Review

Seronegative rheumatoid arthritis: one year in review 2023

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ABSTRACT

In the past 20 years, earlier diagnosis and more intensive management have considerably improved the prognosis of rheumatoid arthritis (RA), with milder disease course achieved in particular in seropositive patients. In contrast, seronegative RA has remained largely neglected, and continues to be surrounded by uncertainties regarding its correct diagnosis, clinical phenotype, optimal treatment strategies and relevant outcomes.

The purpose of this review is to summarise new insights about the pathogenic, clinical and prognostic peculiarities of seronegative RA that emerged during 2022, and that make this disease subset at least partially different from its seropositive counterpart.

Introduction

Rheumatoid arthritis (RA) is increasingly regarded as a syndrome encompassing different clinical phenotypes, variable response to treatments and different outcomes (1, 2). The autoimmune nature of the disease is supported by the recognition of RA-associated autoantibodies, such as rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA) and other anti-modified protein antibodies (AMPA), in most patients (3, 4). These autoantibodies are directly involved in several aspects of RA pathology (3-6) and identify subjects with a more aggressive course of the disease in terms of joint destruction, comorbidities and mortality (6, 7). Although less frequently, some patients with RA however do not show any circulating autoantibody and are thus *bona fide* referred to as 'seronegative' (8). The many diagnostic uncertainties surrounding seronegative RA, together with the common belief of its more benign nature, have greatly hampered a proper understanding of this disease subgroup. However, the recognition that advances in the management of seropositive RA over the past two decades have not led to equally significant improvements in the prognosis of seronegative patients (9) imposes more focused analyses stratified for the autoantibody status.

The aim of this review is to summarise and critically discuss the most relevant data on seronegative RA that have expanded knowledge on this disease subtype during the past year. To this end, we performed a Medline search of English language articles published from 1st January to 31st December 2022, using MESH terms and free text words including RF, ACPA, citrullinated, autoantibodies, seronegative, autoantibody-negative.

Pathogenesis

Genetic susceptibility

The pathogenesis of RA, particularly its seronegative form, is complex and variously influenced by genetic and environmental factors, the microbiota, barrier layers and hormones. The pathogenetic mechanisms involved in seropositive RA appear more homogeneous, with a more substantial contribution of genetic factors, and with a prominent driving role of adaptive immunity (10). In contrast, the development of seronegative RA seems to be related to a lower genetic susceptibility and a more critical role of environmental factors. Moreover, the greater heterogeneity in clinical expression, course and response to therapy suggests it is a more heterogeneous immunopathological entity than seropositive RA (11).

The genetic susceptibility risk of seronegative RA relies on both HLA and non-HLA genes. The results of HLA studies have mainly confirmed lack of association with the most ro-

bust HLA genetic loci reported for RA, the shared epitope, and the presence of mutations in both HLA class I and class II genes, with the stronger association with the ancestral haplotype 8.1, which contains HLA-B*08 with aspartate at position 9 and DRB1*03 with serine at position 11 (11). The importance of non-HLA susceptibility loci was recently confirmed through analyses performed in large populations of patients with seropositive and seronegative RA. A genome-wide association study (GWAS) of 31 313 RA cases (68% seropositive) and ~1 million controls from Northwestern Europe found 25 sequence variants of the Janus Kinase (JAK)/ signal transducer and activator of transcription proteins (STAT) pathway characterising patients with RA, 33 for seropositive and 2 for seronegative (12). However, both signals in seronegative RA were also found in seropositive RA and pointed to causal genes: a missense variant rs2476601-A in PTPN22 and intronic variant rs7731626-A in ANKRD55.2 Another study, applying mendelian randomisation design on 3 GWASs metanalysis, found differences in cytokine patterns of genetic activity between seronegative and seropositive RA (13). However, while the genetic association of interleukin (IL)-1β, IL-1ra, and IL-6 activity with seropositive RA was statistically significant, the association of seronegative RA with IL-2 receptor alpha subunit, IL-8, and IL-18 was not; seronegative RA were few cases and lacked statistical power. Susceptibility to developing RA may also be determined by epigenetic factors, such as DNA methylation and microRNAs (14-16). In a recent review, Chang C *et al*. (17) summarised the roles of miRNAs in the susceptibility, pathogenesis, diagnosis, treatment, and prognosis of RA. Numerous miRNAs, but especially functional genetic variants of miR-499 and miR-146, are abnormally expressed in cells involved in RA, and may putatively explain disease susceptibility independently of seropositivity. However, no studies have specifically addressed the possible epigenetic differences between seropositive and seronegative RA.

