The value of rheumatoid arthritis autoantibodies in disease pathogenesis and treatment prognosis

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CHAPTER 1

Introduction

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a severe autoimmune disease of unknown etiology that symmetrically affects the small diarthrodial joints of the hands and feet. Its prevalence is estimated to affect approximately 0.5-1% of the global population, making it one of the most prevalent autoimmune diseases worldwide (1). RA typically manifests with symptoms such as joint pain, swelling, stiffness, and fatigue, profoundly impacting patients' quality of life and productivity. If left untreated, it leads to progressive joint damage and disability. Moreover, RA is associated with a range of comorbidities, including cardiovascular diseases, osteoporosis, and systemic inflammation, further exacerbating its burden on affected individuals (2). Central to the disease process is the activation of innate and adaptive immune cells, leading to the production of pro-inflammatory cytokines, chemokines, and autoantibodies. Below, the most important autoantibodies are described, with what is currently known about their role in pathogenesis and disease propagation.

AUTOANTIBODIES

RA is broadly classified into seropositive and seronegative RA based on the presence or absence of specific autoreactive antibodies in patients' serum. Seropositive RA is characterized by the presence of rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPAs), whereas seronegative RA lacks these conventional autoantibodies. Seropositive RA is associated with more aggressive disease phenotypes, higher disease activity, increased radiographic progression, and poorer treatment responses compared to seronegative RA (3, 4). Autoantibodies are also thought to play a crucial role in the pathogenesis of RA, and several types have been identified, each with distinct targets and clinical significance, described below.

Rheumatoid factor

RF was the first autoantibody associated with RA and is detected in approximately 60-70% of patients. RF is a hallmark autoantibody in RA, integral to major classification criteria such as the 1987 American College of Rheumatology (ACR) criteria and the 2010 ACR/European League Against Rheumatism (EULAR) criteria (5, 6). RF positivity is associated with more severe disease manifestations, including increased joint damage and extra-articular complications (7). RF targets the Fc region of IgG and comprises various isotypes, with IgM being the most prevalent (8). Although its role in RA pathogenesis is not fully elucidated, RF likely contributes to immune complex formation, particularly in the synovial fluid, where high-affinity RF may perpetuate inflammation by stimulating proinflammatory cytokine production (9, 10).

Anti-citrullinated protein antibodies

ACPAs are highly specific biomarkers for RA, are detected in approximately 70-80% of patients, and occur rarely in individuals without RA (11, 12). ACPAs target citrulline residues on proteins or peptides, a post-translational modification mediated by peptidyl arginine deiminases (PADs), converting arginine to citrulline (13). ACPAs recognize a plethora of citrullinated proteins, including fibrinogen, vimentin, α-enolase, and collagen type II, among others, and show marked isotype usage (14, 15). Their presence overlaps largely with that of RF IgM, and they are similarly incorporated with RA classification criteria mentioned above in the form of the clinical anti-CCP2 or CCP3 test, which measures ACPA directed to a diverse spectrum of citrullinated peptides (16, 17). ACPA positivity correlates with more severe disease phenotypes, increased radiographic progression, and poorer treatment responses (18, 19).

