia. Risk factors for this complication are age (>60 years), previous use of other DMARDs, hypoalbuminemia, the presence of diabetes mellitus, and a history of pleural disease and/or prior ILD related to AR (OR: 7.1), the latter being a great confounding factor when establishing a causal relationship with the drug [148]. It could be difficult to distinguish MTX-induced pneumonitis and pneumonitis related to *Pneumocystis* [149]. In a systematic review, 15 of 3463 RA patients (0.43%) who were receiving MTX developed MTX-induced pneumonitis [150]. Even if rare, this complication needs to be known and should not be neglected. Its treatment includes withdrawal of MTX, supportive therapy, and adjunctive steroids. The outcome is good if the condition is recognized early, and if appropriate treatment is given [146].

Although the overall benefit:risk ratio for MTX is remarkably favourable, as with any drug, adverse events can occur. Based on known adverse events of MTX treatment, we propose that it is good practice to assess the patient for several potential risk factors (Table 2). Among these precautions are the assessment of pre-existing infections, immunosuppression, haematological, kidney or liver diseases,

neoplasms, the cardiovascular risks, potential interactions with concomitant therapies. In parallel the psychological status of the patient should be judged or assessed including the coping strategies and the internal resilience.

Table 2. Preparation of MTX therapy.

| |
|---|
| Diagnosis of rheumatoid/inflammatory arthritis and/or need for treatment |
| Diagnosis of chronic kidney or liver diseases |
| Assessment of cardiovascular risks |
| Assessment of a neoplastic disease |
| Discussion on smoking habits and the advantage of smoking cessation |
| Evaluation/judging of depression and resilience/coping strategies |
| Assessment of anemia, leukopenia, or thrombocytopenia |
| Documentation of concomitant drug therapy |
| Defining therapeutic aim |
| Shared decision making with the patient |
| Evaluation of alcohol-consumption |
| Diagnosis of active/chronic infection with herpes zoster, tuberculosis, hepatitis, HIV, relevant fungal infection |
| Diagnosis of immunodeficiency |
| Documentation of vaccination status |
| Consider testing for tuberculosis |
| Anticonception/assessing wish of conception/family planning |

4.9. Placing MTX as an Anchor Drug

MTX is considered the initial drug of choice in RA pharmacotherapy with subsequent use as an anchor drug when used in combination due to its efficacy (when used optimally), safety, large dose-titratable range, and cost-effectiveness [4,5,94].

If the patient is unable to achieve treatment targets on maximally tolerated doses of oral MTX, and after a switch to the SC route, other treatment strategies must be considered. These include adding other csDMARDs, and adding or switching to biologic therapies or tsDMARDs, depending on the absence or presence of poor prognostic factors [4]. In routine practice, several combinations can be considered with some variations in preference according to country. As an example, physicians may take advantage of the combination with probenecid [151], the synergistic action of MTX and HCQ [69], or the triple combination MTX, HCQ, and SSZ [69,70]. In UK or Canada, rheumatologists are required to use at least two csDMARDs before the application of bDMARDs is approved by the payers [4]. The EULAR Task Force strongly recommends that bDMARDs and tsDMARDs should primarily be added to csDMARDs, such as MTX, since all bDMARDs have better efficacy when combined with MTX than as monotherapy [4]. This may be due to complementary mechanisms of action, pharmacokinetic interactions, and reduction of immunogenicity of the administered biologic agent.

If MTX is well tolerated in combination with concomitant csDMARDs or targeted therapies, the dose does not need to be tapered while waiting to achieve the desired treatment target. In case of poor tolerability, MTX can be used at 7.5–10 mg/week to provide additional efficacy to TNF-inhibitors [77] and intolerance at these low doses leading to discontinuation is very rare. Stopping MTX should be limited to patients with contraindications or very poor tolerance to MTX.

Current application of these guidelines is far from optimal. Analysing a database of 35,640 US patients with RA starting oral MTX, Rohr et al. [152] reported that over the 20,041 patients who had to change the treatment over a 5 year follow-up, only 13% switched to SC MTX. In those who added or switched to a biologic, only 37% were on >15 mg/week of oral MTX when a biologic was started. The addition or switch to a biologic occurred in the first 3 months of MTX therapy in 41% of patients, and in the first 6 months in 51% of patients. As stated by the authors, this study revealed that the MTX was dramatically underutilized as an efficacious anchor drug in clinical practice

4.10. Conception and Pregnancy

This section applies to RA patients who are planning to become pregnant and/or breastfeeding, men planning to conceive and patients who have accidentally conceived while taking MTX.

MTX is pro-abortive and teratogenic but does not decrease fertility [153]. Therefore, MTX at any dose should be avoided during pregnancy [154]. The optimal delay between the last MTX dose and conception is still a matter of debate and may vary between countries. Guidelines from the British Society for Rheumatology (BSR) and the British Health Professionals Society in Rheumatology recommend to stop MTX three months in advance of conception [154]. On the other hand, MTX is recommended to be stopped between 48 h and 6 months before conception, in France and in Spain, respectively. Thus, to avoid medicolegal issues, we recommend following the legal label of your country. In the case of accidental pregnancy on low-dose MTX, the drug should be stopped immediately [154].

MTX cannot be recommended in breastfeeding because [154] MTX is excreted into breast milk and may be toxic for the neonate.

If a woman has received MTX within 3 months prior to conception, or in the case of accidental pregnancy on MTX, folate supplementation should be continued prior to and throughout pregnancy at a dose of 5 mg/day [154].

Data regarding preconception exposure to MTX in men are reassuring, and, indeed, recent BSR guidelines state there is no need for men wishing to father children to stop MTX [154]. A study involving a very large cohort concluded that paternal exposure to MTX within 90 days before pregnancy was not associated with congenital malformations, stillbirths, and preterm birth [155]. Older recommendations suggest to stop MTX three months prior to conception, but this is not evidenced by an understanding of the impact of MTX on spermatogenesis or paternal-mediated teratogenicity, but rather relies on the timeframe of spermatogenesis [156].

4.11. Vaccinations

RA patients are more prone to infections than healthy subjects due to the immune dysfunction associated with the condition. DMARDs inhibit cellular and humoral immunity, thus aggravating the susceptibility to infections. For these reasons, vaccines against preventable diseases should be counselled in patients with RA. Influenza, pneumococcal, and shingles are important for all patients with RA whereas human papilloma virus, hepatitis B virus, and yellow fever (YF) vaccines are relevant only in selected patients, such as those living in or traveling to a YF-endemic country [157]. Protection is far from optimal. In 2012, in the United States, only 28.5% of patients older than 60 with rheumatic diseases were vaccinated against pneumococcal pneumonia; 45.8% were optimally vaccinated against influenza, and only 4.0% of patients were vaccinated against shingles [158].

MTX significantly decreases vaccine response to pneumococcal [159,160] and seasonal influenza vaccines [159] in a dose-dependent manner [161]. A recent randomized controlled trial showed that, in order to impair the response to vaccines, MTX had to be present just before or at the same time of vaccination [162]. It is, therefore, recommended that vaccination be done before a DMARD is started, where practically feasible [5]. However, it may be necessary to prioritize control of the disease. In such circumstances, and for seasonal vaccinations, MTX dosing can be temporarily interrupted. In a prospective randomized parallel-group trial, patients with RA taking a stable dose of MTX were randomly assigned at a ratio of 1:1:1:1 to continue MTX (group 1), suspend MTX for 4 weeks before vaccination (group 2), suspend MTX for 2 weeks before and 2 weeks after vaccination (group 3), or suspend MTX for 4 weeks after vaccination (group 4). All participants were vaccinated with trivalent influenza vaccine. Temporary MTX discontinuation improved the immunogenicity of vaccination. No difference was found between groups three and four [162]. These results were confirmed in another study [161] that demonstrated improvement in immunogenicity of seasonal influenza vaccination in patients receiving methotrexate in whom the drug was withheld for 2 weeks immediately after immunisation, compared with those who continued to receive methotrexate with a satisfactory vaccine response seen in 76% of the former compared with 55% of the latter group. However, there was a

slightly higher incidence of flares in those patients who withheld methotrexate compared with those who had remained on methotrexate (11% vs. 5%). Therefore, suspending MTX administration for 2 weeks after the vaccination seems to be good practice.

Shingles vaccine can be used safely with low-dose MTX (i.e., up to 25 mg/week) [157].

Live vaccines, such as yellow fever, measles/mumps/rubella, varicella, and oral typhoid vaccinations, are contra-indicated in patients receiving MTX. Information remains too scarce to question the current guidelines.

4.12. No Need for MTX Discontinuation in Case of Surgery

Despite the efficacy of DMARDs, 58% of RA patients will ultimately undergo orthopaedic surgery, with nearly 24% undergoing large-joint arthroplasty [163]. Patients receiving DMARD are potentially at higher risk of infections and delay in wound healing due to the anti-inflammatory properties of these compounds. A study involving 388 patients with RA who underwent surgery found that patients who continued MTX through surgery had fewer complications, infections, and RA flares than patients who discontinued MTX for 2 weeks before and after surgery [164]. A special consideration should be given to patients who develop renal dysfunction postoperatively, since the risk of MTX toxicity is increased in cases of renal failure.

4.13. Sharing the Decision Making with the Patient

Adherence to treatment has been shown to depend on the level of appropriate information provided to patients and the amount of good interaction with the rheumatologist [165]. Non-adherent patients flare four times more frequently than adherent patients [100]. Most arthritis patients prefer to be involved in decisions about their medication [166], and this has been linked to higher patient satisfaction.

