

Impact of autoimmune serology test results on RA classification and diagnosis

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ABSTRACT

Rheumatoid arthritis (RA) is the most common systemic autoimmune disease and also the most severe arthritic disorder. The measurement of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) in serum supports the diagnosis of RA, which gained increasing significance over the last 65 years. However, a high variability between RF and ACPA methods has been described, impacting the diagnostic performance of the current ACR/EULAR RA classification criteria.

The great number of commercially available assays, often lacking traceability to an international standard, is a major factor attributing to this in-between assay variability. The adoption of an international standard for ACPA, as is since long available for rheumatoid factor, is therefore highly desirable.

Further harmonization in clinical interpretation of RF/ACPA assays could be obtained by harmonization of the cut-offs, for both the low and high antibody levels, based on predefined specificity in disease controls. Reporting test result specific likelihood ratios (LR) adds value in the interpretation of autoantibody tests. However, a good understanding of the control population used to define antibody test result interval-associated LRs is crucial in defining the diagnostic performance characteristics of antibody serology.

Finally, specificity in RA classification can be improved by refining serological weight scoring taking into account the nature of the antibody, the antibody level and double RF + ACPA positivity.

1. Introduction historical outline of serology testing in RA guidelines

Rheumatoid arthritis (RA) is a chronic, inflammatory joint disease with a worldwide prevalence of about 5 per 1000 adults, mainly affecting women [1–3]. It is characterized by joint inflammation that can lead to cartilage and bone damage if left untreated and can potentially result in disability, reduction of quality of life and increased mortality [4,5]. Since early diagnosis and treatment may prevent or delay progression of joint damage in the majority of patients with early RA, it is important to establish a diagnosis of RA as soon as possible [6–9]. However, identifying a patient with RA among the many patients

showing similar symptoms of arthritis can be challenging, even for expert physicians [10,11]. Historically, autoantibody measurement in serum supports the diagnosis of RA and has gained increasing significance over the last 65 years [12] (Fig. 1).

Despite its relatively low specificity ($\pm 80\%$) [13] and presence in various inflammatory diseases [14], rheumatoid factor (RF) is still the most well-known serological marker of RA, already incorporated in the first diagnostic criteria for RA proposed in 1956 [15,16]. In these criteria, a positive autoimmune serology test result was defined as a positive sheep cell agglutination test [17] or a positive streptococcal agglutination test.

Already two years later, new insights resulted in the publication of

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the 1958 revision of the diagnostic criteria for RA [18]. These revised criteria addressed the specificity of the autoimmune serology, by defining that the agglutination test method used shouldn't be positive in more than 5% of healthy controls in two laboratories. A definite RA diagnosis required five out of 11 criteria, and thus immune serology constituted up to 20% of the criteria needed [18] (Fig. 1).

Based on a computerized analysis of 262 contemporary, consecutively studied patients with RA and 262 control subjects with rheumatic diseases other than RA (non-RA), the American Rheumatism Association, thereafter renamed the American College of Rheumatology (ACR), formulated the 1987 revised RA classification criteria [19]. The definition of a positive RF result was slightly modified to 'abnormal amounts of serum RF by any method for which the result had been positive in <5% of normal control subjects', thereby stating a specificity higher than 95% [12]. RF was incorporated as sole serological marker and constituted one of seven classification criteria. As RA was defined by the presence of four or more of seven criteria, autoimmune serology increased in weight to 25% of the criteria needed for classification [12] (Fig. 1).