Lifestyle and environment

A correct lifestyle is important for preventing both seropositive and seronegative RA. A recent study conducted on 1,219 incident RA cases (776 seropositive, 443 seronegative) demonstrated that a high level of healthy lifestyle index score was associated with a lower RA risk, both in seropositive and seronegative RA, with hazard ratios of 0.85 and 0.87, respectively (18). The healthy lifestyle index analysed 5 different aspects of lifestyle: smoking, alcohol consumption, body mass index, physical activity, and diet; the higher the index, the healthier the lifestyle. Another study followed 108 505 women 25–42 years old without RA for a median follow-up time of 25.3 years and found that long-term weight gain was strongly associated with increased RA risk, with weight gain of ≥20 kg associated with more than a three-fold increased risk (19). There was no difference between seronegative and seropositive RA. Despite these similarities, previous epidemiological studies indirectly suggested a different role for lifestyle, demographic and environmental factors. A decline in the incidence of RF positive RA had been already reported in Finland in 1980–2000 (20) and in the Pima indian population (21) and was more recently confirmed in a large US study (22); although not proven, these changes were attributed to public health measures including smoking cessation. In contrast, seronegative RA cases seem to increase (22-24), possibly due to growing obesity rates and ageing population.

Among environmental factors, considerations can be deducted from studies conducted on serum samples, case-control studies and metanalyses. The first assessed the prevalence and magnitude of antibody response against various bacterial and viral immunogen peptides derived from pathogens previously associated with RA, including *P. gingivalis, A. actinomycetemcomitans, M. avium*, Epstein-Barr virus, and human endogenous retrovirus in the sera of RA patients compared with the general population (25). The study demonstrated a significantly increased humoral response against all tested peptides in patients with RA. Among them, seronegative patients seemed to differ from seropositive RA only for lower titres and prevalence of antibodies against A. actinomycetemcomitans. A Swedish case-control study including 3515 incident RA cases and 5429 matched controls found that some allergic conditions such as atopic dermatitis were specifically associated with increased RA risk in older and ACPA-negative patients (26). Finally, a systematic review and metanalysis of all published epidemiological studies concerning the association between occupational exposure to free crystalline silica and subsequent development of RA confirmed a significant association in both seropositive and seronegative patients (27).

Immunopathogenesis

As a primary site of inflammation in RA, the synovial tissue represents a valuable source of information on the immunopathogenesis of the disease (28). Although few studies have evaluated the differences between seronegative and seropositive RA, it appears that, in the latter, more pronounced lymphoplasmacytic infiltrates are found, whereas monocytes and macrophages predominate in seronegative synovitis (11). A recent spatial transcriptomics study confirmed the differences in the cellular composition of the synovial tissue between seronegative and seropositive RA. Samples from 3 patients with seronegative RA and 3 patients with seropositive RA were analysed, demonstrating that synovitis of seronegative patients lacked robust signals of adaptive immune responses, and was rather characterised by an increased presence of dendritic cells (29). In contrast, tissues of patients with seropositive RA were much more organised into ectopic lymphoid structures. In keeping with these findings, comprehensive immunoprofiling of the synovial $CD4^+$ T cell subsets has convincly shown lower expression of markers of peripheral helper cells and lower levels of inhibitory receptors, such as PD-1, in ACPA-negative compared to ACPA-positive patients, indicating lower signs of activation in the former (30). A milder

imbalance between $CD4^+$ T cell subsets in seronegative patients has been demonstrated also in the peripheral blood in a study comparing 145 ACPA-positive, 145 ACPA-negative RA, and 38 healthy controls (31).