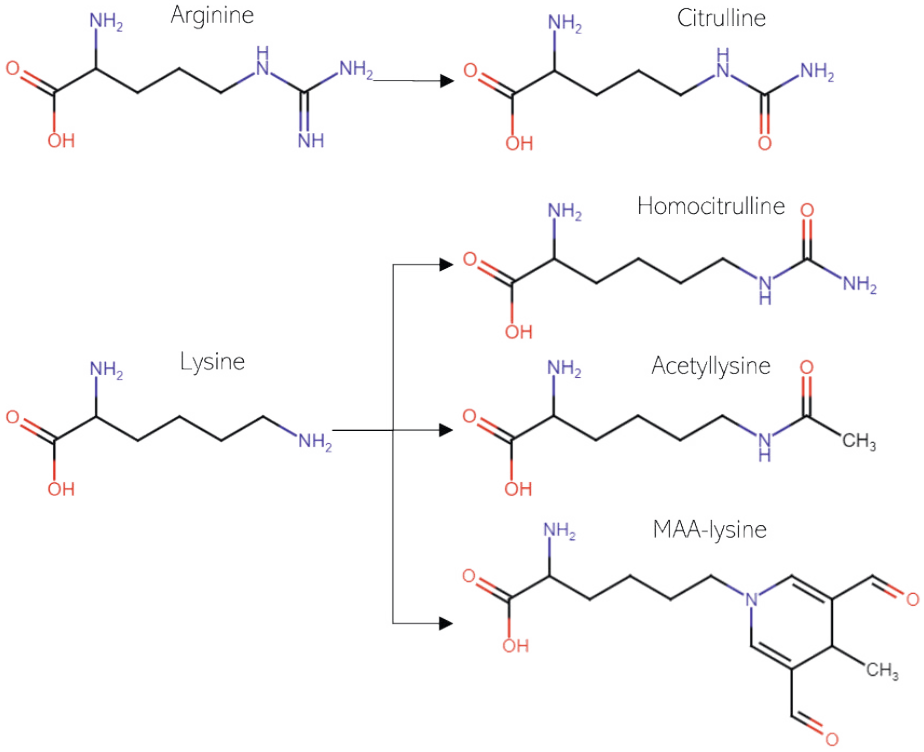
Other anti-modified protein antibodies

Besides RF and ACPAs, a growing number of anti-modified protein antibodies (AMPAs) continue to be discovered which target other post-translation modifications of peptides (Figure 1). Anti-carbamylated protein antibodies (anti-CarP) have emerged as novel autoantibodies in RA and are detected in approximately 30-40% of patients (20). Carbamylation is a chemical modification involving the conversion of lysine residues to homocitrulline in proteins, mediated by myeloperoxidase and other reactive oxygen species in diverse states of inflammation (21). Anti-CarP antibodies recognize carbamylated proteins and have been implicated in RA pathogenesis, although their precise mechanisms of action remain to be elucidated. Anti-CarP positivity has been associated with more severe joint damage and radiographic progression, independent of ACPA status, suggesting their potential as prognostic biomarkers in RA (20, 22, 23).

Anti-acetylated protein antibodies (AAPA) are antibodies targeting acetylated proteins, which are formed by the acetylation of lysine residues (24). Acetylation is a reversible post-translational modification regulated by histone acetyltransferases and histone deacetylases. AAPA positivity has been observed in RA patients, although its clinical significance and functional role in disease pathogenesis is unclear.

Anti-malondialdehyde-acetaldehyde adduct antibodies (anti-MAA) are antibodies targeting adducts formed by the reaction of malondialdehyde (MDA) and acetaldehyde with lysine in proteins(25). These adducts are generated under conditions of oxidative stress and lipid peroxidation, which are prevalent in RA synovium(26, 27). Anti-MAA antibodies have been detected in RA patients, particularly those with severe disease and extra-articular manifestations (25, 28, 29). However, anti-MAA/MDA antibodies are not RA-specific and can be found in many other conditions as well. Nonetheless, they are thought to contribute to RA pathogenesis by promoting inflammation, endothelial dysfunction, and tissue damage, although further studies are needed to determine their exact role.

Figure 1: Post-translational modifications on arginine and lysine



AUTOANTIBODY CHARACTERISTICS IN RA

A growing body of evidence points to the importance of AMPAs and especially ACPAs in the pathogenesis of RA. ACPAs can be detected in the sera of RA patients years before the onset of clinical symptoms, implicating their involvement in the preclinical stages of disease development (30, 31). ACPAs have also been identified in individuals with recent-onset clinically suspect arthralgia, with a positive predictive value exceeding 60%(32). However, at this early stage, ACPA titers are typically low, and their peptide recognition profile is limited(33, 34). As individuals progress towards disease onset, ACPA titers and peptide-recognition profiles undergo substantial expansion, indicative of an active immune response and suggesting a role for ACPAs in precipitating disease onset. The efficacy of selective B-cell depletion therapies in treating RA provides compelling evidence for the involvement of B cells and possibly autoantibodies(35), including ACPAs, in driving the chronicity of RA.

The recognition profile of ACPAs is diverse, with these antibodies targeting a variety of citrullinated antigens. Epitope spreading, characterized by an increase or shift in the antigen recognition profile, may have important pathophysiological consequences as it

does in other autoimmune disease like pemphigus (36), potentially exacerbating disease severity and progression. While specific anti-citrullinated epitope or protein reactivity predictive of disease course in RA have not been conclusively identified, the breadth of ACPA recognition profiles may offer insights into disease heterogeneity and progression.

Isotype switching further enhances the functional diversity of AMPAs in RA patients before disease onset (37-39). Interestingly, the avidity maturation of ACPAs and anti-CarP differs from conventional antibody responses, with these autoantibodies exhibiting relatively low avidity despite extensive isotype switching (40, 41). Little is as of yet known about other AMPA families in this regard. ACPA also exhibit an additional unusual feature of containing additional glycans in the variable antibody domains, notably increased in RA and predictive for disease progression (42, 43). These discrepancy suggests that the regulation of ACPA responses may differ from that of conventional antibody responses, highlighting the unique immunological characteristics of ACPAs in RA. It is expected that similar investigations will following for the characteristics in the other AMPAs.

RISK FACTORS FOR RA AND AUTOANTIBODY FORMATION

Understanding the temporal evolution of autoantibodies described above and its potential role in RA development requires consideration of both genetic and environmental risk factors. Among the various genetic risk factors implicated in RA, human leukocyte antigen (HLA) is the most prominent. Specifically, a common amino acid sequence at position 70-74 of the HLA-DRB1 molecule, referred to as the “shared epitope” (HLA-SE), has been strongly linked to the development of ACPA-positive but not ACPA-negative RA (44). Notably, HLA-SE also does not predispose to the rare state of ACPA-positivity in the absence of RA, but rather associates only with ACPA-positive RA (45). Additionally HLA-SE is specifically associated with the presence of variable-domain glycosylated ACPA during the pre-disease phase (46), which may have various implications (beyond the scope of this introduction) in the process of autoreactive ACPA B-cell survival. Conversely, certain alleles of HLA-DRB1, such as HLA-DRB1*13, have been identified as protective against seropositive RA development (47), suggesting a complex interplay between HLA alleles and disease susceptibility.