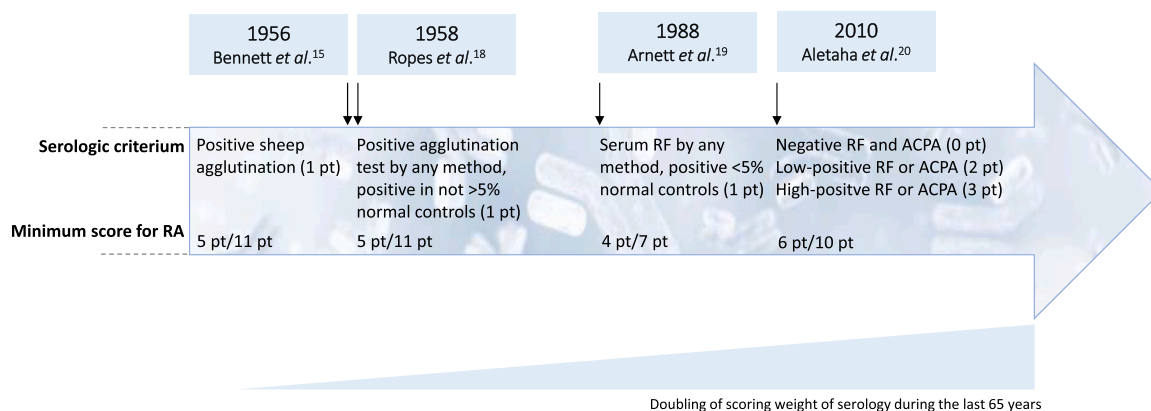
The emerging understanding of the therapeutic 'window of opportunity', starting treatment with disease modifying anti-rheumatic drugs (DMARDs) as early as possible aiming to halt progression of the disease [1,6–9], prompted the development of new RA classification criteria, improving the identification of patients with early disease [20]. In order to enhance sensitivity, the ACR and the European Alliance of Associations for Rheumatology (EULAR) proposed new classification criteria in 2010. Importantly, anti-citrullinated protein antibodies (ACPA) were added to RF as serological marker for RA. ACPA are associated with poorer outcomes and are more specific ($\pm 95\%$) for RA than RF, especially when comparing to relevant disease controls [20–22]. However, sensitivities of RF and ACPA for RA are comparable ($\pm 60\%$) [1,23]. Despite the difference in specificity, similar weights are given to RF and ACPA in the 2010 ACR/EULAR criteria. Application of the 2010 classification criteria provides a score of 0–10, with a score ≥ 6 allowing the classification of RA. The presence of RF or ACPA contributes two points if detectable and three points if present at levels >3 times the upper limit of normal; meaning above the reference range in the individual laboratory, a measure often coinciding with the reference ranges suggested by the individual assay manufacturers. Consequently, autoantibodies may now account for up to 50% of the scores needed to classify as definite RA, emphasizing their increasing importance during the last two

decades (20) (Fig. 1).

2. RA guidelines: diagnostic versus classification criteria - the clinician's perspective

Currently, no diagnostic criteria for RA are in use. Differences between classification and diagnosis have been summarized [11]. Classification criteria are used as a standardized means for including a well-defined set of patients in research studies to ensure better comparability across studies. The process of diagnosis, particularly for complicated multisystem involvement typical of rheumatic diseases such as RA, is a highly complex cognitive process that requires synthesis of many data points, typically beyond a simple algorithm-based set of criteria [11].

In people with suspicion of a rheumatic disease, time to diagnosis as well as change of the initial diagnosis to another 'final' diagnosis is often a challenge. This is pinned down in a study evaluating people diagnosed by a rheumatologist in the late 1990s: the exclusion of a true RA case was very rare, benchmarking this with the final diagnosis 10 years later. In other words, most patients with (early) RA were correctly diagnosed at the first occasion. However many patients initially diagnosed with RA did not carry RA as diagnosis any longer 10 years later [24]. Applying the 1987 revised criteria would have on the one hand excluded more patients with true RA, while on the other hand the number of false diagnoses would have decreased, resulting in a poorer sensitivity but better specificity [25]. Even though the current ACR/EULAR 2010 RA classification criteria [20] must not be considered diagnostic criteria, they can nevertheless facilitate early identification of RA patients thus allowing timely start of therapy with disease-modifying anti-rheumatic drugs (DMARD) [26]. The original report described a sensitivity of 82% and a specificity of 61% [4]. Since the publication of the ACR/EULAR 2010 criteria, several studies compared the performance of these criteria to the ACR 1987 RA criteria [7,27–30]. Several challenges and limitations in interpreting validation studies for criteria that have the purpose of classification of disease need to be considered. Particularly, applying classification criteria to unintended populations could conceivably alter the performance of the criteria. The target population as defined in the ACR/EULAR 2010 RA classification criteria are patients with at least one clinically swollen joint (i.e. evidence of definite synovitis) which cannot be better explained by another diagnosis [20]. A further challenge is that there is no true gold standard to compare with, although the initiation of



(15) Bennett et al. Bull Rheum Dis. 1956;7:121-4
 (18) Ropes et al. Bull Rheum Dis. 1958;9:175-6
 (19) Arnett et al. Arthritis Rheum 1988;31:315-24
 (20) Ann Rheum Dis 2010;69:1580-8

Fig. 1. Historical timeline of the diagnostic and classification criteria for rheumatoid arthritis highlighting the serological domains.