Patients exhibit a complex range of beliefs related to DMARD therapy [167]. Furthermore, the degree to which patients desire to be directed by their physician regarding treatment choice varies tremendously and may evolve over time to become more collaborative [168]. Sympathetic understanding will facilitate the provision of appropriate information to allow the patient to make informed decisions [169]. Furthermore, it is crucial to educate patients about the importance of a T2T strategy, since it has been shown that patient preference is among the leading barriers to treatment adjustment to T2T in RA [170].

The EULAR Task Force recently reiterated the relative safety of MTX and recommended that the frequent fears of patients after reading the package insert should be addressed by providing appropriate information [4]. The overemphasis on the risk of side effects that are widely spread on social media can generally be overcome by an open, balanced, and reassuring discussion regarding the benefits/risks and risk mitigation at the time of the first prescription. The other crucial points to address are the importance of maintained therapy, especially in those patients in remission who want to stop MTX, and patient adherence (in particular, for patients who tell their practitioner that they are taking MTX when, actually, this is not the case). Available data suggest that adherence to RA therapy does not exceed 66% [171], but this proportion is enhanced by the belief in the necessity of the medication. For this reason, when explaining the achievable goals of treatment to the patient, the rheumatologist should emphasise the availability of long-term observational data demonstrating the numerous benefits and good overall tolerability of MTX.

When initiating MTX, we recommend that rheumatologists adopt a shared decision-making approach to the recommended regime. Key points to be emphasised in dialogue with the patient are summarized on Table 3.

Table 3. Key points patients will need to be counselled about when rheumatologists adopt a shared decision-making approach to the recommended regime. NSAIDs, non-steroidal anti-inflammatory drugs.

| Topics to Discuss with Patients |
|--|
| • The choice of routes of administration and the advantages of each |
| • Expected time to experiencing benefits (this is very important as a patient may experience transient tolerability problems such as nausea prior to experiencing and improvement in symptoms and there will therefore be a danger of non-adherence if there has not been appropriate counselling) |
| • Potential toxicities with reassurance about the capability to mitigate these risks through regular and appropriate blood monitoring |
| • The potential tolerability issues, especially nausea with appropriate reassurance that this can be lessened or mitigated with use of folic acid supplementation or parenteral administration |
| • Women of childbearing potential need to be counselled about pregnancy and family planning. |
| • Vaccinations and how to ensure optimum outcomes of vaccination |
| • Alcohol consumption |
| • Interactions between MTX and other medication with particular advice about NSAIDs |

5. Conclusions

In conclusion, MTX is a remarkable drug with a unique place in the pharmacotherapeutic management of RA. Key recommendations for clinical use are listed in Table 4. In the last 2 decades, the cumulative experience of rheumatologists has helped to refine the possibility of achieving the best outcomes with MTX. It has a large dose titratable range and can be administered as a once weekly dose regimen by either oral or subcutaneous routes. Parenteral administration of MTX has the advantages of maximising bioavailability, reducing gastrointestinal intolerance, and potentially enhancing compliance and adherence. MTX is recommended as the first-line csDMARD treatment for RA and can also be used in combination with other csDMARDs, bDMARDs, or tsDMARDs. In the case of concomitant use with targeted therapies, MTX facilitates optimum achievable efficacy, and also has the advantage, when used with bDMARDs, of reducing ADA_b responses to administered biologic. MTX is also highly cost-effective and has a favourable benefit/risk profile overall. Nonetheless, adverse events associated with MTX are well-described and tolerability issues can be a troublesome complication of this treatment. However, with appropriate risk-mitigation strategies, including folic acid supplementation, blood monitoring, and patient education, one can optimise achievable outcomes.

Table 4. Key recommendations for clinical use of MTX. csDMARD, conventional synthetic disease-modifying antirheumatic drugs; bDMARDs, biological DMARDs; tsDMARDs, targeted synthetic DMARDs; SC, subcutaneous.

| Key Points for Clinical Use of MTX |
|--|
| Modify predictors of response to MTX |
| Encourage smoking cessation |
| Limit alcohol consumption |
| Ensure appropriate education on how to optimise outcomes |
| Manage anxiety and depression |
| Start at the right dose |
| Generally 10–15 mg/week, but should be personalized |
| There is no need to start with higher dose |
| Choose between oral and parenteral route. |

Table 4. Cont.

| Key Points for Clinical Use of MTX |
|--|
| Escalate as quickly as tolerated |
| Titrate dose according to individual clinical response Generally aim to reach a dose of at least 20 mg/week after 6 months Consider local recommendations and clinical context |
| Switch to SC route for doses higher than 15 mg/week to enhance MTX bioavailability |
| Prevent side effects |
| Start folic acid supplementation at a dose of at least 5 mg/week and up to 5 mg/day other than the day of methotrexate (or folinic acid at a dose lower than 7.5 mg/week) |
| Instruct the patient to not take folic acid on the day of MTX administration |
| Folinic acid should be administered on a weekly basis the day after MTX administration |
| Counsel patients about risk mitigation through blood monitoring according to local protocols |
| Be aware of potential drug to drug interactions |
| Instruct the patients to seek advice from their rheumatologist about compatibility with methotrexate of self-medicated drugs or drugs prescribed by another physician |
| In case of poor tolerability |
| Diminish gastrointestinal side effects by switching to SC route |
| In case of inadequate response |
| Enhance MTX bioavailability by splitting oral dose or switching to SC route |
| Consider combination with csDMARD, bDMARDs or tsDMARDs Do not stop MTX nor reduce MTX dose unless there are toxicity or tolerability concerns |
| Give time for MTX achieving maximum clinical benefit |
| Use intra-articular or systemic glucocorticoids as bridging therapies |
| Educate the patient and adopt a shared decision-making approach |

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References

1. Heinimann, K.; von Kempis, J.; Sauter, R.; Schiff, M.; Sokka-Isler, T.; Schulze-Koops, H.; Muller, R. Long-Term Increase of Radiographic Damage and Disability in Patients with RA in Relation to Disease Duration in the Era of Biologics. Results from the SCQM Cohort. *J. Clin. Med.* **2018**, *7*, 57. [[CrossRef](#)] [[PubMed](#)]
2. Smolen, J.S.; Breedveld, F.C.; Burmester, G.R.; Bykerk, V.; Dougados, M.; Emery, P.; Kvien, T.K.; Navarro-Compan, M.V.; Oliver, S.; Schoels, M.; et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann. Rheum. Dis.* **2016**, *75*, 3–15. [[CrossRef](#)]
3. Brinkmann, G.H.; Norvang, V.; Norli, E.S.; Grovle, L.; Haugen, A.J.; Lexberg, A.S.; Rodevand, E.; Bakland, G.; Nygaard, H.; Kroll, F.; et al. Treat to target strategy in early rheumatoid arthritis versus routine care—A comparative clinical practice study. *Semin. Arthritis Rheum.* **2018**. [[CrossRef](#)]
4. Smolen, J.S.; Landewe, R.; Bijlsma, J.; Burmester, G.; Chatzidionysiou, K.; Dougados, M.; Nam, J.; Ramiro, S.; Voshaar, M.; van Vollenhoven, R.; 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*, 960–977. [[CrossRef](#)]
5. Singh, J.A.; Saag, K.G.; Bridges, S.L., Jr.; Akl, E.A.; Bannuru, R.R.; Sullivan, M.C.; Vaysbrot, E.; McNaughton, C.; Osani, M.; Shmerling, R.H.; et al. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Care Res. (Hoboken)* **2016**, *68*, 1–25. [[CrossRef](#)] [[PubMed](#)]
6. Bykerk, V.P.; Akhavan, P.; Hazlewood, G.S.; Schieir, O.; Dooley, A.; Harouei, B.; Khraishi, M.; Leclercq, S.A.; Legare, J.; Mosher, D.P.; et al. Canadian Rheumatology Association recommendations for pharmacological management of rheumatoid arthritis with traditional and biologic disease-modifying antirheumatic drugs. *J. Rheumatol.* **2012**, *39*, 1559–1582. [[CrossRef](#)]
7. Lau, C.S.; Chia, F.; Harrison, A.; Hsieh, T.Y.; Jain, R.; Jung, S.M.; Kishimoto, M.; Kumar, A.; Leong, K.P.; Li, Z.; et al. APLAR rheumatoid arthritis treatment recommendations. *Int. J. Rheum. Dis.* **2015**, *18*, 685–713. [[CrossRef](#)] [[PubMed](#)]
8. Felson, D.T.; Smolen, J.S.; Wells, G.; Zhang, B.; van Tuyl, L.H.; Funovits, J.; Aletaha, D.; Allaart, C.F.; Bathon, J.; Bombardieri, S.; 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*, 404–413. [[CrossRef](#)]
9. Kavanaugh, A.; Fleischmann, R.M.; Emery, P.; Kupper, H.; Redden, L.; Guerette, B.; Santra, S.; Smolen, J.S. Clinical, functional and radiographic consequences of achieving stable low disease activity and remission with adalimumab plus methotrexate or methotrexate alone in early rheumatoid arthritis: 26-week results from the randomised, controlled OPTIMA study. *Ann. Rheum. Dis.* **2013**, *72*, 64–71. [[CrossRef](#)]
10. Radner, H.; Smolen, J.S.; Aletaha, D. Remission in rheumatoid arthritis: Benefit over low disease activity in patient-reported outcomes and costs. *Arthritis Res. Ther.* **2014**, *16*, R56. [[CrossRef](#)]
11. Linde, L.; Hetland, M.L.; Ostergaard, M. Drug survival and reasons for discontinuation of intramuscular methotrexate: A study of 212 consecutive patients switching from oral methotrexate. *Scand. J. Rheumatol.* **2006**, *35*, 102–106. [[CrossRef](#)]
12. Thiele, K.; Huscher, D.; Bischoff, S.; Spathling-Mestekemper, S.; Backhaus, M.; Aringer, M.; Kohlmann, T.; Zink, A.; German Collaborative Arthritis Centres. Performance of the 2011 ACR/EULAR preliminary remission criteria compared with DAS28 remission in unselected patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2013**, *72*, 1194–1199. [[CrossRef](#)]
13. Grigor, C.; Capell, H.; Stirling, A.; McMahon, A.D.; Lock, P.; Vallance, R.; Kincaid, W.; Porter, D. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): A single-blind randomised controlled trial. *Lancet* **2004**, *364*, 263–269. [[CrossRef](#)]
14. Verstappen, S.M.; Jacobs, J.W.; van der Veen, M.J.; Heurkens, A.H.; Schenk, Y.; ter Borg, E.J.; Blaauw, A.A.; Bijlsma, J.W.; The Utrecht Rheumatoid Arthritis Cohort Study Group. 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*, 1443–1449. [[CrossRef](#)]
15. Mueller, R.B.; Spaeth, M.; von Restorff, C.; Ackerman, C.; Schulze-Koops, H.; Von Kempis, J. Superiority of a Treat-to Target Strategy over conventional treatment with csDMARD and corticosteroids: A multi-center randomized controlled trial in RA patients with an inadequate response to conventional synthetic DMARDs, and new therapy with certolizumab pegol. *J. Clin. Med.* **2019**, *8*, 302.