Despite being outside the scope of this review, it is worth mentioning the opportunity to disentangle some possible pathogenetic mechanisms of seronegative RA offered by the adverse events of specific immunotherapies. Examples have emerged primarily for drugs capable of altering the IL-4 and IL-13 axes, such as dupilumab, and immune checkpoint inhibitors (ICIs) used for cancer. Dupilumab is a monoclonal antibody approved for atopic dermatitis, asthma and chronic rhinitis. It blocks the membrane receptor that IL-4 shares with IL-13, reducing Th2 responses. Relevantly, the alteration induced by dupilumab may determine a compensatory immunological switch with activation of the IL-23/IL-17 axis (32), and the development of clinical manifestations typical of seronegative arthritis (32, 33). By interfering with immune cell-inhibitory costimulatory molecules, such as PD, PD-1 and CTLA-4, ICIs activate T lymphocytes against cancer cells. In some patients, ICIs can trigger several immune-related adverse events, including chronic arthritis. Arthritis in course of ICIs is mostly polyarticular and seronegative in 80% of the cases, thus hardly distinguishable from seronegative RA (34). Pathogenetically, ICIs can induce Th17 cell activation and proliferation, changes in CD8 immune-effectors profile, and impaired regulatory T cell survival (35, 36). A specific role of the IL-17 axis also in seronegative RA, if any, remains to be investigated.

Novel antibodies

Research in recent years has also shown that newly discovered autoantibodies are present in a significant percentage of seropositive and seronegative RA patients, highlighting a driving role of adaptive immunity also in these latter (3, 4). These are mainly AMPA, *i.e.*, antibodies directed against proteins that have undergone posttranslational modifications. Sidiras *et al*. (37) identified eleven novel carbamylated autoantigens in synovial fluid and serum of RA patients using a combined proteomics approach. Among them, specific antibodies against carbamylated hemopexin and alpha-2-macroglobulin allowed the diagnosis of 60% seronegative RA patients in the early arthritis cohort analysed. Another study investigating the presence and significance of anti-mitochondrial antibodies (AMA) found AMA levels to be elevated in up to 26% of RA patients (38). AMA levels correlated significantly with erosive disease and joint space narrowing, independent of ACPA positivity. Furthermore, antibodies to an outer mitochondrial membrane protein, MFN1, predicted the development of erosive disease in seronegative RA. A multiplex immunoassay with peptides from disease-related proteins in joints of RA patients detected a set of five peptides composed principally by new ACPA (cross)reactivities but also included a peptide without citrulline, that identified 22.5% (n=125) of seronegative patients (n=556) with 99% specificity (39).

It is interesting to mention that signs of B cell activation and antibody production have also been recently described in the classical seronegative arthitides arising in course of ICIs. Cappelli *et al*. (40) showed that more than 11% of patients with seronegative ICI-induced arthritis had anti-RA33 antibodies, whereas none of the patients not developing arthritis had these antibodies. Similarly, a significant increase in transitional B cells and specific autoantibodies to joint-related proteins was documented in a prospective cohort of patients treated with ICI for melanoma who developed inflammatory arthritis (41). Altogether, these finding point at an autoimmune contribution to disease pathogenesis also in a set of conditions traditionally considered seronegative.

Pre-clinical disease

In seropositive RA, the typical evolution is that genetically predisposed individuals may develop systemic autoimmunity under the effect of environmental, endogenous and stochastic factors; of them, some develop joint symptoms eventually followed by overt arthritis (42). The natural history of seronegative RA is in contrast more difficult to decipher, and disease extrinsication is generally considered more abrupt (43). The recent adiministration of the Symptoms in Persons at Risk of Rheumatoid Arthritis (SPARRA) questionnaire to seropositive and seronegative individuals at risk of developing RA from four European centres partially confirmed this evidence (44). It was indeed shown that specific symptoms details such as pattern of joint pain, frequency of joint swelling, presence of tingling sensations, and frequency of feeling fatigued provided useful additional information to estimate risk of developing clinical arthritis in subjects with autoantibodies; in contrast, in the seronegative subgroup, informative items were pattern of symptom development that increased rapidly and muscle weakness, but the prediction model was inaccurate. Similarly, results from imaging studies have shown that subclinical synovial and extra-synovial inflammation is infrequent in the pre-arthritis phase of subjects with clinically suspect arthralgia (CSA) lacking autoantibodies. Among 577 CSA, 80% of whom autoantibody-negative, intrametatarsal bursitis on magnetic resonance imaging (MRI) was present and specific for RA development in 28% of ACPA-positive compared to 9% of ACPA-negative individulas ($p<0.001$) (45). In keeping with the very intangible pre-clinical phase of seronegative RA, recent proteomic analyses have shown that multiple serum analytes, including C-reactive protein (CRP) levels, serum amyloid A, and soluble PD-1, effectively identify imminent cases of RA before diagnosis in ACPA-positive (n=69), but not ACPA-negative (n=50) subjects from a cohort of US military personnel (46).