Environmental risk factors, such as tobacco smoking and inhalant exposures, also demonstrate stronger associations with ACPA-positive RA(48-50). Of particular interest is the gene-environment interaction between HLA-SE alleles and smoking (51), suggesting a potential role for smoking-induced citrullination in RA pathogenesis. Other evidence implicates mucosal sites like the periodontium and the microbiome of the intestinal tract(52, 53), as well as female sex hormones(54) as potential factors in autoimmune initiation in RA.

TREATMENT CHALLENGES

RA is not a new disease. RA has been demonstrated in paleopathological studies of human remains dating back to 4000BCE, and findings suggestive of RA appear in 17th century Dutch art (55, 56). Despite its long history, the disease processes underlying RA remained virtually untreatable until as recently as 50 years ago, and patients could expect only limited symptomatic relief from the debilitating pain, loss of function, and systemic complications that the disease causes. Since then, huge advances have been made in effective therapies that not only address symptoms, but also halt the persistent, uncontrolled inflammation and associated disease progression, termed disease modifying anti-rheumatic drugs (DMARDs) (57). Novel biologic and small-molecule agents have further augmented the rheumatologists therapeutic arsenal, together with a treat-to-target approach, and disease remission is achievable in an increasing numbers of patients (58).

However, all of these treatments have side-effects that are often not insignificant. A growing need exists to allow patients to attempt to taper their DMARDs but remain symptom free, termed drug free remission (DFR)(59). However, only 10-20% of patients are able to achieve and retain this lofty treatment goal (60), and disease flare following unsuccessful DMARD tapering may exacerbate disease burden, though thankfully, patients who experience a disease flare are virtually always able to recapture remission (61). Identifying factors predicting disease flare post-DMARD tapering could optimize clinical decision-making.

Sustained DFR is also interesting from a pathophysiological standpoint, since it most closely approximates the holy grail of RA treatment outcomes: a cure. If DFR is sustained, perhaps it identifies a form of a true state of disease remission, without the need for treatment, in which the underlying autoimmune processes that initiated and propagated disease, have been durably dampened and immune tolerance has been re-established. Identifying factors which are prognostically favorable for this outcome, could then shed light on which mechanisms in RA are important in perpetuating disease, and improve treatment by targeting those mechanisms (62).

AMPA AND TREATMENT OUTCOMES

Given their presence and evolution over time in pre-diseases states, it is understandable that there is much interest in the prognostic value of autoantibody characteristics in RA once RA has manifested. Autoantibody seropositivity is associated with poorer treatment outcomes and radiographic damage (18, 19, 63). However, given the diversity of the RA autoantibody profile, with its varied autoantigen recognition and extensive isotype switching, the breadth of this profile may also be important. Furthermore, serum autoantibody levels may fluctuate. In other autoimmune diseases

like systemic lupus erythematosus, these fluctuations correlate with disease activity, and are monitored over time to inform clinical decision-making (64). If such fluctuations in serum autoantibody concentrations also occur in RA, they may hold promise as accessible biomarkers for disease course prediction.

Unfortunately, conflicting studies regarding the relationship between autoantibody fluctuations and disease activity, often not accounting for immunosuppressive treatment intensity, complicate interpretation. Most studies have shown that RF (IgM, IgA, and IgG) levels decrease after treatment initiation with different DMARD classes, while anti-CCP2 (IgG) levels decrease only marginally, rebound after decreasing, or do not decrease at all, but did not account for treatment effects (65-71). Virtually nothing was known, at the start of the studies described in this thesis, about the prognostic value of changes in AMPA characteristics (isotype usage, epitope recognition, and seroconversion to negative, to name a few) over time, and newer autoantibodies like anti-CarP, Similar reflections apply to AAPAs, and anti-MAA and also these also warranted consideration.

Understanding the clinical implications of changes in autoantibody levels is crucial. If autoantibody levels predict future disease activity, measuring pre-treatment values or monitoring changes over time could guide treatment decisions. Additionally, investigating the association between autoantibody changes, immunosuppression, and disease activity could provide insights into the role of B cell autoimmune response in RA persistence.

CALPROTECTIN: A NEW PROGNOSTIC BIOMARKER

Not only AMPAs have been investigated as possible biomarkers for predicting RA course: calprotectin, a heterodimeric complex of S100 calcium-binding proteins (MRP-8 and MRP-14), shows promise as a biomarker in RA (72, 73). Although conceptually similar to conventional inflammatory indices like erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), it may serve as a more sensitive marker of the disease activity in rheumatic illnesses because it directly reflects inflammation in synovium and significantly increases during active inflammation (74, 75). Multiple studies recently reviewed suggest its potential in various inflammatory disorders, including RA, ankylosing spondylitis, psoriatic arthritis, and systemic lupus erythematosus (76). Interestingly, calprotectin has also been implicated in predicting disease relapses in juvenile idiopathic arthritis (77, 78). Theoretically, its presence in synovial tissue and its ability to enter systemic circulation may make it a more specific and reliable marker for residual joint inflammation than hepatocyte-dependent acute-phase reactants like CRP (79). Perhaps by measuring calprotectin in patients in stable remission under DMARDs, we may patients at risk of future disease flare after DMARD tapering and facilitate risk stratification to predict whether DMARD tapering will be successful.