methotrexate treatment or other DMARD represents a good proxy [26]. A systematic review revealed that the ACR/EULAR 2010 RA classification criteria resulted in an increase in sensitivity of 11% at the cost of a slight decrement in specificity of 4% compared with the 1987 criteria [26]. Biliavska et al. documented that 20.5% of 'false positive' (non-RA with a score ≥ 6) patients were RF and/or ACPA positive, most of them showing high positivity [27]. Using the initiation of MTX as gold standard for diagnostic accuracy may be a convenient proxy, since many patients with rheumatic conditions mimicking RA would be considered for a first DMARD treatment with MTX [31]. This enlightens a fundamental difference between diagnosis and classification since a diagnosis would always be established with the intent to treat based on protocols used nowadays.

There remains an ongoing debate on the differences between seronegative and seropositive RA. A recent study showed that outcomes in RA have improved over the past twenty years. However a significant improvement in outcomes of RA patients diagnosed after 2010, i.e. in the treat to target decade [32] in comparison to the 1990s was only observed in seropositive patients [33]. This exemplifies that seronegative RA patients might benefit from continuous re-evaluation of diagnosis, taking into account that during the course of their disease diagnosis might change [34], as well as careful treatment decisions. Following the intent to treat approach for diagnosis of RA remains highly independent of RF positivity, since 25% of both seronegative as well as seropositive patients develop erosions within the first year of diagnosis [35,36]. Taking into account that serostatus is highly weighted in the 2010 ACR/EULAR criteria, it is not surprising that seronegative patients take longer until being diagnosed and also show higher disease activity [37,38]. The differences in disease inflammation variables, like higher number of swollen joints, but also composite scores, may lead to partly different (long-term) outcomes. This is not related to radiologic progression, but to disease activity and disease burden, which is easier to re-balance the sooner it is targeted [39,40]. Ideally rheumatologists would like to start the most effective treatment for patients within their window of opportunity to prevent refractory disease [41]. One of the best predictors of insufficient long-term outcomes, like functional disability, but also ongoing disease activity, are high scores in the Health Assessment Questionnaire (HAQ) [42,43]. Seropositive patients had numerically lower HAQ scores [37] and were more likely to achieve remission using the ACR/EULAR Boolean remission definition [38].

2.1. This leads us to the crucial question: when does someone start to have RA?

Rheumatologists are placed in the dilemma of starting treatment early enough, to prevent disability and comorbidities and restore a good state of health-related quality of life, however at the risk of subscribing potentially harmful treatment. There is a strong interest to better understand the process and signs of early development of RA, to identify and treat those individuals (at-risk individuals for progression to RA) before the onset of RA, hoping to prevent the patient from acquiring the diagnosis of RA. The new concept of extension of the different phases of RA before the onset of the clinical diagnosis [44,45], introduced an even greater challenge but also the opportunity to eventually catch a person at risk to develop RA early enough to prevent the onset of what we currently consider the condition RA. In short, the different phases describe the activation of the immune system through environmental factors based on a genetic background, which then leads to systemic autoimmunity, thereafter general and musculoskeletal symptoms, eventually converge to a diagnosis of RA [46]. Current study programmes investigating and treating at-risk individuals phrase inclusion criteria for their participants quite differently. While there is general agreement that the presence of ACPA and/or RF in at-risk patients has substantial predictive value for development of inflammatory arthritis, the detailed implications of imaging findings for risk estimation to

progress to RA are under vigorous investigation [47–52]. Since the detection of synovitis by means of ultrasound may be considered as endpoint (arthritis, independent of the assessment method), detection at baseline might obviously be an exclusion criterion [49]. However, others highlight this finding as predictor for clinical arthritis, since, besides ACPA positivity, the presence of synovitis as determined by ultrasound coincided with higher odds of developing clinical arthritis [47]. In the latter and other studies, ultrasound changes were not considered as exclusion criterion and tenosynovitis and synovitis were both identified as baseline predictors for RA development [53]. In evaluation of at-risk individuals, the application of ultrasound in routine assessments is useful but the interpretation needs always to be put in the overall context of the patient [51]. Since no sufficient evidence for the treatment of at-risk individuals yet exists, it only makes sense to recognize someone as patient with RA when the rheumatologist can intervene in the disease course based on evidence that the benefits of this intervention outweighs its risks. This notion is backed up, since many people with undifferentiated arthritis, with the majority being seronegative, do not progress to RA but recover to a state of health often without the need of a DMARD treatment [54].