Take-home messages

- Pathogenetic studies in seronegative RA are hampered by the difficulties in creating definitive diagnostic groups (11).
- Genetic studies confirm that most of the susceptibility loci identified in seropositive RA cannot be replicated in seronegative patients (12, 13).

- Endogeneous and environmental factors contribute to disease pathogenesis, but specific risk factors could differ between seropositive and seronegative RA (18, 19, 22, 23).
- Differences in the pathogenic pathways between the two subsets of the disease can be partially captured from the analysis of the synovial tissue (29, 30).
- The pre-clinical history of seronegative RA appears shorter and more 'abrupt' than that of seropositive RA (43, 44).

Epidemiology

The correct estimate of the true prevalence and incidence of seronegative RA is troublesome and likely biased by the selection criteria as well as by the natural history of this subset of the disease. It is indeed well established that, in national and international registries of established RA, the proportion of seronegative patients, mainly based on RF testing, is as low as 10-40% among all RA cases in historical cohorts (47) and has remained around 20-30% in more recent times (48). Most of these registries however collect information on patients escalated to biological (b) and/or targeted synthetic (ts) disease modifying anti-rheumatic drugs (DMARDs). The perception that absence of autoantibodies 'protects' against unfavourable outcomes might obviously have enriched these registries of seropositive patients. However, the proportion of seronegative RA is low also in non-selected cohorts of established RA receiving different types of treatments (49, 50), possibly due to the milder/self-remitting nature of some forms of rheumatoid-like seronegative polyarthritides. Data from early arthritis cohorts in contrast describe a different picture, with as many as 50-60% of the patients fulfilling RA classification criteria despite lacking autoantibodies (51, 52). Naturally, such a high proportion may reflect the inclusion of false-positive cases. However, these data need to be interpreted also considering the proposed changing epidemiology of RA, with increasing incidence of seronegative forms in more recent years, as alredy discussed (20-23). In this perspective, changes possibly attributable to ageing population deserve discussion. Based on the inclusion rate of patients with RA in the Leiden Early Arthritis Cohort between 1994 and 2015, Matthijssen *et al*. (23) calculated an increase of nearly 3% per year in the crude incidence of ACPA-negative RA; when adjusting incidence rates for the changes in age, much of the increase appeared related to ageing (adjusted incidence increase 1.7% per year). Interestingly, the higher prevalence of seronegative RA seems to mostly affect women, as recently indicated by a cohort study (53) and a metanalysis of 84 publications that included 87 RA cohorts (54), suggesting possible gender-related/specific differences in RA risk factors.

Take-home messages

- The frequency of seronegative RA within general RA cohorts greatly varies depending on several factors, including disease duration, persistence and progression (48).
- Due to changes in demographic and environmental risk factors, the incidence of seronegative RA is increasing (22, 23).

Classification, diagnosis and differential diagnosis

Since the release and dissemination of the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria in 2010 (55), the clinical and serological phenotype of patients with RA significantly changed (56). In the context of the score-based algorithm, autoantibody positivity provides a high contribution in the final sum of at least 6 points. Accordingly, it might be relatively simple to categorise patients with seropositive status. The same cannot be said for patients negative for autoantibodies since it is required a much larger number of involved joints to achieve the score of 6 (57). The 2010 criteria are therefore being criticised because of low sensitivity for seronegative forms (58). The analysis of time-trends between 2005 and 2017 in a large early arthritis Italian cohort recently demonstrated that, in seronegative patients, the proportion identified within 12 weeks progressively decreased from 37.9% to 25.6% (p=0.08) (59). Of note, the reduction in the rate of early referral after 2010 was prominent in patients classified as RA solely based on the 2010 criteria.