16. Kyburz, D.; Gabay, C.; Michel, B.A.; Finckh, A. The long-term impact of early treatment of rheumatoid arthritis on radiographic progression: A population-based cohort study. *Rheumatology (Oxford)* **2011**, *50*, 1106–1110. [[CrossRef](#)]
17. Weinblatt, M.E. Methotrexate in rheumatoid arthritis: A quarter century of development. *Trans. Am. Clin. Climatol. Assoc.* **2013**, *124*, 16–25.
18. Weinblatt, M.E.; Coblyn, J.S.; Fox, D.A.; Fraser, P.A.; Holdsworth, D.E.; Glass, D.N.; Trentham, D.E. Efficacy of low-dose methotrexate in rheumatoid arthritis. *N. Engl. J. Med.* **1985**, *312*, 818–822. [[CrossRef](#)]
19. Brown, P.M.; Pratt, A.G.; Isaacs, J.D. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. *Nat. Rev. Rheumatol.* **2016**, *12*, 731–742. [[CrossRef](#)]
20. Lebbe, C.; Beyeler, C.; Gerber, N.J.; Reichen, J. Intraindividual variability of the bioavailability of low dose methotrexate after oral administration in rheumatoid arthritis. *Ann. Rheum. Dis.* **1994**, *53*, 475–477. [[CrossRef](#)]
21. Schiff, M.H.; Jaffe, J.S.; Freundlich, B. Head-to-head, randomised, crossover study of oral versus subcutaneous methotrexate in patients with rheumatoid arthritis: Drug-exposure limitations of oral methotrexate at doses ≥ 15 mg may be overcome with subcutaneous administration. *Ann. Rheum. Dis.* **2014**, *73*, 1549–1551. [[CrossRef](#)]
22. Hoekstra, M.; Haagsma, C.; Neef, C.; Proost, J.; Knuij, A.; van de Laar, M. Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. *J. Rheumatol.* **2004**, *31*, 645–648.
23. Dalrymple, J.M.; Stamp, L.K.; O'Donnell, J.L.; Chapman, P.T.; Zhang, M.; Barclay, M.L. Pharmacokinetics of oral methotrexate in patients with rheumatoid arthritis. *Arthritis Rheum.* **2008**, *58*, 3299–3308. [[CrossRef](#)]
24. Dervieux, T.; Greenstein, N.; Kremer, J. Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis. *Arthritis Rheum.* **2006**, *54*, 3095–3103. [[CrossRef](#)]
25. Stamp, L.K.; O'Donnell, J.L.; Chapman, P.T.; Zhang, M.; Frampton, C.; James, J.; Barclay, M.L. Determinants of red blood cell methotrexate polyglutamate concentrations in rheumatoid arthritis patients receiving long-term methotrexate treatment. *Arthritis Rheum.* **2009**, *60*, 2248–2256. [[CrossRef](#)]
26. Tishler, M.; Caspi, D.; Graff, E.; Segal, R.; Peretz, H.; Yaron, M. Synovial and serum levels of methotrexate during methotrexate therapy of rheumatoid arthritis. *Br. J. Rheumatol.* **1989**, *28*, 422–423. [[CrossRef](#)]
27. Herman, R.A.; Veng-Pedersen, P.; Hoffman, J.; Koehnke, R.; Furst, D.E. Pharmacokinetics of low-dose methotrexate in rheumatoid arthritis patients. *J. Pharm. Sci.* **1989**, *78*, 165–171. [[CrossRef](#)]
28. Chladek, J.; Martinkova, J.; Simkova, M.; Vaneckova, J.; Koudelkova, V.; Nozickova, M. Pharmacokinetics of low doses of methotrexate in patients with psoriasis over the early period of treatment. *Eur. J. Clin. Pharmacol.* **1998**, *53*, 437–444. [[CrossRef](#)]
29. Dervieux, T.; Furst, D.; Lein, D.O.; Capps, R.; Smith, K.; Caldwell, J.; Kremer, J. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: Results of a multicentred cross sectional observational study. *Ann. Rheum. Dis.* **2005**, *64*, 1180–1185. [[CrossRef](#)]
30. Stamp, L.K.; Barclay, M.L.; O'Donnell, J.L.; Zhang, M.; Drake, J.; Frampton, C.; Chapman, P.T. Effects of changing from oral to subcutaneous methotrexate on red blood cell methotrexate polyglutamate concentrations and disease activity in patients with rheumatoid arthritis. *J. Rheumatol.* **2011**, *38*, 2540–2547. [[CrossRef](#)]
31. Bressolle, F.; Bologna, C.; Kinowski, J.M.; Sany, J.; Combe, B. Effects of moderate renal insufficiency on pharmacokinetics of methotrexate in rheumatoid arthritis patients. *Ann. Rheum. Dis.* **1998**, *57*, 110–113. [[CrossRef](#)] [[PubMed](#)]
32. Chan, E.S.; Cronstein, B.N. Methotrexate—How does it really work? *Nat. Rev. Rheumatol.* **2010**, *6*, 175–178. [[CrossRef](#)] [[PubMed](#)]
33. Wessels, J.A.; van der Kooij, S.M.; le Cessie, S.; Kievit, W.; Barerra, P.; Allaart, C.F.; Huizinga, T.W.; Guchelaar, H.J.; Pharmacogenetics Collaborative Research, G. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. *Arthritis Rheum.* **2007**, *56*, 1765–1775. [[CrossRef](#)] [[PubMed](#)]
34. Lopez-Rodriguez, R.; Ferreiro-Iglesias, A.; Lima, A.; Bernardes, M.; Pawlik, A.; Paradowska-Gorycka, A.; Swierkot, J.; Slezak, R.; Gonzalez-Alvaro, I.; Narvaez, J.; et al. Evaluation of a clinical pharmacogenetics model to predict methotrexate response in patients with rheumatoid arthritis. *Pharmacogenom. J.* **2018**, *18*, 539–545. [[CrossRef](#)] [[PubMed](#)]