CANCER IMMUNOTHERAPY & AUTOIMMUNITY

While the field of rheumatology and RA boomed with new autoantibody discoveries, breakthroughs were also being made in a completely different field: cancer immunotherapy, in the form of immune checkpoint inhibitors (ICIs). Broadly speaking, ICIs work by blocking the body's natural mechanisms for T-cell inhibition, be it via cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), or its ligand PD-L1, to promote an antitumor immune response. Ipilimumab, an anti-CTLA-4 monoclonal antibody, was the first of its kind in advanced metastatic melanoma (80), and it and its brothers have provided significant survival benefits over traditional chemotherapies for a wide range of advanced malignancies (81).

What is intriguing about this anti-cancer therapy is that by inducing immune antitumor effects, ICIs also induce unintended autoimmune side-effects in as many as 80% of patients, termed immune-related adverse events (irAEs)(82, 83). These irAEs can occur in virtually any organ system, can be severe enough to warrant ICI cessation or even cause long-term damage, and are difficult to predict(84). Because of this phenomenon, irAEs draw comparisons to many traditional autoimmune diseases, such as vitiligo, ulcerative colitis, Hashimoto thyroiditis, and even rheumatoid arthritis. In irAEs following ICI treatment, we have, in effect, a human model for induction of autoimmunity, and its potential overlap in the field of rheumatic autoimmune studies is promising indeed. At the start of the studies described in this thesis, the nature of the autoimmune response induced following ICI in general, and the autoantibody response more specifically, was still ill defined and therefore important to investigate both from a clinical- and basic/translational scientific view.

OUTLINE OF THIS THESIS

Main aims

- To characterize the relationship of genetic and environmental risk factors with the AMPA profile in different populations of RA
- To describe the longitudinal changes in characteristics of the AMPA profile, and investigate its prognostic value regarding early treatment outcomes and drug-free remission
- To assess calprotectin as a marker of residual inflammation in patients in disease remission and as clinical tool in tapering DMARDs
- To investigate autoantibody formation and its relation to irAEs in ICIs

As described above, RA autoantibodies play diverse roles in the pathogenesis of RA, reflecting the complex interplay between genetic predisposition, environmental factors, and dysregulated immune responses. Understanding the intricate mechanisms

underlying RA pathogenesis, heterogeneity, and autoantibody profiles is crucial for advancing precision medicine approaches, improving patient outcomes, and developing targeted therapies tailored to individual disease subtypes and phenotypes.

In **Chapter 2,** we examined AMPAs in four different seropositive RA populations from across the world. These populations were vastly different in terms of genetic background, smoking habits, and environmental exposures. By studying the differences in the AMPA profile of these patient – or, perhaps more interestingly, their commonalities – and the relationship of AMPA to known RA risk factors, we were able to investigate whether divergent or common pathways are at play in the development of seropositive RA.

The studies described in **chapters 3 through 5** concern the theragnostic value of various elements of the AMPA profile. Because seropositivity is such an evident poor prognostic factor for various treatment outcomes, we measured various aspects of AMPAs in seropositive RA in an attempt to understand which part of the AMPA profile most strongly confers this poor prognosis. For this, we used data and serum from patients from the Induction therapy with Methotrexate and Prednisone in Rheumatoid Or Very Early arthritic Disease (IMPROVED) study, a multicentre, randomized controlled trial that enrolled 610 patients with untreated RA or undifferentiated arthritis (85). We measured fourteen different RA autoantibodies, comprising eight isotypes and six fine specificities within four AMPA families, and attempted to dissect whether the presence of one, many, or a specific combination of many AMPAs was related to prognosis. Specifically, we focused on early response to DMARD therapy and long-term ability to achieve drug-free remission.

The investigations in **Chapter 4 and 5** characterize the changes of autoantibodies over time, and the possible prognostic value thereof. In these chapters, the concept of immunological remission, described above, is central. At the moment, DFR is the closest proxy for disease cure available in RA, but true curation has not yet been achieved. Autoantibodies, intimately linked with disease initiation and pathogenesis, may change over time, but details are lacking. It is possible that changes in these AMPAs may set apart a group of patients in whom the underlying immunopathology has been favourably modulated, that is, patients in true immunological remission (86), and that this state of remission is favorable for achieving long term DFR. Such a relationship could yield a meaningful prognostic marker for drug tapering decisions, and it could elucidate pathways that lead to long-term resolution of the pathophysiology underlying RA.

wIn **Chapter 4**, this relationship was examined in the form of seroconversion from positive to negative for the fourteen autoantibodies mentioned above, while in **Chapter 5**, emphasis was put in level changes over time, and a direct relationship with immunosuppression was also examined.

In a similar theme, **Chapter 6** focused on the value of calprotectin as a marker of residual inflammation in RA patients in remission to identify patients with favorable chances for DFR. This study made use of the previously mentioned IMPROVED study and of similarly designed RETRO study (acronym for the Reduction of Therapy in Patients with Rheumatoid Arthritis in Ongoing Remission)(87). We evaluated whether calprotectin alone, or its addition to other clinical predictors associated with disease flare within 1 year of study-randomized tapering decisions.