Together with sensitivity, specificity remains an issue for any classification criteria, and careful exclusion of other possible forms of arthritis is mandatory. The risk of misdiagnosis with the 2010 criteria is particularly high in seronegative patients, in whom self-limiting arthritis may occur in nearly 30% of the cases (60). In this setting, the application of the 1987 criteria (61) for classification purposes might be more specific. Using data from the BARFOT (Better Anti-Rheumatic PharmacOTherapy) early arthritis cohort (n=2543), only 3% of ACPA-negative patients fulfilling the 1987 criteria at disease presentation were misdiagnosed over a follow-up of 5 years, as recently shown (62). The differential diagnosis of seronegative polyarthritides remains however troublesome and includes inflammatory, infectious and non-rheumatic disease. As an example, calcium pyrophosphate deposition disease (CPPD) often mimicks seronegative RA. In a recent paper, Krekeler *et al*. (63) retrospectively evaluated the prevalence of CPPD in a large cohort of patients with chronic inflammatory arthritis. Among them, the prevalence of CPPD on hands radiographs was significantly higher in seronegative versus seropositive RA. Of note, no clinical nor laboratory feature could precisely discriminate between polyarticular CPPD and RA in that cohort, and synovial fluid analysis was often not available. Indeed, more than 25% of patient primarily diagnosed with seronegative RA fulfilled the diagnostic criteria for CPPD. Another crucial diagnostic issue is to differentiate between spondylarthritis (SpA) and seronegative RA. Mease *et al*. (64) recently conducted a systematic literature review to compare clinical features between SpA and RA. Among 79 records analysed published between 1997 and 2020, the Authors pointed out at differences that may help to distinguish between the two entities.

For instance, enthesitis was present significantly more often in SpA compared to RA and, among the latter, seronegative patients displayed higher entheseal abnormalities. In addition, skin or nails psoriasis and dactylitis were almost exclusively reported in SpA patients. At the same time, the presence of destructive peripheral arthritis, high disability, increased risk for cardiovascular comorbidities and pain were described as quite overlapping between the diseases, thus not helping in differential diagnosis. Interestingly, the Authors noted some differences also comparing early versus late and seropositive versus seronegative RA. In particular, autoantibody-negativity was associated with greater fatigue, less frequent metacarpophalangeal (MCP), interphalangeal and ankle joints involvement, and more common enthesopathy at ultrasonography (US).

Take-home messages

- Classification criteria developed for RA may have insufficient sensitivity and specificity for seronegative forms (58, 59).
- Due to the many mimickers, improvement in sensitivity may come at the expenses of reduced specificity (60, 63).
- Differential diagnosis remains mostly based on clinical experience (64).

Imaging

Imaging techniques such as US and MRI potentially offer several opportunities in patients with seronegative RA, including improvement of early and differential diagnosis and better identification of the anatomical lesions that sustain the disease process.

Literature dating back more than 30 years ago had suggested that, notwithstanding the overall clinical similarities between seropositive and seronegative RA, a proportion of patients in this latter subgroup could be characterised by larger joint and carpal involvement despite relative sparing of the small joints of the hands and feet (65). Interestingly, this evidence appears now somehow confirmed in a small Mexican study that compared US findings of twelve-joints, including the elbows, knees and ankles, in 21 seronegative and 49 seropositive patients (66). The Authors reported a non-significant trend for higher frequency of grey-scale (GS) and power Doppler (PD) synovitis at the knees in the former (76% and 38%, respectively, *vs*. 57% and 18%; p=0.13 and p=0.08). In contrast, fewer seronegative patients showed involvement of the 2nd MCP joint (38% *vs*. 71%, p=0.008), which was very rarely PD-positive (9% *vs*. 53%, p<0.001). The less frequent involvement of the small joints of the hands could in part explain the lower US scores found in seronegative vs seropositive RA patients in the same range of swollen joints when imaging assessment is restricted to the wrist, the MCPs 2/3 and proximal interphalangeals 2/3 of the dominant hand (67). Wang *et al*. (68) reported on the differential US patterns of hand joints and tendons in seronegative RA compared to osteoarthritis (OA) of similar age and gender and with relatively short disease duration (approximatively 10 months in both groups). The study is limited by the small sample size (83 RA, 40 OA), absence of a control group of seropositive RA and, more importantly, lack of information on the number of clinically swollen joints. However, the analysis confirmed the important role of tenosynovitis in RA. Patients with seronegative RA were indeed more frequently characterised by abnormal tendon GS scores (39% *vs*. 8%), which were more commonly of grade 2 as compared to grade 1 in OA. Furthermore, PD scores ≥2 were found in 23% of RA tendons and in none of OA, underscoring the inflammatory vs degenerative nature of the two diseases. Although one of the most challenging differential diagnoses of seronegative RA is represented by psoriatic arthritis (PsA), no US studies on the topic were published in 2022. In contrast, novel use of MRI shows promise in clinical practice and reaserch. By applying computing systems based on artificial neural networks to MRI sequences, Folle *et al*. (69), in a cohort of 135 seronegative RA, 190 seropositive RA, 177 PsA, and 147 psoriasis was able to demonstrate good discriminatory capacity for RA (both seropositive and seronegative) *vs*. PsA, and moderate for seropositive *vs*. seronegative RA. These findings are also relevant from a pathophysiological perspective, underscoring the existence of 'true' differences between RA (as a whole) and PsA.