35. Hughes, C.D.; Scott, D.L.; Ibrahim, F.; Investigators, T.P. Intensive therapy and remissions in rheumatoid arthritis: A systematic review. *BMC Musculoskelet. Disord.* **2018**, *19*, 389. [[CrossRef](#)] [[PubMed](#)]
36. Smolen, J.S.; Aletaha, D.; Bijlsma, J.W.; Breedveld, F.C.; Boumpas, D.; Burmester, G.; Combe, B.; Cutolo, M.; de Wit, M.; Dougados, M.; et al. Treating rheumatoid arthritis to target: Recommendations of an international task force. *Ann. Rheum. Dis.* **2010**, *69*, 631–637. [[CrossRef](#)]
37. Van Nies, J.A.; Tsonaka, R.; Gaujoux-Viala, C.; Fautrel, B.; van der Helm-van Mil, A.H. 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*, 806–812. [[CrossRef](#)] [[PubMed](#)]
38. O'Dell, J.R.; Curtis, J.R.; Mikuls, T.R.; Cofield, S.S.; Bridges, S.L., Jr.; Ranganath, V.K.; Moreland, L.W.; Investigators, T.T. Validation of the methotrexate-first strategy in patients with early, poor-prognosis rheumatoid arthritis: Results from a two-year randomized, double-blind trial. *Arthritis Rheum.* **2013**, *65*, 1985–1994. [[CrossRef](#)]
39. O'Dell, J.R.; Cohen, S.B.; Thorne, J.C.; Kremer, J. Treatment of rheumatoid arthritis in the USA: Premature use of tumor necrosis factor inhibition and underutilization of concomitant methotrexate. *Open Access Rheumatol.* **2018**, *10*, 97–101. [[CrossRef](#)]
40. Mueller, R.B.; Graninger, W.; Sidiropoulos, P.; Goger, C.; von Kempis, J. Median time to low disease activity is shorter in tocilizumab combination therapy with csDMARDs as compared to tocilizumab monotherapy in patients with active rheumatoid arthritis and inadequate responses to csDMARDs and/or TNF inhibitors: Sub-analysis of the Swiss and Austrian patients from the ACT-SURE study. *Clin. Rheumatol.* **2017**, *36*, 2187–2192. [[CrossRef](#)]
41. Moreland, L.W.; O'Dell, J.R.; Paulus, H.E.; Curtis, J.R.; Bathon, J.M.; St Clair, E.W.; Bridges, S.L., Jr.; Zhang, J.; McVie, T.; Howard, G.; et al. A randomized comparative effectiveness study of oral triple therapy versus etanercept plus methotrexate in early aggressive rheumatoid arthritis: The treatment of Early Aggressive Rheumatoid Arthritis Trial. *Arthritis Rheum.* **2012**, *64*, 2824–2835. [[CrossRef](#)]
42. Goekoop-Ruiterman, Y.P.; de Vries-Bouwstra, J.K.; Allaart, C.F.; van Zeben, D.; Kerstens, P.J.; Hazes, J.M.; Zwinderman, A.H.; Roday, H.K.; Han, K.H.; Westedt, M.L.; et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): A randomized, controlled trial. *Arthritis Rheum.* **2005**, *52*, 3381–3390. [[CrossRef](#)]
43. Muller, R.B.; von Kempis, J.; Haile, S.R.; Schiff, M.H. Effectiveness, tolerability, and safety of subcutaneous methotrexate in early rheumatoid arthritis: A retrospective analysis of real-world data from the St. Gallen cohort. *Semin. Arthritis Rheum.* **2015**, *45*, 28–34. [[CrossRef](#)]
44. Van Vollenhoven, R.F.; Ernestam, S.; Geborek, P.; Petersson, I.F.; Coster, L.; Waltbrand, E.; Zickert, A.; Theander, J.; Thorner, A.; Hellstrom, H.; et al. Addition of infliximab compared with addition of sulfasalazine and hydroxychloroquine to methotrexate in patients with early rheumatoid arthritis (Swefot trial): 1-year results of a randomised trial. *Lancet* **2009**, *374*, 459–466. [[CrossRef](#)]
45. Gabriel, S.E.; Michaud, K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis Res. Ther.* **2009**, *11*, 229. [[CrossRef](#)]
46. Young, A.; Koduri, G.; Batley, M.; Kulinskaya, E.; Gough, A.; Norton, S.; Dixey, J.; Early Rheumatoid Arthritis Study (ERAS) Group. Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis. *Rheumatology (Oxford)* **2007**, *46*, 350–357. [[CrossRef](#)]
47. Gonzalez, A.; Maradit Kremers, H.; Crowson, C.S.; Nicola, P.J.; Davis, J.M., 3rd; Thorne, T.M.; Roger, V.L.; Gabriel, S.E. The widening mortality gap between rheumatoid arthritis patients and the general population. *Arthritis Rheum.* **2007**, *56*, 3583–3587. [[CrossRef](#)]
48. Choi, H.K.; Hernan, M.A.; Seeger, J.D.; Robins, J.M.; Wolfe, F. Methotrexate and mortality in patients with rheumatoid arthritis: A prospective study. *Lancet* **2002**, *359*, 1173–1177. [[CrossRef](#)]
49. Wasko, M.C.; Dasgupta, A.; Hubert, H.; Fries, J.F.; Ward, M.M. Propensity-adjusted association of methotrexate with overall survival in rheumatoid arthritis. *Arthritis Rheum.* **2013**, *65*, 334–342. [[CrossRef](#)]
50. Ridker, P.M.; Everett, B.M.; Pradhan, A.; MacFadyen, J.G.; Solomon, D.H.; Zaharris, E.; Mam, V.; Hasan, A.; Rosenberg, Y.; Iturriaga, E.; et al. Low-Dose Methotrexate for the Prevention of Atherosclerotic Events. *N. Engl. J. Med.* **2019**, *380*, 752–762. [[CrossRef](#)]
51. Atzeni, F.; Boiardi, L.; Salli, S.; Benucci, M.; Sarzi-Puttini, P. Lung involvement and drug-induced lung disease in patients with rheumatoid arthritis. *Expert Rev. Clin. Immunol.* **2013**, *9*, 649–657. [[CrossRef](#)]
52. Suda, T. Up-to-Date Information on Rheumatoid Arthritis-Associated Interstitial Lung Disease. *Clin. Med. Insights Circ. Respir. Pulm. Med.* **2015**, *9*, 155–162. [[CrossRef](#)]

53. Rojas-Serrano, J.; Herrera-Bringas, D.; Perez-Roman, D.I.; Perez-Dorame, R.; Mateos-Toledo, H.; Mejia, M. Rheumatoid arthritis-related interstitial lung disease (RA-ILD): Methotrexate and the severity of lung disease are associated to prognosis. *Clin. Rheumatol.* **2017**, *36*, 1493–1500. [[CrossRef](#)]
54. England, B.R.; Sayles, H.; Michaud, K.; Thiele, G.M.; Poole, J.A.; Caplan, L.; Sauer, B.C.; Cannon, G.W.; Reimold, A.; Kerr, G.S.; et al. Chronic lung disease in U.S. Veterans with rheumatoid arthritis and the impact on survival. *Clin. Rheumatol.* **2018**, *37*, 2907–2915. [[CrossRef](#)]
55. Conway, R.; Carey, J.J. Methotrexate and lung disease in rheumatoid arthritis. *Paininerva Med.* **2017**, *59*, 33–46. [[CrossRef](#)]
56. Conway, R.; Low, C.; Coughlan, R.J.; O'Donnell, M.J.; Carey, J.J. Methotrexate and lung disease in rheumatoid arthritis: A meta-analysis of randomized controlled trials. *Arthritis Rheumatol.* **2014**, *66*, 803–812. [[CrossRef](#)]
57. Conway, R.; Low, C.; Coughlan, R.J.; O'Donnell, M.J.; Carey, J.J. Methotrexate use and risk of lung disease in psoriasis, psoriatic arthritis, and inflammatory bowel disease: Systematic literature review and meta-analysis of randomised controlled trials. *BMJ* **2015**, *350*, h1269. [[CrossRef](#)]
58. Mason, A.; Holmes, C.; Edwards, C.J. Inflammation and dementia: Using rheumatoid arthritis as a model to develop treatments? *Autoimmun. Rev.* **2018**, *17*, 919–925. [[CrossRef](#)]
59. Judge, A.; Garriga, C.; Arden, N.K.; Lovestone, S.; Prieto-Alhambra, D.; Cooper, C.; Edwards, C.J. Protective effect of antirheumatic drugs on dementia in rheumatoid arthritis patients. *Alzheimers Dement. (N. Y.)* **2017**, *3*, 612–621. [[CrossRef](#)]
60. Lazaro, E.; Morel, J. Management of neutropenia in patients with rheumatoid arthritis. *Jt. Bone Spine* **2015**, *82*, 235–239. [[CrossRef](#)]
61. Allen, L.S.; Groff, G. Treatment of Felty's syndrome with low-dose oral methotrexate. *Arthritis Rheum.* **1986**, *29*, 902–905. [[CrossRef](#)]
62. Isasi, C.; Lopez-Martin, J.A.; Angeles Trujillo, M.; Andreu, J.L.; Palacio, S.; Mulero, J. Felty's syndrome: Response to low dose oral methotrexate. *J. Rheumatol.* **1989**, *16*, 983–985. [[PubMed](#)]
63. Finckh, A.; Bansback, N.; Marra, C.A.; Anis, A.H.; Michaud, K.; Lubin, S.; White, M.; Sizto, S.; Liang, M.H. Treatment of very early rheumatoid arthritis with symptomatic therapy, disease-modifying antirheumatic drugs, or biologic agents: A cost-effectiveness analysis. *Ann. Intern. Med.* **2009**, *151*, 612–621. [[CrossRef](#)]
64. Fitzpatrick, R.; Scott, D.G.; Keary, I. Cost-minimisation analysis of subcutaneous methotrexate versus biologic therapy for the treatment of patients with rheumatoid arthritis who have had an insufficient response or intolerance to oral methotrexate. *Clin. Rheumatol.* **2013**, *32*, 1605–1612. [[CrossRef](#)]
65. Mainman, H.; McClaren, E.; Heycock, C.; Saravanan, V.; Hamilton, J.; Kelly, C. When should we use parenteral methotrexate? *Clin. Rheumatol.* **2010**, *29*, 1093–1098. [[CrossRef](#)]
66. Deighton, C.; Hyrich, K.; Ding, T.; Ledingham, J.; Lunt, M.; Luqmani, R.; Kiely, P.; Bukhari, M.; Abernethy, R.; Ostor, A.; et al. BSR and BHPR rheumatoid arthritis guidelines on eligibility criteria for the first biological therapy. *Rheumatology (Oxford)* **2010**, *49*, 1197–1199. [[CrossRef](#)]
67. Carmichael, S.J.; Beal, J.; Day, R.O.; Tett, S.E. Combination therapy with methotrexate and hydroxychloroquine for rheumatoid arthritis increases exposure to methotrexate. *J. Rheumatol.* **2002**, *29*, 2077–2083. [[PubMed](#)]
68. Schapink, L.; van den Ende, C.H.M.; Gevers, L.; van Ede, A.E.; den Broeder, A.A. The effects of methotrexate and hydroxychloroquine combination therapy vs methotrexate monotherapy in early rheumatoid arthritis patients. *Rheumatology (Oxford)* **2018**, *58*, 131–134. [[CrossRef](#)]
69. Hazlewood, G.S.; Barnabe, C.; Tomlinson, G.; Marshall, D.; Devoe, D.J.; Bombardier, C. Methotrexate monotherapy and methotrexate combination therapy with traditional and biologic disease modifying anti-rheumatic drugs for rheumatoid arthritis: A network meta-analysis. *Cochrane Database Syst. Rev.* **2016**, CD010227. [[CrossRef](#)]
70. Peper, S.M.; Lew, R.; Mikuls, T.; Brophy, M.; Rybin, D.; Wu, H.; O'Dell, J. Rheumatoid Arthritis Treatment After Methotrexate: The Durability of Triple Therapy Versus Etanercept. *Arthritis Care Res. (Hoboken)* **2017**, *69*, 1467–1472. [[CrossRef](#)] [[PubMed](#)]
71. Quach, L.T.; Chang, B.H.; Brophy, M.T.; Soe Thwin, S.; Hannagan, K.; O'Dell, J.R. Rheumatoid arthritis triple therapy compared with etanercept: Difference in infectious and gastrointestinal adverse events. *Rheumatology (Oxford)* **2017**, *56*, 378–383. [[CrossRef](#)]
72. Fleischmann, R.; Tongbram, V.; van Vollenhoven, R.; Tang, D.H.; Chung, J.; Collier, D.; Urs, S.; Ndirangu, K.; Wells, G.; Pope, J. Systematic review and network meta-analysis of the efficacy and safety of tumour necrosis