At the time of the publication of **chapter 7**, little was known about autoantibody production in irAEs, and only case studies post-ICI treatment had been published. We set about characterizing the presence of a wide spectrum of organ-specific autoantibodies before and after ICI treatment, and analyzed whether the development of autoantibodies was related to treatment outcomes or toxicity. Understanding how irAEs develop has two-fold significance: first, organ-specific autoantibodies have the potential to serve as biomarkers for the development of irAEs, and second, investigating their development in patients with irAEs may provide insights into the role of autoantibodies in the onset of new autoimmune conditions, such as rheumatoid arthritis.

REFERENCES

1. Finckh A, Gilbert B, Hodkinson B, Bae SC, Thomas R, Deane KD, et al. Global epidemiology of rheumatoid arthritis. Nature reviews Rheumatology. 2022;18(10):591-602.
2. Figus FA, Piga M, Azzolin I, McConnell R, Iagnocco A. Rheumatoid arthritis: Extra-articular manifestations and comorbidities. Autoimmunity reviews. 2021;20(4):102776.
3. Willemze A, Trouw LA, Toes RE, Huizinga TW. The influence of ACPA status and characteristics on the course of RA. Nature reviews Rheumatology. 2012;8(3):144-52.
4. De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Meheus L, et al. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Annals of the rheumatic diseases. 2004;63(12):1587-93.
5. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis and rheumatism. 1988;31(3):315-24.
6. Smolen JS, Landewe R, Bijlsma J, Burmester G, Chatzidionysiou K, Dougados M, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Annals of the rheumatic diseases. 2017;76(6).
7. Ingegnoli F, Castelli R, Gualtierotti R. Rheumatoid factors: clinical applications. Disease markers. 2013;35(6):727-34.
8. Artandi SE, Calame KL, Morrison SL, Bonagura VR. Monoclonal IgM rheumatoid factors bind IgG at a discontinuous epitope comprised of amino acid loops from heavy-chain constant-region domains 2 and 3. Proc Natl Acad Sci USA. 1992;89(94-98).
9. Mathsson L, Lampa J, Mullazehi M, Ronnelid J. Immune complexes from rheumatoid arthritis synovial fluid induce FcgammaRIIa dependent and rheumatoid factor correlated production of tumour necrosis factor-alpha by peripheral blood mononuclear cells. Arthritis research & therapy. 2006;8(3):R64.
10. Van Snick JL, Van Roost E, Markowetz B, Cambiaso CL, Masson PL. Enhancement by IgM rheumatoid factor of in vitro ingestion by macrophages and in vivo clearance of aggregated IgG or antigen-antibody complexes. Eur J Immunol. 1978;8(4):279-85.
11. Demoruelle MK, Parish MC, Derber LA, Kolfenbach JR, Hughes-Austin JM, Weisman MH, et al. Performance of anti-cyclic citrullinated Peptide assays differs in subjects at increased risk of rheumatoid arthritis and subjects with established disease. Arthritis and rheumatism. 2013;65(9):2243-52.
12. van Zanten A, Arends S, Roozendaal C, Limburg PC, Maas F, Trouw LA, et al. Presence of anticitrullinated protein antibodies in a large population-based cohort from the Netherlands. Annals of the rheumatic diseases. 2017;76(7):1184-90.
13. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays. 2003;25(11):1106-18.
14. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. The Journal of clinical investigation. 1998;101(1):273-81.
15. Ioan-Facsinay A, Willemze A, Robinson DB, Peschken CA, Markland J, van der Woude D, et al. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. Arthritis and rheumatism. 2008;58(10):3000-8.

16. Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. Annals of the rheumatic diseases. 2006;65(7):845-51.

17. Pruijn GJ, Wiik A, van Venrooij WJ. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. Arthritis research & therapy. 2010;12(1):203.

18. van der Kooij SM, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Guler-Yuksel M, Zwinderman AH, Kerstens PJ, et al. Drug-free remission, functioning and radiographic damage after 4 years of response-driven treatment in patients with recent-onset rheumatoid arthritis. Annals of the rheumatic diseases. 2009;68(6):914-21.

19. Wevers-de Boer K, Visser K, Heimans L, Ronday HK, Molenaar E, Groenendael JH, et al. Remission induction therapy with methotrexate and prednisone in patients with early rheumatoid and undifferentiated arthritis (the IMPROVED study). Annals of the rheumatic diseases. 2012;71(9):1472-7.

20. Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GM, van Veelen PA, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(42):17372-7.

21. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Horkko S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nature medicine. 2007;13(10):1176-84.

22. Ajeganova S, van Steenbergen HW, Verheul MK, Forslind K, Hafstrom I, Toes RE, et al. The association between anti-carbamylated protein (anti-CarP) antibodies and radiographic progression in early rheumatoid arthritis: a study exploring replication and the added value to ACPA and rheumatoid factor. Annals of the rheumatic diseases. 2017;76(1):112-8.

23. Jiang X, Trouw LA, van Wesemael TJ, Shi J, Bengtsson C, Kallberg H, et al. Anti-CarP antibodies in two large cohorts of patients with rheumatoid arthritis and their relationship to genetic risk factors, cigarette smoking and other autoantibodies. Annals of the rheumatic diseases. 2014;73(10):1761-8.