At the anatomical level, imaging may reveal important peculiarities of seronegative RA, in which extra-synovial compartments may have a prominent involvement. The best discriminatory capacity for relevant outcomes in seronegative patients appears to be offered by tenosynovitis. A recent longitdinal study on 390 ACPA-negative UA patients revealed that MRI-detected tenosynovitis was associated with RA development with an AUC of 0.795 (70). Application of MRI increased the positive predictive value (PPV) from 19% to 28%, with the highest performance improvement compared to clinical assessment in patients with seronegative oligoarthritis (PPV from 19% to 27%). These findings were independently confirmed in a US study of 19 bilateral joints and 16 bilateral tendon compartments in 150 UA patients, 74% of whom were ACPA-negative (71). After applying principal component analysis to identify non-redudant variables that accounted for the largest proportion of the variance, digit flexor tendon GS independently predicted seronegative persistent arthritis, with a Nagelkerke R^2 value of 0.304. Relevantly, in patients with seronegative RA, tenosynovitis would appear not only as earliest and most specific site of disease localisation, but also the most sensitive to change. Longitudinal MRI analyses in 198 RA patients (47% of whom ACPA-negative) from the Leiden Early Arthritis Clinic have indeed revealed that, among seronegative patients undergoing sustained drug-free remission, tenosynovitis and osteitis decreased significantly, whilst synovitis scores remained mostly unchanged (72). In contrast, the small group of ACPA-positive patients experiencing disease resolution showed lower MRI inflammatory scores already at disease presentation, and a greater reduction of synovitis over time. It is important to emphasise that, in our view, these data

do not dispute the central role of synovitis also in seronegative RA. However, similarly to other seronegative arthritides such as PsA, the involvement of extra-synovial compartmets might be equally important.

Take-home messages

- Conventional imaging techniques have thus far failed to demonstrate specific lesions that could distinguish the various forms of chronic polyarthritis (66, 67).
- The precocious and common involment of tendons in seronegative RA might underlie specific pathogenetic processes (70, 71).

Response to therapy

The association between autoantibody status and response to conventional synthetic (cs), b or ts DMARDs is controversial for most drugs.

Methotrexate (MTX) is believed to have similar efficacy in seropositive and seronegative RA patients, to the point of being recommended as the anchor drug regardless of autoantibody status (73). The speed and magnitude of clinical response could however be lower in seronegative patients (74, 75). Recently published studies reinforced the hypothesis of greater drug efficacy in seropositive RA. Duong *et al*. (76) performed machine learning data analysis of 4 randomised controlled trials (RCTs) involving of 775 patients with early RA of ≤24 months duration. Outcome of interest was improvement of disease activity at 24 weeks. Together with lower 28-joints disease activity score (DAS28) and disability scores at baseline, ACPA positivity was predictive of better response. The non-complete appropriateness for seronegative RA is also suggested by recent studies independently demonstrating that MTX reduces withdrawal rates of tumor necrosis factor inhibitors (TNFis) due to ineffectiveness in seropositive, but not seronegative RA patients (77, 78).