- factor inhibitor-methotrexate combination therapy versus triple therapy in rheumatoid arthritis. *RMD Open* **2017**, *3*, e000371. [[CrossRef](#)]
73. Bathon, J.M.; Martin, R.W.; Fleischmann, R.M.; Tesser, J.R.; Schiff, M.H.; Keystone, E.C.; Genovese, M.C.; Wasko, M.C.; Moreland, L.W.; Weaver, A.L.; et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N. Engl. J. Med.* **2000**, *343*, 1586–1593. [[CrossRef](#)]
74. Breedveld, F.C.; Weisman, M.H.; Kavanaugh, A.F.; Cohen, S.B.; Pavelka, K.; van Vollenhoven, R.; Sharp, J.; Perez, J.L.; Spencer-Green, G.T. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum.* **2006**, *54*, 26–37. [[CrossRef](#)]
75. Emery, P.; Breedveld, F.C.; Hall, S.; Durez, P.; Chang, D.J.; Robertson, D.; Singh, A.; Pedersen, R.D.; Koenig, A.S.; Freundlich, B. Comparison of methotrexate monotherapy with a combination of methotrexate and etanercept in active, early, moderate to severe rheumatoid arthritis (COMET): A randomised, double-blind, parallel treatment trial. *Lancet* **2008**, *372*, 375–382. [[CrossRef](#)]
76. Emery, P.; Fleischmann, R.; van der Heijde, D.; Keystone, E.C.; Genovese, M.C.; Conaghan, P.G.; Hsia, E.C.; Xu, W.; Baratele, A.; Beutler, A.; et al. The effects of golimumab on radiographic progression in rheumatoid arthritis: Results of randomized controlled studies of golimumab before methotrexate therapy and golimumab after methotrexate therapy. *Arthritis Rheum.* **2011**, *63*, 1200–1210. [[CrossRef](#)] [[PubMed](#)]
77. Burmester, G.R.; Kivitz, A.J.; Kupper, H.; Arulmani, U.; Florentinus, S.; Goss, S.L.; Rathmann, S.S.; Fleischmann, R.M. Efficacy and safety of ascending methotrexate dose in combination with adalimumab: The randomised CONCERTO trial. *Ann. Rheum. Dis.* **2015**, *74*, 1037–1044. [[CrossRef](#)]
78. Kaeley, G.S.; MacCarter, D.K.; Goyal, J.R.; Liu, S.; Chen, K.; Griffith, J.; Kupper, H.; Garg, V.; Kalabic, J. Similar Improvements in Patient-Reported Outcomes Among Rheumatoid Arthritis Patients Treated with Two Different Doses of Methotrexate in Combination with Adalimumab: Results From the MUSICA Trial. *Rheumatol. Ther.* **2018**, *5*, 123–134. [[CrossRef](#)]
79. Bandres Ciga, S.; Salvatierra, J.; Lopez-Sidro, M.; Garcia-Sanchez, A.; Duran, R.; Vives, F.; Raya-Alvarez, E. An examination of the mechanisms involved in secondary clinical failure to adalimumab or etanercept in inflammatory arthropathies. *J. Clin. Rheumatol.* **2015**, *21*, 115–119. [[CrossRef](#)]
80. Jani, M.; Barton, A.; Warren, R.B.; Griffiths, C.E.; Chinoy, H. The role of DMARDs in reducing the immunogenicity of TNF inhibitors in chronic inflammatory diseases. *Rheumatology (Oxford)* **2014**, *53*, 213–222. [[CrossRef](#)]
81. Jani, M.; Chinoy, H.; Warren, R.B.; Griffiths, C.E.; Plant, D.; Fu, B.; Morgan, A.W.; Wilson, A.G.; Isaacs, J.D.; Hyrich, K.; et al. Clinical utility of random anti-tumor necrosis factor drug-level testing and measurement of antidrug antibodies on the long-term treatment response in rheumatoid arthritis. *Arthritis Rheumatol.* **2015**, *67*, 2011–2019. [[CrossRef](#)]
82. Bartelds, G.M.; Krieckaert, C.L.; Nurmohamed, M.T.; van Schouwenburg, P.A.; Lems, W.F.; Twisk, J.W.; Dijkmans, B.A.; Aarden, L.; Wolbink, G.J. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA* **2011**, *305*, 1460–1468. [[CrossRef](#)]
83. Pascual-Salcedo, D.; Plasencia, C.; Ramiro, S.; Nuno, L.; Bonilla, G.; Nagore, D.; Ruiz Del Agua, A.; Martinez, A.; Aarden, L.; Martin-Mola, E.; et al. Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis. *Rheumatology (Oxford)* **2011**, *50*, 1445–1452. [[CrossRef](#)]
84. Balsa, A.; Sanmarti, R.; Rosas, J.; Martin, V.; Cabeza, A.; Gomez, S.; Montoro, M. Drug immunogenicity in patients with inflammatory arthritis and secondary failure to tumour necrosis factor inhibitor therapies: The REASON study. *Rheumatology (Oxford)* **2018**, *57*, 688–693. [[CrossRef](#)] [[PubMed](#)]
85. Vogelzang, E.H.; Kneepkens, E.L.; Nurmohamed, M.T.; van Kuijk, A.W.; Rispen, T.; Wolbink, G.; Krieckaert, C.L. Anti-adalimumab antibodies and adalimumab concentrations in psoriatic arthritis; an association with disease activity at 28 and 52 weeks of follow-up. *Ann. Rheum. Dis.* **2014**, *73*, 2178–2182. [[CrossRef](#)]
86. Bartelds, G.M.; Wijbrandts, C.A.; Nurmohamed, M.T.; Stapel, S.; Lems, W.F.; Aarden, L.; Dijkmans, B.A.; Tak, P.P.; Wolbink, G.J. Clinical response to adalimumab: Relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann. Rheum. Dis.* **2007**, *66*, 921–926. [[CrossRef](#)]