24. Juarez M, Bang H, Hammar F, Reimer U, Dyke B, Sahbudin I, et al. Identification of novel antiacetylated vimentin antibodies in patients with early inflammatory arthritis. Annals of the rheumatic diseases. 2016;75(6):1099-107.

25. Thiele GM, Duryee MJ, Anderson DR, Klassen LW, Mohring SM, Young KA, et al. Malondialdehyde-acetaldehyde adducts and anti-malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. Arthritis & rheumatology. 2015;67(3):645-55.

26. Gronwall C, Amara K, Hardt U, Krishnamurthy A, Steen J, Engstrom M, et al. Autoreactivity to malondialdehyde-modifications in rheumatoid arthritis is linked to disease activity and synovial pathogenesis. Journal of autoimmunity. 2017;84:29-45.

27. Mikuls TR, Duryee MJ, Rahman R, Anderson DR, Sayles HR, Hollins A, et al. Enrichment of malondialdehyde-acetaldehyde antibody in the rheumatoid arthritis joint. Rheumatology. 2017;56(10):1794-803.

28. England BR, Duryee MJ, Roul P, Mahajan TD, Singh N, Poole JA, et al. Malondialdehyde-Acetaldehyde Adducts and Antibody Responses in Rheumatoid Arthritis-Associated Interstitial Lung Disease. Arthritis & rheumatology. 2019;71(9):1483-93.

29. Mikuls TR, Duryee MJ, England BR, Anderson DR, Hearth-Holmes M, Su K, et al. Malondialdehyde-acetaldehyde antibody concentrations in rheumatoid arthritis and other rheumatic conditions. Int Immunopharmacol. 2018;56:113-8.

30. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis and rheumatism. 2003;48(10):2741-9.

31. van de Stadt LA, de Koning MH, van de Stadt RJ, Wolbink G, Dijkmans BA, Hamann D, et al. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. Arthritis and rheumatism. 2011;63(11):3226-33.

32. van Steenbergen HW, Mangnus L, Reijnierse M, Huizinga TW, van der Helm-van Mil AH. Clinical factors, anticitrullinated peptide antibodies and MRI-detected subclinical inflammation in relation to progression from clinically suspect arthralgia to arthritis. Annals of the rheumatic diseases. 2016;75(10):1824-30.

33. Bos WH, van de Stadt LA, Sohrabian A, Ronnelid J, van Schaardenburg D. Development of anti-citrullinated protein antibody and rheumatoid factor isotypes prior to the onset of rheumatoid arthritis. Arthritis research & therapy. 2014;16(2):405.

34. van de Stadt LA, van der Horst AR, de Koning MH, Bos WH, Wolbink GJ, van de Stadt RJ, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. Annals of the rheumatic diseases. 2011;70(1):128-33.

35. Edwards JC, Cambridge G. Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology. 2001;40(2):205-11.

36. Rock B, Martins CR, Theofilopoulos AN, Balderas RS, Anhalt GJ, Labib RS, et al. The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). N Engl J Med. 1989;320(22):1463-9.

37. Verpoort KN, Jol-van der Zijde CM, Papendrecht-van der Voort EA, Ioan-Facsinay A, Drijfhout JW, van Tol MJ, et al. Isotype distribution of anti-cyclic citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. Arthritis and rheumatism. 2006;54(12):3799-808.

38. van Delft MAM, Verheul MK, Burgers LE, Derksen V, van der Helm-van Mil AHM, van der Woude D, et al. The isotype and IgG subclass distribution of anti-carbamylated protein antibodies in rheumatoid arthritis patients. Arthritis research & therapy. 2017;19(1):190.

39. Brink M, Hansson M, Mathsson-Alm L, Wijayatunga P, Verheul MK, Trouw LA, et al. Rheumatoid factor isotypes in relation to antibodies against citrullinated peptides and carbamylated proteins before the onset of rheumatoid arthritis. Arthritis research & therapy. 2016;18:43.

40. van Delft MAM, Verheul MK, Burgers LE, Rantapaa-Dahlqvist S, van der Helm-van Mil AHM, Huizinga TWJ, et al. The anti-carbamylated protein antibody response is of overall low avidity despite extensive isotype switching. Rheumatology. 2018;57(9):1583-91.

41. Suwannalai P, Scherer HU, van der Woude D, Ioan-Facsinay A, Jol-van der Zijde CM, van Tol MJ, et al. Anti-citrullinated protein antibodies have a low avidity compared with antibodies against recall antigens. Annals of the rheumatic diseases. 2011;70(2):373-9.

42. Hafkenscheid L, de Moel E, Smolik I, Tanner S, Meng X, Jansen BC, et al. N-Linked Glycans in the Variable Domain of IgG Anti-Citrullinated Protein Antibodies Predict the Development of Rheumatoid Arthritis. Arthritis & rheumatology. 2019;71(10):1626-33.

43. Rombouts Y, Willemze A, van Beers JJ, Shi J, Kerkman PF, van Toorn L, et al. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. Annals of the rheumatic diseases. 2016;75(3):578-85.