Few more data are recently available about response to second-line therapies. A systematic literature review published in 2022 on 99 laboratory markers from 41 studies failed to show an association between RF and ACPA and response to TNFis (79). This data is however not confirmed in all trials, as seropositivity has been described to be a risk factor for worse response to TNFis in some studies (80, 81). Although the possible differences in TNFi outcomes in relation to autoantibody subgroups have not been further addressed, indirect evidence that this class of drugs is effective in seronegative RA comes from the aforementioned studies indicating good retention rates irrespective of the concomitant use of MTX (77, 78). Furthermore, a recent analysis from Shipa *et al*. (82) suggested that cycling between TNFis rather than swapping to other mechanisms of action is effective in seronegative but not seropositive RA. The authors evaluated drug survival of 435 RA patients refractory to a first TNFi and subsequently treated with a second TNFi or a different bDMARD, including abatacept, tocilizumab or rituximab. Over 2 years, the retention rate for biologics with a different mechanism of action was longer compared to that of a second TNFi only in seropositive RA, whilst seronegative patients did not show any advantage in drug survival with the use of non-TNFis.

Anti-lymphocyte therapy was previously shown to be more effective in seropositive compared to seronegative RA patients (83, 84). More recently, Norris-Grey *et al*. (85) assessed long-term persistence of rituximab and investigated possible predictors of drug discontinuation. Data from 404 patients were retrieved from medical records, under a real-life treatment setting. Of note, most patients had long-standing disease before the first cycle of treatment with rituximab (median disease duration: 10 years), and had already failed at least 1 bDMARD; moreover, the large majority (>90%) was seropositive (for RF, ACPA or both). Major reasons for stopping rituximab were primary or secondary failure, and autoantibody negativity appeared to be an independent predictor of drug discontinuation. Similarly, the already cited work from Shipa *et al*. (82) showed prolonged retention rate in RA patients treated with rituximab, but only in the presence of autoantibodies. Alten *et al*. (86) reported long-term retention rate of subcutaneous abatacept in RA patients from the Abatacept SubCutaneOus in Routine clinical practice (ASCORE) prospective multicenter trial. Among 2892 studied patients, 47% was still on abatacept therapy at 2 years. Higher retention of abatacept was observed in patients with lower exposure to previous biologics and in those with RF and/or ACPA positivity. This data suggests a lower persistence in treatment for seronegative RA, thus a lower efficacy or a higher burden of side effects in this subgroup of patients.

Only little information is currently available regarding predictive factors for response to JAK inhibitors (JAKis) in RA. One single *post-hoc* analysis of five RCTs previously showed that tofacitinib response was higher in seropositive compared to seronegative patients at 3 months (87). A similar trend was recently described by Sugawara and colleagues (88). The Authors retrospectively collected data from 132 patients with RA treated with tofacitinib or baricitnib. Through a cluster analysis, the study population was divided into 3 subgroups according to common clinical, serological and radiological characteristics. The primary outcome was the evaluation of inadequate response to JAKis, defined both as non-response (achieving neither American College of Rheumatology 20 - ACR20 response nor ΔDAS28>1.2 at 12 weeks), and intolerance. Interestingly, the authors identified a specific subgroup of patients – negative for autoantibodies and interstitial lung disease, positive for advanced joint destruction – that was particularly prone to interrupt treatment. Furthermore, in the whole population, univariate analysis showed a tendency of inadequate response to JAKi in 16% seropositive and 44% seronegative patients. Better response of seropositive RA to JAKis was also found in the Korean nationwide database including 300 patients receiving tofacitinib (89). In logistic regression analysis, the only variables that predicted lower drug discontinuation were positivity of RF (OR 0.06, 95% CI: 0.01–0.55) and ACPA (OR 0.11, 95% CI 0.02–0.71). Data remain however controversial. Results from the CorEvitas' RA Registry including 429 tofacitinib initiators

rather failed to demonstrate significant differences in relation to ACPA status for several outcomes, including changes in Clinical Disease Activity Index, modified Health Assessment Questionnaire (HAQ), patient global assessment (PGA) scores, and proportion of patients achieving a clinical response (90).

Take-home messages

- Methotrexate is effective in both seropositive and seronegative RA; however, its benefits are more convincent in seropositive forms (76).
- Drugs targeting pro-inflammatory cytokines such as TNFis convey similar benefits in seropositive and seronegative patients (77, 78).
- Drugs targeting adaptive immunity have lower retention in seronegative patients (85, 86).
- The impact of autoantibody-positivity on the effectiveness of JAKis has been poorly analysed and remains debated (88-90).