In the end, the relevance of serological tests in the diagnostic process depends on the pre-test probability and the reassurance of diagnostic thoughts that the additional knowledge of ACPA and RF levels of a particular patient bring along. In a typical clinical scenario of a 55-year-old woman with morning stiffness, symmetric swelling of the wrists \pm affection of small joints, maybe even with a history of smoking, a clinician would consider the diagnosis of RA, independent of serology, although he or she would assume a high likelihood of retrieving positive ACPA or RF tests simultaneously. ACPA and/or RF positivity or levels play a more crucial role in the diagnostic assessment of individuals with less typical symptoms, that don't fit the descriptions in rheumatology textbooks. In a 40-year-old non-smoking male with asymmetrical swelling of only few untypical joints the pre-test probability is considered to be below 30%, i.e. in the middle part of the left logarithmic vertical scale in Fig. 2, and to the left on the x-axes depicting pre-test probabilities in Fig. 3c and d, whereas in the first scenario described above it might be considered above 70% (corresponding to the lower part of the left horizontal scale in Fig. 2, and to the right on the x-axes depicting pre-test probabilities in Fig. 3c and d). It is particularly in those less clinically clear cases that accurate and reproducible serologic tests are helpful (Figs. 2 and 3).

Taken together, in this part of the review we aimed to summarize the importance of serological testing in RA management, highlighting pitfalls of such testing and their impact on RA diagnostics and current RA classification criteria [12,55,56.].

3. Pitfalls in serology testing affecting current RA classification criteria

3.1. In-between assay variability in RF/ACPA testing

RF and ACPA analyses are central biomarkers in the classification of RA (20). Nevertheless, a high variability between RF and ACPA methods has been described [55,56,57]. A first factor attributing to this in-between assay variability is the growing number of *in vitro* diagnostic methods with a wide variety of formats, reagents, and signal detection systems.

RF are autoantibodies that bind to epitopes within the constant region (Fc) of immunoglobulin type G (IgG). Different assays are used to detect RF. The original tests using hemagglutination of sensitized sheep red blood cells [17,58], with reagents prepared in-house by each laboratory, were further developed to more stable agglutination tests with latex-containing particles of uniform size instead of sheep red blood cells [58,59]. Hemagglutination tests are currently considered to be obsolete and were replaced by more or less automated immunoassays. Nephelometric and turbidimetric RF assays measure RF by RF-induced