44. du Montcel ST, Michou L, Petit-Teixeira E, Osorio J, Lemaire I, Lasbleiz S, et al. New classification of HLA-DRB1 alleles supports the shared epitope hypothesis of rheumatoid arthritis susceptibility. Arthritis and rheumatism. 2005;52(4):1063-8.

45. Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis and rheumatism. 2005;52(11):3433-8.

46. Kissel T, van Wesemael TJ, Lundquist A, Kokkonen H, Kawakami A, Tamai M, et al. Genetic predisposition (HLA-SE) is associated with ACPA-IgG variable domain glycosylation in the predisease phase of RA. Annals of the rheumatic diseases. 2022;81(1):141-3.

47. van der Woude D, Lie BA, Lundstrom E, Balsa A, Feitsma AL, Houwing-Duistermaat JJ, et al. Protection against anti-citrullinated protein antibody-positive rheumatoid arthritis is predominantly associated with HLA-DRB1*1301: a meta-analysis of HLA-DRB1 associations with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in four European populations. Arthritis and rheumatism. 2010;62(5):1236-45.

48. Ebel AV, Lutt G, Poole JA, Thiele GM, Baker JF, Cannon GW, et al. Association of Agricultural, Occupational, and Military Inhalants With Autoantibodies and Disease Features in US Veterans With Rheumatoid Arthritis. Arthritis & rheumatology. 2021;73(3):392-400.

49. Stolt P, Kallberg H, Lundberg I, Sjogren B, Klareskog L, Alfredsson L, et al. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. Annals of the rheumatic diseases. 2005;64(4):582-6.

50. Deane KD, Demoruelle MK, Kelmenson LB, Kuhn KA, Norris JM, Holers VM. Genetic and environmental risk factors for rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2017;31(1):3-18.

51. Kallberg H, Padyukov L, Plenge RM, Ronnelid J, Gregersen PK, van der Helm-van Mil AH, et al. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. Am J Hum Genet. 2007;80(5):867-75.

52. Bingham CO, 3rd, Moni M. Periodontal disease and rheumatoid arthritis: the evidence accumulates for complex pathobiologic interactions. Current opinion in rheumatology. 2013;25(3):345-53.

53. Brewer RC, Lanz TV, Hale CR, Sepich-Poore GD, Martino C, Swafford AD, et al. Oral mucosal breaks trigger anti-citrullinated bacterial and human protein antibody responses in rheumatoid arthritis. Science translational medicine. 2023;15(684):eabq8476.

54. Cutolo M, Villaggio B, Craviotto C, Pizzorni C, Seriolo B, Sulli A. Sex hormones and rheumatoid arthritis. Autoimmunity reviews. 2002;1(5):284-9.

55. Hinojosa-Azaola A, Alcocer-Varela J. Art and rheumatology: the artist and the rheumatologist's perspective. Rheumatology. 2014;53(10):1725-31.

56. Entezami P, Fox DA, Clapham PJ, Chung KC. Historical perspective on the etiology of rheumatoid arthritis. Hand Clin. 2011;27(1):1-10.

57. Kievit W, Fransen J, de Waal Malefijt MC, den Broeder AA, van Riel PL. Treatment changes and improved outcomes in RA: an overview of a large inception cohort from 1989 to 2009. Rheumatology. 2013;52(8):1500-8.

58. Ramiro S, Landewe RB, van der Heijde D, Sepriano A, FitzGerald O, Ostergaard M, et al. Is treat-to-target really working in rheumatoid arthritis? a longitudinal analysis of a cohort of patients treated in daily practice (RA BIODAM). Annals of the rheumatic diseases. 2020;79(4):453-9.

59. Ajeganova S, van Steenbergen HW, van Nies JA, Burgers LE, Huizinga TW, van der Helm-van Mil AH. Disease-modifying antirheumatic drug-free sustained remission in rheumatoid arthritis: an increasingly achievable outcome with subsidence of disease symptoms. Annals of the rheumatic diseases. 2016;75(5):867-73.

60. Verstappen M, van Mulligen E, de Jong PHP, van der Helm-Van Mil AHM. DMARD-free remission as novel treatment target in rheumatoid arthritis: A systematic literature review of achievability and sustainability. RMD Open. 2020;6(1).

61. van Mulligen E, Weel AE, Hazes JM, van der Helm-van Mil A, de Jong PHP. Tapering towards DMARD-free remission in established rheumatoid arthritis: 2-year results of the TARA trial. Annals of the rheumatic diseases. 2020;79(9):1174-81.

62. Ajeganova S, Huizinga T. Sustained remission in rheumatoid arthritis: latest evidence and clinical considerations. Ther Adv Musculoskelet Dis. 2017;9(10):249-62.

63. Nijjar JS, Morton FR, Bang H, Buckley CD, van der Heijde D, Gilmour A, et al. The impact of autoantibodies against citrullinated, carbamylated, and acetylated peptides on radiographic progression in patients with new-onset rheumatoid arthritis: an observational cohort study. Lancet Rheumatol. 2021;3(4):e284-e93.

64. Fanouriakis A, Kostopoulou M, Andersen J, Aringer M, Arnaud L, Bae SC, et al. EULAR recommendations for the management of systemic lupus erythematosus: 2023 update. Annals of the rheumatic diseases. 2024;83(1):15-29.