Outcomes

Despite being considered overall 'milder' compared to its seropositive counterpart, seronegative RA has extremely viariable clinical outcomes. A proportion of patients requires life long DMARD therapy and is characterised by persistent or progressive disease (9). On the other side, sustained drug-free remission, which is very uncommon in seropositive RA, can be achieved in up 40% of the patients lacking autoantibodies (91). If clinical prognosis cannot be predicted by demographic and clinical variables alone (92), serum biomarkers and early assessment of response appear to offer relevant discriminatory ability. In a recently published study on 266 early RA patients from the Leiden Early Arthritis cohort, 50% of whom ACPA-negative, higher baseline levels of CRP (≥15 mg/l) and early achievement of DAS remission identified a subgroup of seronegative patients with 80% probability of achieving drug-free remission, compared with 45% chances in subjects lacking these combined characteristics (93). Further adding information to the diverse clinical outcomes of seronegative RA, Cagnotto *et al*. (94), based on two different Swedish early RA cohorts recruited in different time periods, described particularly favourable outcomes in terms of clinical remission and response to therapy among male seronegative patients. In contrast, the proportion of patients in remission was low in seronegative females, although barriers limiting the achievement of a satisfactory response were mainly confined to subjective components of the DAS28 and acute phase reactants.

It is well established that seronegative RA patients are characterised by a less severe course of the disease in terms of joint and systemic bone damage (95-97). A recent post-hoc analysis of the BARFOT study evaluated the presence of joint erosions by conventional radiography at different time points in 608 patients with early RA (98). Despite a similar course of DAS28, PGA, VAS pain and HAQ, 24% of the patients never developed erosions over 8 years of follow-up. Relevantly, the proportion of never-erosive RA was 14% among ACPA-positive subjects, and 30% among ACPA-negatives; in multivariable analysis, absence of ACPA was the strongest predictor of erosion-free status over and above disease duration and activity. In addition with maintaing better joint integrity, seronegative patients were confirmed to experience less bone density loss over time than seropositives, with significant 3-years decrease of bone mineral density at the femoral neck but not the total hip and lumbar spine (99).

Collectively, the evidence that a large proportion of seronegative RA achieves adequate control of inflammatory activity and never develops erosions reassuringly confirms the more 'benign' nature of this subgroup of the disease, but also raises uncertainties about which outcomes should be assessed in these patients. It is indeed common experience that, in clinical practice, seronegative patients are less often satisfied with their treatment (100), more frequently complain of non-nociceptive pain (101) and develop concomitant fibromyalgia (102). Poor self-perception of the disease is often the limiting factor to the achievement of remission (103), and painful symptoms apparently dissociate from objective inflammation early during the patients' history (104). As a matter of fact, the management of chronic pain remains the greatest challenge in seronegative RA (9). No studies have specifically addressed neither the magnitude of persistent pain nor its management based on autoantibody characteristics, and the poor response to immunosuppressive treatmet escalation is more theoretical than scientifically proven. In a recently published *post hoc* analysis of pooled data from nine RCTs of tofacitinib in RA and PsA, pain reduction was significant even in those patients with abrogated inflammation (no swollen joints and CRP <6 mg/l) after 3 months (105). Disappointingly, this data refers to the typical RA population enrolled in RCTs, with high disease activity and autoantibody-positivity in >90% of the cases. Whether apparently non-inflammatory pain may improve with DMARDs also in seronegative patients needs to be specifically analysed. The lower rates of pain improvement in PsA compared to RA in the aforementioned study (105), toghether with the possibile clinical and pathophysiological similarities between seronegative RA and PsA, do not appear encouraging.

Take-home messages

- Many seronegative patients achieve satisfatory suppression of joint and systemic inflammation, and many remain erosion-free (93, 98).
- Despite such favourable outcomes, many seronegative patients miss their target because of persistent pain (103, 104).

Conclusions

The scientific community has been struggling for years to try to identify the peculiarities of seronegative RA compared to its seropositive counterpart. Research is hampered by the many diagnostic and management difficulties offered by this subgroup of the disease. We have tried to summarise and critically present the most relevant literature published in the last year and hope to continue updating the review with further discovieries to come.

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