87. Ungar, B.; Kopylov, U.; Engel, T.; Yavzori, M.; Fudim, E.; Picard, O.; Lang, A.; Williet, N.; Paul, S.; Chowers, Y.; et al. Addition of an immunomodulator can reverse antibody formation and loss of response in patients treated with adalimumab. *Aliment. Pharmacol. Ther.* **2017**, *45*, 276–282. [[CrossRef](#)]
88. Qiu, Y.; Mao, R.; Chen, B.L.; Zhang, S.H.; Guo, J.; He, Y.; Zeng, Z.R.; Ben-Horin, S.; Chen, M.H. Effects of Combination Therapy With Immunomodulators on Trough Levels and Antibodies Against Tumor Necrosis Factor Antagonists in Patients With Inflammatory Bowel Disease: A Meta-analysis. *Clin. Gastroenterol. Hepatol.* **2017**, *15*, 1359.e6–1372.e6. [[CrossRef](#)]
89. Krieckaert, C.L.; Nurmohamed, M.T.; Wolbink, G.J. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. *Ann. Rheum. Dis.* **2012**, *71*, 1914–1915. [[CrossRef](#)] [[PubMed](#)]
90. Kaeley, G.S.; Evangelisto, A.M.; Nishio, M.J.; Goss, S.L.; Liu, S.; Kalabic, J.; Kupper, H. Methotrexate dosage reduction upon adalimumab initiation: Clinical and ultrasonographic outcomes from the randomized noninferiority MUSICA trial. *J. Rheumatol.* **2016**, *43*, 1480–1489. [[CrossRef](#)] [[PubMed](#)]
91. Klareskog, L.; van der Heijde, D.; de Jager, J.P.; Gough, A.; Kalden, J.; Malaise, M.; Martin Mola, E.; Pavelka, K.; Sany, J.; Settas, L.; et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: Double-blind randomised controlled trial. *Lancet* **2004**, *363*, 675–681. [[CrossRef](#)]
92. Quintin, E.; Scoazec, J.Y.; Marotte, H.; Miossec, P. Rare incidence of methotrexate-specific lesions in liver biopsy of patients with arthritis and elevated liver enzymes. *Arthritis Res. Ther.* **2010**, *12*, R143. [[CrossRef](#)]
93. Simms, R.W.; Kwok, C.K.; Anderson, L.G.; Erlandson, D.M.; Greene, J.M.; Kelleher, M.; O'Dell, J.R.; Partridge, A.J.; Roberts, W.N.; Robbins, M.L. Guidelines for monitoring drug therapy in rheumatoid arthritis: American College of Rheumatology Ad Hoc Committee on Clinical Guidelines. *Arthritis Rheum.* **1996**, *39*, 723–731.
94. Visser, K.; Katchamart, W.; Loza, E.; Martinez-Lopez, J.; Salliot, C.; Trudeau, J.; Bombardier, C.; Carmona, L.; Van der Heijde, D.; Bijlsma, J.; et al. Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: Integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. *Ann. Rheum. Dis.* **2009**, *68*, 1086–1093.
95. Price, S.; James, C.; Deighton, C. Methotrexate use and alcohol. *Clin. Exp. Rheumatol.* **2010**, *28*, S114–S116.
96. Humphreys, J.H.; Warner, A.; Costello, R.; Lunt, M.; Verstappen, S.M.M.; Dixon, W.G. Quantifying the hepatotoxic risk of alcohol consumption in patients with rheumatoid arthritis taking methotrexate. *Ann. Rheum. Dis.* **2017**, *76*, 1509–1514. [[CrossRef](#)]
97. Kremer, J.M.; Weinblatt, M.E. Quantifying the hepatotoxic risk of alcohol consumption in patients with rheumatoid arthritis taking methotrexate. *Ann. Rheum. Dis.* **2018**, *77*, e4. [[CrossRef](#)]
98. Bernatsky, S.; Ehrmann Feldman, D. Discontinuation of methotrexate therapy in older patients with newly diagnosed rheumatoid arthritis: Analysis of administrative health databases in Quebec, Canada. *Drugs Aging* **2008**, *25*, 879–884. [[CrossRef](#)]
99. Van den Bemt, B.J.F.; van den Hoogen, F.H.J.; Benraad, B.; Hekster, Y.A.; van Riel, P.L.C.M.; van Lankveld, W. Adherence Rates and Associations with Nonadherence in Patients with Rheumatoid Arthritis Using Disease Modifying Antirheumatic Drugs. *J. Rheumatol.* **2009**, *36*, 2164–2170. [[CrossRef](#)]
100. Contreras-Yanez, I.; Ponce De Leon, S.; Cabiedes, J.; Rull-Gabayet, M.; Pascual-Ramos, V. Inadequate therapy behavior is associated to disease flares in patients with rheumatoid arthritis who have achieved remission with disease-modifying antirheumatic drugs. *Am. J. Med. Sci.* **2010**, *340*, 282–290. [[CrossRef](#)]
101. Anderson, J.J.; Wells, G.; Verhoeven, A.C.; Felson, D.T. Factors predicting response to treatment in rheumatoid arthritis: The importance of disease duration. *Arthritis Rheum.* **2000**, *43*, 22–29. [[CrossRef](#)]
102. Saevarsdottir, S.; Wallin, H.; Seddighzadeh, M.; Ernestam, S.; Geborek, P.; Petersson, I.F.; Bratt, J.; van Vollenhoven, R.F.; SWEFOT Trial Investigators Group. Predictors of response to methotrexate in early DMARD naive rheumatoid arthritis: Results from the initial open-label phase of the SWEFOT trial. *Ann. Rheum. Dis.* **2011**, *70*, 469–475. [[CrossRef](#)]
103. Teitsma, X.M.; Jacobs, J.W.G.; Welsing, P.M.J.; de Jong, P.H.P.; Hazes, J.M.W.; Weel, A.; Petho-Schramm, A.; Borm, M.E.A.; van Laar, J.M.; Lafeber, E.; et al. Inadequate response to treat-to-target methotrexate therapy in patients with new-onset rheumatoid arthritis: Development and validation of clinical predictors. *Ann. Rheum. Dis.* **2018**, *77*, 1261–1267. [[CrossRef](#)]

104. Romao, V.C.; Canhao, H.; Fonseca, J.E. Old drugs, old problems: Where do we stand in prediction of rheumatoid arthritis responsiveness to methotrexate and other synthetic DMARDs? *BMC Med.* **2013**, *11*, 17. [[CrossRef](#)]
105. Saevarsdottir, S.; Wedren, S.; Seddighzadeh, M.; Bengtsson, C.; Wesley, A.; Lindblad, S.; Askling, J.; Alfredsson, L.; Klareskog, L. Patients with early rheumatoid arthritis who smoke are less likely to respond to treatment with methotrexate and tumor necrosis factor inhibitors: Observations from the Epidemiological Investigation of Rheumatoid Arthritis and the Swedish Rheumatology Register cohorts. *Arthritis Rheum.* **2011**, *63*, 26–36. [[CrossRef](#)]
106. Crowson, C.S.; Rollefstad, S.; Ikdahl, E.; Kitas, G.D.; van Riel, P.; Gabriel, S.E.; Matteson, E.L.; Kvien, T.K.; Douglas, K.; Sandoo, A.; et al. Impact of risk factors associated with cardiovascular outcomes in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2018**, *77*, 48–54. [[CrossRef](#)]
107. Joseph, R.M.; Movahedi, M.; Dixon, W.G.; Symmons, D.P. Smoking-Related Mortality in Patients With Early Rheumatoid Arthritis: A Retrospective Cohort Study Using the Clinical Practice Research Datalink. *Arthritis Care Res. (Hoboken)* **2016**, *68*, 1598–1606. [[CrossRef](#)]
108. Sergeant, J.C.; Hyrich, K.L.; Anderson, J.; Kopec-Harding, K.; Hope, H.F.; Symmons, D.P.M.; Co-Investigators, R.; Barton, A.; Verstappen, S.M.M. Prediction of primary non-response to methotrexate therapy using demographic, clinical and psychosocial variables: Results from the UK Rheumatoid Arthritis Medication Study (RAMS). *Arthritis Res. Ther.* **2018**, *20*, 147. [[CrossRef](#)]
109. Hamilton, R.A.; Kremer, J.M. Why intramuscular methotrexate may be more efficacious than oral dosing in patients with rheumatoid arthritis. *Br. J. Rheumatol.* **1997**, *36*, 86–90. [[CrossRef](#)]
110. Pichlmeier, U.; Heuer, K.U. Subcutaneous administration of methotrexate with a prefilled autoinjector pen results in a higher relative bioavailability compared with oral administration of methotrexate. *Clin. Exp. Rheumatol.* **2014**, *32*, 563–571.
111. Braun, J.; Kastner, P.; Flaxenberg, P.; Wahrisch, J.; Hanke, P.; Demary, W.; von Hinuber, U.; Rockwitz, K.; Heitz, W.; Pichlmeier, U.; et al. Comparison of the clinical efficacy and safety of subcutaneous versus oral administration of methotrexate in patients with active rheumatoid arthritis: Results of a six-month, multicenter, randomized, double-blind, controlled, phase IV trial. *Arthritis Rheum.* **2008**, *58*, 73–81. [[CrossRef](#)]
112. Hazlewood, G.S.; Thorne, J.C.; Pope, J.E.; Lin, D.; Tin, D.; Boire, G.; Haraoui, B.; Hitchon, C.A.; Keystone, E.C.; Jamal, S.; et al. The comparative effectiveness of oral versus subcutaneous methotrexate for the treatment of early rheumatoid arthritis. *Ann. Rheum. Dis.* **2016**, *75*, 1003–1008. [[CrossRef](#)]
113. Gaujoux-Viala, C.; Rincheval, N.; Dougados, M.; Combe, B.; Fautrel, B. Optimal methotrexate dose is associated with better clinical outcomes than non-optimal dose in daily practice: Results from the ESPOIR early arthritis cohort. *Ann. Rheum. Dis.* **2017**, *76*, 2054–2060. [[CrossRef](#)]
114. Atsumi, T.; Yamamoto, K.; Takeuchi, T.; Yamanaka, H.; Ishiguro, N.; Tanaka, Y.; Eguchi, K.; Watanabe, A.; Origasa, H.; Yasuda, S.; et al. The first double-blind, randomised, parallel-group certolizumab pegol study in methotrexate-naïve early rheumatoid arthritis patients with poor prognostic factors, C-OPERA, shows inhibition of radiographic progression. *Ann. Rheum. Dis.* **2016**, *75*, 75–83. [[CrossRef](#)]
115. Wevers-de Boer, K.; Visser, K.; Heimans, L.; Runday, H.K.; Molenaar, E.; Groenendaal, J.H.; Peeters, A.J.; Westedt, M.L.; Collee, G.; de Sonnaville, P.B.; 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*, 1472–1477. [[CrossRef](#)]
116. Bergstra, S.A.; Allaart, C.F.; Stijnen, T.; Landewe, R.B.M. Meta-Regression of a Dose-Response Relationship of Methotrexate in Mono- and Combination Therapy in Disease-Modifying Antirheumatic Drug-Naïve Early Rheumatoid Arthritis Patients. *Arthritis Care Res. (Hoboken)* **2017**, *69*, 1473–1483. [[CrossRef](#)]
117. Rezaei, H.; Saevarsdottir, S.; Forslind, K.; Albertsson, K.; Wallin, H.; Bratt, J.; Ernestam, S.; Geborek, P.; Pettersson, I.F.; van Vollenhoven, R.F. In early rheumatoid arthritis, patients with a good initial response to methotrexate have excellent 2-year clinical outcomes, but radiological progression is not fully prevented: Data from the methotrexate responders population in the SWEFOT trial. *Ann. Rheum. Dis.* **2012**, *71*, 186–191. [[CrossRef](#)]
118. Smolen, J.S.; Aletaha, D. Rheumatoid arthritis therapy reappraisal: Strategies, opportunities and challenges. *Nat. Rev. Rheumatol.* **2015**, *11*, 276–289. [[CrossRef](#)]
119. Emery, P.; Bingham, C.O., 3rd; Burmester, G.R.; Bykerk, V.P.; Furst, D.E.; Mariette, X.; van der Heijde, D.; van Vollenhoven, R.; Arendt, C.; Mountian, I.; et al. Certolizumab pegol in combination with dose-optimised methotrexate