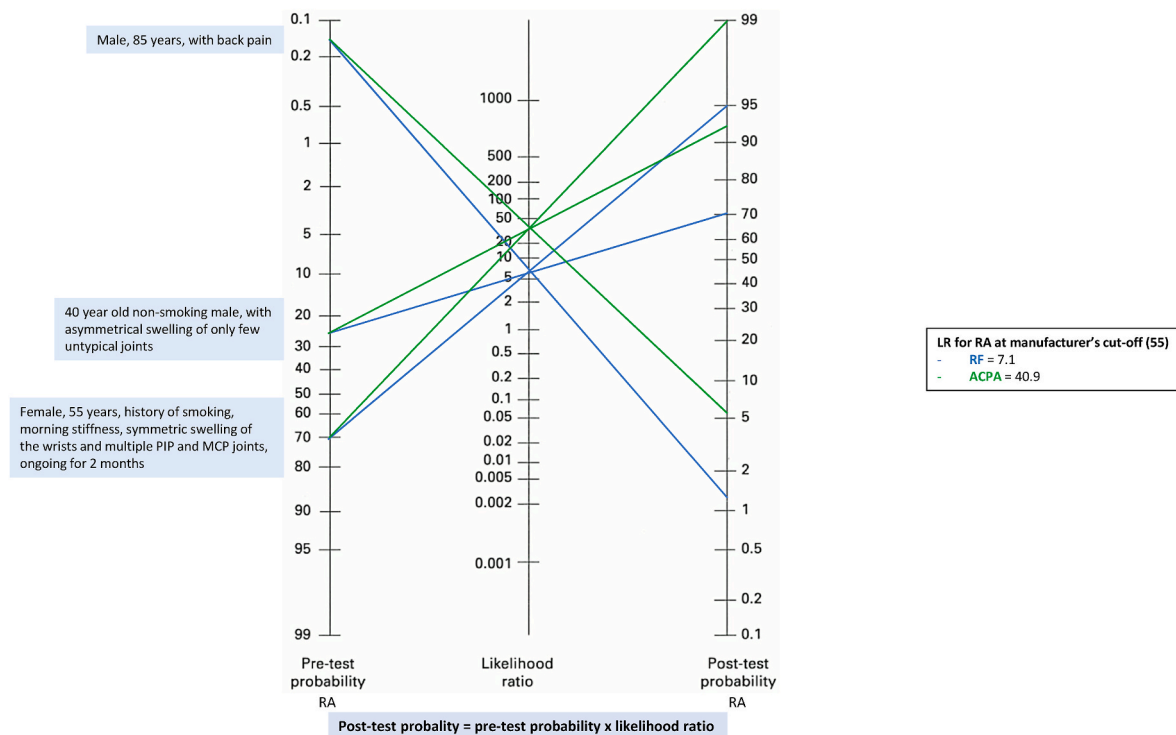


Fig. 2. Bayes theorem in clinical practice using the Fagan nomogram. Examples of patients presenting with different pre-test probabilities for rheumatoid arthritis are shown on the left-hand side. The likelihood ratios for RF and ACPA for assays from Thermo Fisher Scientific using the manufacturer's cut-off were obtained from Ref. [55]. A line drawn from the pretest probability (left) through the LR of the test result (center) gives the posttest probability (right).

agglutination of IgG-coupled particles [60]. Although the technique is non-isotype specific, the agglutination principle favours detection of IgM RF. Enzyme-linked immunosorbent assays (ELISAs) use isotype-specific (IgA, IgM or IgG) secondary antibodies to detect RF bound to wells coated with IgG [61]. These fundamental differences in measuring principle may cause considerable in-between assay variability. Furthermore, RF assays use different sources of IgG as the target antigen (rabbit IgG, human IgG or human Fc domains) to which the RFs bind. Other factors that are likely to cause in-between assay variability are the polyclonal nature of the RF response and the concentration of the target IgG-antigen [62].

Regarding ACPA detection, different generations of tests are currently available. Initially, ACPA were detected by ELISA using citrullinated recombinant rat filaggrin [63]. Subsequently, sensitivity of ACPA tests was enhanced without compromising specificity by using synthetic cyclic citrullinated peptides (CCP), most commonly the proprietary version 2 (CCP2) [22]. Later, a third generation ACPA (CCP3) test was designed by combinatorial peptide engineering which contains multiple citrullinated epitopes displayed in a conformational structure aiming to increase epitope exposure and immunoreactivity [64]. In addition, ACPA tests measuring antibodies to other citrullinated proteins and peptides (with different diagnostic performance) have been described, as recently reviewed by Rönnelid et al. In Europe, the anti-CCP2 test is the most commonly used test (12). It is manufactured and distributed by different companies and in different assay formats.

3.2. Lack of traceability to international reference preparations for RF and ACPA

Harmonization among assays relies on the availability of reference materials [65]. The calibration material should resemble the specificity, affinity and avidity of the patient sample to ensure that it behaves in the same way as the patient sample with respect to the method used [65].

Most RF assays are calibrated against an international recognized

standard [12,55], namely the WHO International standard W1066 or the British NIBSC standard of human RA serum 64/002 standard. Both standards are the same material [66,67] derived from a polyclonal pool of serum from 197 patients [68]. In 2008, the Antibody Standardization Committee (ASC) provided a reference human ACPA for *in vitro* immunodiagnostic use in solid phase enzyme immunoassays [69], distributed by the Centers for Disease Control and Prevention (CDC) and currently available via the Plasma Services Group (www.plasmaservicesgroup.com). The ACPA CDC standard originates from a single patient donor and never became WHO cleared. Although a preliminary evaluation of the ACPA CDC standard revealed a reduction of in-between assay variability [70], none of the commercial ACPA assays has been calibrated against this preparation. Due to the role of the ACPA antibody level in the classification, diagnosis [71,72], risk stratification and prognosis of RA patients [73,74], the international Working Group on the Harmonization of Autoantibody test (WG-HAT) of the International Federations of Clinical Chemistry and Laboratory Medicine (IFCC) proposed ACPA as one of the antibodies for which the production of a commutable reference material is needed [65]. Therefore, the NIBSC recently prepared a candidate ACPA standard named 18/204 which consists of a serum pool of five RA patients. The candidate material was evaluated by NIBSC in 2019–2020. An independent preliminary evaluation showed that when NIBSC 18/204 was used as a calibrator, an improvement in the alignment of ACPA results across several of the evaluated commercial ACPA assays was obtained (Van Hoovels et al., *manuscript submitted*).