65. Bobbio-Pallavicini F, Caporali R, Bugatti S, Montecucco C. What can we learn from treatment-induced changes in rheumatoid factor and anti-citrullinated Peptide antibodies? The Journal of rheumatology. 2008;35(10):1903-5.

66. Bohler C, Radner H, Smolen JS, Aletaha D. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. Annals of the rheumatic diseases. 2013;72(2):241-4.

67. Bos WH, Bartelds GM, Wolbink GJ, de Koning MH, van de Stadt RJ, van Schaardenburg D, et al. Differential response of the rheumatoid factor and anticitrullinated protein antibodies during adalimumab treatment in patients with rheumatoid arthritis. The Journal of rheumatology. 2008;35(10):1972-7.

68. Cambridge G, Leandro MJ, Edwards JC, Ehrenstein MR, Salden M, Bodman-Smith M, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. Arthritis and rheumatism. 2003;48(8):2146-54.

69. Jansen D, Emery P, Smolen JS, Westhovens R, Le Bars M, Connolly SE, et al. Conversion to seronegative status after abatacept treatment in patients with early and poor prognostic rheumatoid arthritis is associated with better radiographic outcomes and sustained remission: post hoc analysis of the AGREE study. RMD Open. 2018;4(1):e000564.

70. Mikuls TR, O'Dell JR, Stoner JA, Parrish LA, Arend WP, Norris JM, et al. Association of rheumatoid arthritis treatment response and disease duration with declines in serum levels of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibody. Arthritis and rheumatism. 2004;50(12):3776-82.

71. Wunderlich C, Oliviera I, Figueiredo CP, Rech J, Schett G. Effects of DMARDs on citrullinated peptide autoantibody levels in RA patients-A longitudinal analysis. Seminars in arthritis and rheumatism. 2017;46(6):709-14.

72. Odink K, Cerletti N, Bruggen J, Clerc RG, Tarcsay L, Zwadlo G, et al. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature. 1987;330(6143):80-2.

73. Vogl T, Eisenblatter M, Voller T, Zenker S, Hermann S, van Lent P, et al. Alarmin S100A8/S100A9 as a biomarker for molecular imaging of local inflammatory activity. Nat Commun. 2014;5:4593.

74. Bae SC, Lee YH. Calprotectin levels in rheumatoid arthritis and their correlation with disease activity: a meta-analysis. Postgrad Med. 2017;129(5):531-7.

75. van Lent PL, Grevers L, Blom AB, Sloetjes A, Mort JS, Vogl T, et al. Myeloid-related proteins S100A8/S100A9 regulate joint inflammation and cartilage destruction during antigen-induced arthritis. Annals of the rheumatic diseases. 2008;67(12):1750-8.

76. Abildtrup M, Kingsley GH, Scott DL. Calprotectin as a biomarker for rheumatoid arthritis: a systematic review. The Journal of rheumatology. 2015;42(5):760-70.

77. Altobelli E, Angeletti PM, Petrocelli R, Lapergola G, Farello G, Cannataro G, et al. Serum Calprotectin a Potential Biomarker in Juvenile Idiopathic Arthritis: A Meta-Analysis. J Clin Med. 2021;10(21).

78. La C, Le PQ, Ferster A, Goffin L, Spruyt D, Lauwerys B, et al. Serum calprotectin (S100A8/A9): a promising biomarker in diagnosis and follow-up in different subgroups of juvenile idiopathic arthritis. RMD Open. 2021;7(2).

79. Youssef P, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, et al. Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane. The Journal of rheumatology. 1999;26(12):2523-8.

80. Mansh M. Ipilimumab and cancer immunotherapy: a new hope for advanced stage melanoma. Yale J Biol Med. 2011;84(4):381-9.

81. Bagchi S, Yuan R, Engleman EG. Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. Annu Rev Pathol. 2021;16:223-49.

82. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. BMC Med. 2015;13:211.

83. Arnaud-Coffin P, Maillet D, Gan HK, Stelmes JJ, You B, Dalle S, et al. A systematic review of adverse events in randomized trials assessing immune checkpoint inhibitors. Int J Cancer. 2019;145(3):639-48.

84. Martins F, Sofiya L, Sykiotis GP, Lamine F, Maillard M, Fraga M, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. Nat Rev Clin Oncol. 2019;16(9):563-80.

85. Heimans L, Akdemir G, Boer KV, Goekoop-Ruiterman YP, Molenaar ET, van Groenendael JH, et al. Two-year results of disease activity score (DAS)-remission-steered treatment strategies aiming at drug-free remission in early arthritis patients (the IMPROVED-study). Arthritis research & therapy. 2016;18:23.

86. Schett G, Emery P, Tanaka Y, Burmester G, Pisetsky DS, Naredo E, et al. Tapering biologic and conventional DMARD therapy in rheumatoid arthritis: current evidence and future directions. Annals of the rheumatic diseases. 2016;75(8):1428-37.

87. Haschka J, Englbrecht M, Hueber AJ, Manger B, Kleyer A, Reiser M, et al. Relapse rates in patients with rheumatoid arthritis in stable remission tapering or stopping antirheumatic therapy: interim results from the prospective randomised controlled RETRO study. Annals of the rheumatic diseases. 2016;75(1):45-51.