- in DMARD-naïve patients with early, active rheumatoid arthritis with poor prognostic factors: 1-year results from C-EARLY, a randomised, double-blind, placebo-controlled phase III study. *Ann. Rheum. Dis.* **2017**, *76*, 96–104. [[CrossRef](#)]
120. Li, R.; Zhao, J.X.; Su, Y.; He, J.; Chen, L.N.; Gu, F.; Zhao, C.; Deng, X.R.; Zhou, W.; Hao, Y.J.; et al. High remission and low relapse with prolonged intensive DMARD therapy in rheumatoid arthritis (PRINT): A multicenter randomized clinical trial. *Medicine (Baltimore)* **2016**, *95*, e3968. [[CrossRef](#)]
 121. Lambert, C.M.; Sandhu, S.; Lochhead, A.; Hurst, N.P.; McRorie, E.; Dhillon, V. Dose escalation of parenteral methotrexate in active rheumatoid arthritis that has been unresponsive to conventional doses of methotrexate: A randomized, controlled trial. *Arthritis Rheum.* **2004**, *50*, 364–371. [[CrossRef](#)]
 122. Daïen, C.; Hua, C.; Gaujoux-Viala, C.; Cantagrel, A.; Dubremetz, M.; Dougados, M.; Fautrel, B.; Mariette, X.; Nayral, N.; Richez, C.; et al. Update of French Society for Rheumatology Recommendations for Managing Rheumatoid Arthritis. *Jt. Bone Spine* **2019**, *86*, 135–150. [[CrossRef](#)]
 123. Dhaon, P.; Das, S.K.; Srivastava, R.; Agarwal, G.; Asthana, A. Oral Methotrexate in split dose weekly versus oral or parenteral Methotrexate once weekly in Rheumatoid Arthritis: A short-term study. *Int. J. Rheum. Dis.* **2018**, *21*, 1010–1017. [[CrossRef](#)]
 124. Rutkowska-Sak, L.; Rell-Bakalarska, M.; Lisowska, B. Oral vs. subcutaneous low-dose methotrexate treatment in reducing gastrointestinal side effects. *Reumatol./Rheumatol.* **2009**, *47*, 207–211.
 125. Kruger, K.; Wollenhaupt, J.; Albrecht, K.; Alten, R.; Backhaus, M.; Baerwald, C.; Bolten, W.; Braun, J.; Burkhardt, H.; Burmester, G.; et al. German 2012 guidelines for the sequential medical treatment of rheumatoid arthritis. Adapted EULAR recommendations and updated treatment algorithm. *Z. Rheumatol.* **2012**, *71*, 592–603. [[CrossRef](#)]
 126. Chakravarty, K.; McDonald, H.; Pullar, T.; Taggart, A.; Chalmers, R.; Oliver, S.; Mooney, J.; Somerville, M.; Bosworth, A.; Kennedy, T.; et al. BSR/BHPR guideline for disease-modifying anti-rheumatic drug (DMARD) therapy in consultation with the British Association of Dermatologists. *Rheumatology (Oxford)* **2008**, *47*, 924–925. [[CrossRef](#)]
 127. Bingham, S.J.; Buch, M.H.; Lindsay, S.; Pollard, A.; White, J.; Emery, P. Parenteral methotrexate should be given before biological therapy. *Rheumatology (Oxford)* **2003**, *42*, 1009–1010. [[CrossRef](#)]
 128. Rozin, A.; Schapira, D.; Balbir-Gurman, A.; Braun-Moscovici, Y.; Markovits, D.; Militianu, D.; Nahir, M.A. Relapse of rheumatoid arthritis after substitution of oral for parenteral administration of methotrexate. *Ann. Rheum. Dis.* **2002**, *61*, 756–757. [[CrossRef](#)]
 129. Węgrzyn, J.; Adeleine, P.; Miossec, P. Better efficacy of methotrexate given by intramuscular injection than orally in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **2004**, *63*, 1232–1234. [[CrossRef](#)]
 130. Harris, E.; Ng, B. Using subcutaneous methotrexate to prolong duration of methotrexate therapy in rheumatoid arthritis. *Eur. J. Rheumatol.* **2018**, *5*, 85–91. [[CrossRef](#)]
 131. Bakker, M.F.; Jacobs, J.W.; Welsing, P.M.; van der Werf, J.H.; Linn-Rasker, S.P.; van der Veen, M.J.; Lafeber, F.P.; Bijlsma, J.W.; Utrecht Arthritis Cohort Study Group. Are switches from oral to subcutaneous methotrexate or addition of ciclosporin to methotrexate useful steps in a tight control treatment strategy for rheumatoid arthritis? A post hoc analysis of the CAMERA study. *Ann. Rheum. Dis.* **2010**, *69*, 1849–1852. [[CrossRef](#)] [[PubMed](#)]
 132. Hameed, B.; Jones, H. Subcutaneous methotrexate is well tolerated and superior to oral methotrexate in the treatment of rheumatoid arthritis. *Int. J. Rheum. Dis.* **2010**, *13*, e83–e84. [[CrossRef](#)] [[PubMed](#)]
 133. Ng, B.; Chu, A. Factors associated with methotrexate dosing and therapeutic decisions in veterans with rheumatoid arthritis. *Clin. Rheumatol.* **2014**, *33*, 21–30. [[CrossRef](#)] [[PubMed](#)]
 134. Pasma, A.; den Boer, E.; van't Spijker, A.; Timman, R.; van den Bemt, B.; Busschbach, J.J.; Hazes, J.M. Nonadherence to disease modifying antirheumatic drugs in the first year after diagnosis: Comparing three adherence measures in early arthritis patients. *Rheumatology (Oxford)* **2016**, *55*, 1812–1819. [[CrossRef](#)] [[PubMed](#)]
 135. Axelsen, M.B.; Eshed, I.; Horslev-Petersen, K.; Stengaard-Pedersen, K.; Hetland, M.L.; Moller, J.; Junker, P.; Podenphant, J.; Schlemmer, A.; Ellingsen, T.; et al. A treat-to-target strategy with methotrexate and intra-articular triamcinolone with or without adalimumab effectively reduces MRI synovitis, osteitis and tenosynovitis and halts structural damage progression in early rheumatoid arthritis: Results from the OPERA randomised controlled trial. *Ann. Rheum. Dis.* **2015**, *74*, 867–875. [[CrossRef](#)] [[PubMed](#)]
 136. Alarcon, G.S.; Tracy, I.C.; Blackburn, W.D., Jr. Methotrexate in rheumatoid arthritis. Toxic effects as the major factor in limiting long-term treatment. *Arthritis Rheum.* **1989**, *32*, 671–676. [[CrossRef](#)]