3.3. Definition of cut-off values

In the ACR/EULAR 2010 RA classification criteria, a low positive RF or a low positive ACPA contributes two points whereas a high positive (>3-times the cut-off in the clinical laboratory performing the test, which is often the cut-off suggested by the manufacturer) RF or ACPA contributes 3 points; a score of ≥ 6 points coincides with the classification of a patient with RA (Fig. 1) [20]. Unfortunately, manufacturers

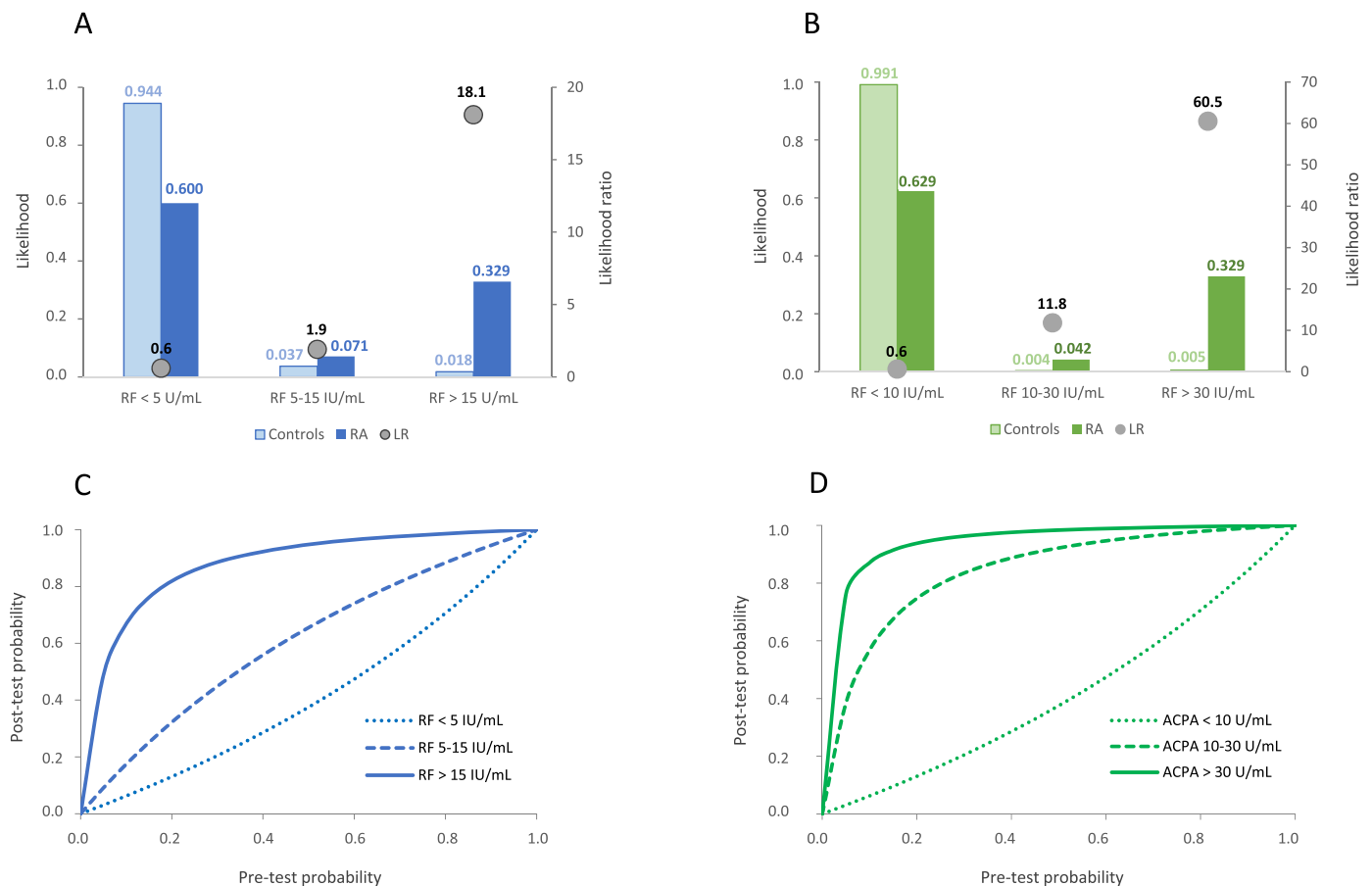


Fig. 3. **A** Likelihoods of rheumatoid factor (RF) test results (<5 IU/mL (cut-off), 5–15 IU/mL, > 15 IU/mL) for rheumatoid arthritis (dark blue bars) and controls (light blue bars) are given on the left y-axis. The likelihood ratios are displayed as grey filled circles on the second right y axis; **B** Likelihoods of anti-cyclic citrullinated peptide (ACPA) test results (<10 IU/mL (cut-off), 10–30 IU/mL, > 30 IU/mL) for rheumatoid arthritis (dark green bars) and controls (light green bars) are given on the left-hand axis. The likelihood ratios are displayed as grey filled circles on the second right y axis; **C** Post-test probability for rheumatoid arthritis as a function of pre-test probability and of RF test result (<5 IU/mL, 5–15 IU/mL, > 15 IU/mL); **D** Post-test probability for rheumatoid arthritis as a function of pre-test probability and of ACPA result (<10 IU/mL, 10–30 IU/mL, > 30 IU/mL). The cut-offs and corresponding LRs for RA of the RF and ACPA assays of Thermo Fisher Scientific as published in Ref. [55] were used.

apply arbitrary cut-off values which may substantially differ between the assays. This has been illustrated for RF assays by the differences in results obtained when analyzing the W1066 standard [55]. When ratios of cut-offs suggested by the manufacturer and the available international standards were compared, an up to 15-fold difference between RF assays [55] and a 2–5-fold difference between ACPA assays [55,70] was obtained. Consequently, diagnostic performances differed among assays, with large differences in sensitivities and specificities. Furthermore, this inconsistency in cut-off definition resulted in different percentages of patients being classified as RA, depending on the RF and ACPA assays used, as previously described [55,57].