137. Van Ede, A.E.; Laan, R.F.; Rood, M.J.; Huizinga, T.W.; van de Laar, M.A.; van Denderen, C.J.; Westgeest, T.A.; Romme, T.C.; de Rooij, D.J.; Jacobs, M.J.; et al. Effect of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid arthritis: A forty-eight week, multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum.* **2001**, *44*, 1515–1524. [[CrossRef](#)]
138. Leeb, B.F.; Witzmann, G.; Ogris, E.; Studnicka-Benke, A.; Andel, I.; Schweitzer, H.; Smolen, J.S. Folic acid and cyanocobalamin levels in serum and erythrocytes during low-dose methotrexate therapy of rheumatoid arthritis and psoriatic arthritis patients. *Clin. Exp. Rheumatol.* **1995**, *13*, 459–463. [[PubMed](#)]
139. Shea, B.; Swinden, M.V.; Tanjong Ghogomu, E.; Ortiz, Z.; Katchamart, W.; Rader, T.; Bombardier, C.; Wells, G.A.; Tugwell, P. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *Cochrane Database Syst. Rev.* **2013**, CD000951. [[CrossRef](#)] [[PubMed](#)]
140. Hartman, M.; van Ede, A.; Severens, J.L.; Laan, R.F.; van de Putte, L.; van der Wilt, G.J. Economic evaluation of folate supplementation during methotrexate treatment in rheumatoid arthritis. *J. Rheumatol.* **2004**, *31*, 902–908.
141. Schmajuk, G.; Tonner, C.; Miao, Y.; Yazdany, J.; Gannon, J.; Boscardin, W.J.; Daikh, D.I.; Steinman, M.A. Folic Acid Supplementation Is Suboptimal in a National Cohort of Older Veterans Receiving Low Dose Oral Methotrexate. *PLoS ONE* **2016**, *11*, e0168369. [[CrossRef](#)] [[PubMed](#)]
142. Liu, L.; Liu, S.; Wang, C.; Guan, W.; Zhang, Y.; Hu, W.; Zhang, L.; He, Y.; Lu, J.; Li, T.; et al. Folate Supplementation for Methotrexate Therapy in Patients With Rheumatoid Arthritis: A Systematic Review. *J Clin. Rheumatol.* **2018**. [[CrossRef](#)] [[PubMed](#)]
143. Mazaud, C.; Fardet, L. Relative risk of and determinants for adverse events of methotrexate prescribed at a low dose: A systematic review and meta-analysis of randomized placebo-controlled trials. *Br. J. Dermatol.* **2017**, *177*, 978–986. [[CrossRef](#)] [[PubMed](#)]
144. Stamp, L.K.; O'Donnell, J.L.; Frampton, C.; Drake, J.; Zhang, M.; Barclay, M.; Chapman, P.T. A Pilot Randomized Controlled Double-Blind Trial of High- Versus Low-Dose Weekly Folic Acid in People With Rheumatoid Arthritis Receiving Methotrexate. *J. Clin. Rheumatol.* **2018**. [[CrossRef](#)]
145. Svanstrom, H.; Lund, M.; Melbye, M.; Pasternak, B. Concomitant use of low-dose methotrexate and NSAIDs and the risk of serious adverse events among patients with rheumatoid arthritis. *Pharmacoepidemiol. Drug Saf.* **2018**, *27*, 885–893. [[CrossRef](#)]
146. Iyyadurai, R.; Carey, R.A.; Satyendra, S. Low-dose methotrexate-induced acute interstitial pneumonitis: Report of two cases from South India and review of literature. *J. Fam. Med. Prim. Care* **2016**, *5*, 875–878. [[CrossRef](#)]
147. Kremer, J.M.; Alarcon, G.S.; Weinblatt, M.E.; Kaymakcian, M.V.; Macaluso, M.; Cannon, G.W.; Palmer, W.R.; Sundry, J.S.; St Clair, E.W.; Alexander, R.W.; et al. Clinical, laboratory, radiographic, and histopathologic features of methotrexate-associated lung injury in patients with rheumatoid arthritis: A multicenter study with literature review. *Arthritis Rheum.* **1997**, *40*, 1829–1837. [[CrossRef](#)]
148. Alarcon, G.S.; Kremer, J.M.; Macaluso, M.; Weinblatt, M.E.; Cannon, G.W.; Palmer, W.R.; St Clair, E.W.; Sundry, J.S.; Alexander, R.W.; Smith, G.J.; et al. Risk factors for methotrexate-induced lung injury in patients with rheumatoid arthritis. A multicenter, case-control study. *Ann. Intern. Med.* **1997**, *127*, 356–364. [[CrossRef](#)]
149. Tokuda, H.; Sakai, F.; Yamada, H.; Johkoh, T.; Imamura, A.; Dohi, M.; Hiramata, M.; Yamada, T.; Kamatani, N.; Kikuchi, Y.; et al. Clinical and radiological features of Pneumocystis pneumonia in patients with rheumatoid arthritis, in comparison with methotrexate pneumonitis and Pneumocystis pneumonia in acquired immunodeficiency syndrome: A multicenter study. *Intern. Med.* **2008**, *47*, 915–923. [[CrossRef](#)]
150. Maetzel, A.; Wong, A.; Strand, V.; Tugwell, P.; Wells, G.; Bombardier, C. Meta-analysis of treatment termination rates among rheumatoid arthritis patients receiving disease-modifying anti-rheumatic drugs. *Rheumatology (Oxford)* **2000**, *39*, 975–981. [[CrossRef](#)]
151. Furst, D.E. Practical clinical pharmacology and drug interactions of low-dose methotrexate therapy in rheumatoid arthritis. *Br. J. Rheumatol.* **1995**, *34* (Suppl. 2), 20–25. [[CrossRef](#)]
152. Rohr, M.K.; Mikuls, T.R.; Cohen, S.B.; Thorne, J.C.; O'Dell, J.R. Underuse of Methotrexate in the Treatment of Rheumatoid Arthritis: A National Analysis of Prescribing Practices in the US. *Arthritis Care Res. (Hoboken)* **2017**, *69*, 794–800. [[CrossRef](#)]
153. Boots, C.E.; Gustofson, R.L.; Feinberg, E.C. Does methotrexate administration for ectopic pregnancy after in vitro fertilization impact ovarian reserve or ovarian responsiveness? *Fertil. Steril.* **2013**, *100*, 1590–1593. [[CrossRef](#)]

154. Flint, J.; Panchal, S.; Hurrell, A.; van de Venne, M.; Gayed, M.; Schreiber, K.; Arthanari, S.; Cunningham, J.; Flanders, L.; Moore, L.; et al. BSR and BHPR guideline on prescribing drugs in pregnancy and breastfeeding—Part I: Standard and biologic disease modifying anti-rheumatic drugs and corticosteroids. *Rheumatology (Oxford)* **2016**, *55*, 1693–1697. [CrossRef]
155. Eck, L.K.; Jensen, T.B.; Mastrogiannis, D.; Torp-Pedersen, A.; Askaa, B.; Nielsen, T.K.; Poulsen, H.E.; Jimenez-Solem, E.; Andersen, J.T. Risk of Adverse Pregnancy Outcome After Paternal Exposure to Methotrexate Within 90 Days Before Pregnancy. *Obstet. Gynecol.* **2017**, *129*, 707–714. [CrossRef]
156. Gutierrez, J.C.; Hwang, K. The toxicity of methotrexate in male fertility and paternal teratogenicity. *Expert Opin. Drug Metab. Toxicol.* **2017**, *13*, 51–58. [CrossRef]
157. Friedman, M.A.; Winthrop, K.L. Vaccines and Disease-Modifying Antirheumatic Drugs: Practical Implications for the Rheumatologist. *Rheum. Dis. Clin. N. Am.* **2017**, *43*, 1–13. [CrossRef]
158. Hmamouchi, I.; Winthrop, K.; Launay, O.; Dougados, M. Low rate of influenza and pneumococcal vaccine coverage in rheumatoid arthritis: Data from the international COMORA cohort. *Vaccine* **2015**, *33*, 1446–1452. [CrossRef]
159. Hua, C.; Barnette, T.; Combe, B.; Morel, J. Effect of methotrexate, anti-tumor necrosis factor alpha, and rituximab on the immune response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis: A systematic review and meta-analysis. *Arthritis Care Res. (Hoboken)* **2014**, *66*, 1016–1026. [CrossRef]
160. Van Aalst, M.; Langedijk, A.C.; Spijker, R.; de Bree, G.J.; Grobusch, M.P.; Goorhuis, A. The effect of immunosuppressive agents on immunogenicity of pneumococcal vaccination: A systematic review and meta-analysis. *Vaccine* **2018**, *36*, 5832–5845. [CrossRef]
161. Park, J.K.; Lee, Y.J.; Shin, K.; Ha, Y.J.; Lee, E.Y.; Song, Y.W.; Choi, Y.; Winthrop, K.L.; Lee, E.B. Impact of temporary methotrexate discontinuation for 2 weeks on immunogenicity of seasonal influenza vaccination in patients with rheumatoid arthritis: A randomised clinical trial. *Ann. Rheum. Dis.* **2018**, *77*, 898–904. [CrossRef]
162. Park, J.K.; Lee, M.A.; Lee, E.Y.; Song, Y.W.; Choi, Y.; Winthrop, K.L.; Lee, E.B. Effect of methotrexate discontinuation on efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis: A randomised clinical trial. *Ann. Rheum. Dis.* **2017**, *76*, 1559–1565. [CrossRef]
163. Goodman, S.M. Rheumatoid arthritis: Perioperative management of biologics and DMARDs. *Semin. Arthritis Rheum.* **2015**, *44*, 627–632. [CrossRef]
164. Grennan, D.M.; Gray, J.; Loudon, J.; Fear, S. Methotrexate and early postoperative complications in patients with rheumatoid arthritis undergoing elective orthopaedic surgery. *Ann. Rheum. Dis.* **2001**, *60*, 214–217. [CrossRef]
165. Viller, F.; Guillemin, F.; Briançon, S.; Moum, T.; Suurmeijer, T.; van den Heuvel, W. Compliance to drug treatment of patients with rheumatoid arthritis: A 3 year longitudinal study. *J. Rheumatol.* **1999**, *26*, 2114–2122.
166. Nota, I.; Drossaert, C.H.; Taal, E.; Vonkeman, H.E.; van de Laar, M.A. Patient participation in decisions about disease modifying anti-rheumatic drugs: A cross-sectional survey. *BMC Musculoskelet. Disord.* **2014**, *15*, 333. [CrossRef]
167. Fraenkel, L.; Nowell, W.B.; Michel, G.; Wiedmeyer, C. Preference phenotypes to facilitate shared decision-making in rheumatoid arthritis. *Ann. Rheum. Dis.* **2018**, *77*, 678–683. [CrossRef]
168. Mathews, A.L.; Coleska, A.; Burns, P.B.; Chung, K.C. Evolution of Patient Decision-Making Regarding Medical Treatment of Rheumatoid Arthritis. *Arthritis Care Res. (Hoboken)* **2016**, *68*, 318–324. [CrossRef]
169. Fraenkel, L.; Peters, E.; Charpentier, P.; Olsen, B.; Errante, L.; Schoen, R.T.; Reyna, V. Decision tool to improve the quality of care in rheumatoid arthritis. *Arthritis Care Res. (Hoboken)* **2012**, *64*, 977–985. [CrossRef]
170. Zak, A.; Corrigan, C.; Yu, Z.; Bitton, A.; Fraenkel, L.; Harrold, L.; Smolen, J.S.; Solomon, D.H. Barriers to treatment adjustment within a treat to target strategy in rheumatoid arthritis: A secondary analysis of the TRACTION trial. *Rheumatology (Oxford)* **2018**, *57*, 1933–1937. [CrossRef]
171. Scheiman-Elazary, A.; Duan, L.; Shourt, C.; Agrawal, H.; Ellashof, D.; Cameron-Hay, M.; Furst, D.E. The Rate of Adherence to Antiarthritis Medications and Associated Factors among Patients with Rheumatoid Arthritis: A Systematic Literature Review and Metaanalysis. *J. Rheumatol.* **2016**, *43*, 512–523. [CrossRef]



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