Cut-off values among companies should be aligned, by defining cut-offs based on a predefined specificity in a control cohort. There is a general trend that cut-off values of RF tests are set at a lower specificity than for ACPA, due to the inherent lower diagnostic performance of RF assays [12,13,55]. Using a single cut-off value only provides the clinician with dichotomous information. However, as has been shown for RF and ACPA, higher RF and ACPA levels are more frequently found in patients with RA [55,72]. The ratio between the likelihood of a test result occurring in diagnosed persons to the likelihood of the same test result occurring in controls is defined as the likelihood ratio (LR) [75]. For dichotomous tests, this value corresponds to the ratio of true positivity in diagnosed persons to false positivity in controls. In addition, test result interval-specific LRs bear more clinically relevant information than the LR derived from a single cut-off [75–78]. Using an

antibody-concentration dependent LR on a nomogram, one can calculate the post-test probability of disease (Fig. 2). However, the same antibody test result can have different implications in different patients, depending on the pre-test probability of disease for these patients (Figs. 2 and 3). Therefore, also because of the clinical heterogeneity of RA, clinical experience is a key determinant in interpreting antibody test results [79]. An alternative approach is to display the relation between pre- and posttest probabilities by a curve (Fig. 3). Such graphical representation has been shown to be a good way to convey diagnostic information [77]. Whatever the approach, the emphasis is not on getting the exact posttest probability but rather on developing an understanding of the meaning of a quantitative laboratory result [76].

As described earlier in our review, the composition of the control population is crucial in defining the diagnostic performance characteristics of RA antibody serology. One can appreciate that for the same antibody levels, higher LRs for RA will be obtained using a healthy control population compared to using a rheumatological diseased control population. This finding underlines i) the difficulty of comparing results of studies based on different (diseased) control cohorts and ii) the necessity of comparing antibody tests by using the same or similar well-defined and preferably universally available study cohorts.

3.4. Scoring weights of serological test results

Several reports in literature agree that the higher the concentration

of RF or ACPA, the higher the LR for RA [55,72]. This is in line with the serological scoring in the current ACR/EULAR 2010 RA classification criteria (20). However, no differentiation is made between the nature of the antibody, assuming that the diagnostic value of RF and ACPA is equivalent, which is not the case because ACPA has a higher specificity and likelihood ratio (LR) for RA than RF [55,72,80–82] (Figs. 2 and 3). Furthermore, the highest LRs were consistently found for double positivity for RF and ACPA [55]. This finding is in line with the demonstration that the combined presence of RF IgM and ACPA mediates increased production of pro-inflammatory cytokines *in vitro* and is associated with elevated systemic inflammation and disease activity in RA [83,84]. Co-occurrence of RF and ACPA is known to be a powerful predictor of future RA and has been associated with a worse disease phenotype with increased risk of recurrence when tapering DMARD therapy [85–88]. Nevertheless, the classification criteria do not consider a difference in contribution for combined positivity of RF and ACPA. By adapting serological weight factors based on inherent diagnostic performance characteristics of RF and ACPA, antibody level and combined positivity, a gain in diagnostic specificity in RA classification is to be expected. Low diagnostic specificity is a major shortcoming in the current RA classification criteria as shown by Biliavska et al. [27].

4. Suggestions for improvement of RF/ACPA test performance in RA diagnostics and classification

Despite the rising importance of autoimmune serology in RA over the last 65 years, there is an important lack of harmonization between the currently available RF and ACPA assays [55,56,57]. The Clinical and Laboratory Institute refers to a definition of “(method) harmonization” as “the process of recognizing, understanding and explaining differences while taking steps to achieve uniformity of results, or at the minimum, a means of conversion of results such that different groups can use the data obtained from assays interchangeably” [89]. In autoimmunity however, harmonization initiatives are more challenging than in routine clinical chemistry, because of the nature of the entities measured [90]. Autoantibodies are complex analytes that can vary between patients and even within a patient during the course of an autoimmune disease [91]. Currently, different international organizations are active in the standardization of autoantibodies in rheumatic and related diseases: the European Autoimmunity Standardization Initiative (EASI), the international Consensus on Antinuclear Antibody Patterns (ICAP), the National Institute of Biological Standards and Control (NIBSC), the Autoantibody Standardization Committee (ACS), a subcommittee of the International Union of Immunology Societies (IUIS), the European Consensus Finding Study Group on Autoantibodies (ECFSG), a.k.a. the EULAR Autoantibody Study Group, and a dedicated WG-HAT of the IFCC, together with the Joint Research Center of the European Commission [92]. Understanding the variability among antibody assays and working on solutions for harmonization in a constructive dialogue between laboratory professionals, clinicians and manufacturers is essential for the success of ongoing harmonization projects.

Adoption of an international standard for ACPA, as available for RF, is highly desirable and would facilitate comparison between different assays [70]. However, the use of a standard doesn't solve all issues as a poor correlation between methods due to antigen source and specificity of the sample will not be improved by the introduction of a common reference material [65]. When methods provide poorly correlating results, commutable standards can be used to put these methods on the same scale, but this will not reduce variability between non-correlating methods. This is clearly shown for RF: although a reference serum has been available since 1968 and most RF assays are traceable to the same reference material, standardization of RF determination across companies has not yet been achieved [12,55]. Thus, despite the availability of reference materials, there remains a need to harmonize RF/ACPA determinations.

Further harmonization in clinical interpretation of RF/ACPA assays

could be obtained by harmonization of the cut-offs of assays, for both the low and high antibody levels, based on predefined specificity in disease controls [12,55]. This is of particular importance because serological scoring in the 2010 ACR/EULAR classification criteria of RA (20) is based on i) the manufacturer's cut-off and ii) an arbitrarily chosen cutoff for high antibody positivity e.g. ‘3 times the manufacturer's cut-off’. Ideally, those cut-offs are established in close cooperation with lab experts, rheumatologists and diagnostic manufacturers, in order to align interpretation across companies. Furthermore, it is important that laboratory professionals and clinicians become more familiar with the concept of LRs and that they develop an intuitive feeling for the clinical relevance of a LR [76].

Finally, there is some evidence that specificity in RA classification, without compromising sensitivity, can be improved by refining the serological weight scoring, taking into account the nature of the antibody (RF versus ACPA), the antibody levels and occurrence of combined RF/ACPA positivity. There is now increasing awareness that efforts should be undertaken to harmonize interpretation of RF and ACPA test results in order to further improve classification and diagnosis of RA.

Credit author statement

Lieve van Hoovels: Conceptualization, Writing - Original draft preparation Paul Studenic: Writing- Original draft preparation. Daniela Sieghart: Writing- Original draft preparation. Guenter Steiner: Conceptualization, Writing- Reviewing and Editing, Supervision Xavie Bossuyt: Conceptualization, Writing- Reviewing and Editing Johan Rönnelid: Conceptualization, Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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