Short Review

The Role of Serology Testing in the Diagnostic Work-Up of Rheumatoid Arthritis

Running Title: Serology for Rheumatoid Arthritis

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Abstract:

The diagnosis of Rheumatoid Arthritis (RA) requires a combination of clinical, laboratory and imaging investigations. The ACR/EULAR classification criteria were presented in 2010 with the aim of improving the identification of individuals with RA. Because of its positive predictive value for RA, anticitrullinated peptide antibodies (ACPA) testing was newly introduced in the criteria in 2010, but with the same weight as the less specific test Rheumatoid Factor. This review addresses evidence, knowledge gaps, advantages and disadvantages of different laboratory testing paradigms in RA diagnosis. The goal is to support physicians in appreciating nuances of laboratory testing, to take informed decisions about who should be tested, when these tests should be utilized, and which tests are most appropriate for a diagnostic work-up.

Key Words: Rheumatoid Arthritis, Serology

1. Introduction

Rheumatoid Arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation that ultimately leads to irreversible joint destruction. RA affects approximately 0.5-1.0% of the general population[1], and has an incidence as high as 40.9 per 100,000 in the Western world.[2] In RA, precise and early diagnosis represents a pivotal factor, due to its chronic and progressive nature: in fact, joint damage with erosion may happen in 75% of the patients as early as two years after diagnosis.[3, 4] The current American College of Rheumatology (ACR) / European League against Rheumatism (EULAR) classification criteria[5,6] were introduced in 2010 with the aim of updating the older criteria from 1987.[7] The criteria are based on a combination of clinical, laboratory and imaging investigations; each of these sub-criteria contributes with points to a final score, which may range in between 0 and

10. Scores equal or above 6 are needed for a RA classification. Despite the fact that they were originally conceived for research classification purposes, these criteria are widely used in clinical practice as the "gold standard" for the diagnosis of RA.[8] Two systematic literature reviews (SLR) and meta-analyses (MA)[9, 10] were recently performed to assess the overall diagnostic accuracy of the criteria: the pooled sensitivity and specificity are 68% (±17%) and 69% (±15%) respectively according to[9], while they range 73-76% and 61-74% according to[10]. Overall, these classification criteria are characterized by fair sensitivity and fair specificity.

Because of its positive predictive value for RA, anticitrullinated peptide antibodies (ACPA) testing was newly introduced in 2010 in the criteria. Specifically, ACPA and Rheumatoid Factor (RF) testing belong to the same sub-criterion, and are given the same weight in contributing to the final score. A positive CCP result or RF result does not necessarily equate to a RA diagnosis; in fact, these tests may contribute as many as 3 points to the 6-10 points required for a classification of RA (Table 1).

Table 1:   2010 ACR / EULAR classification criteria [5, 6].

Test result	Points
RF and CCP negative	0
RF and/or CCP low positive	2
RF and/or CCP high positive	3

According to a recent study[8], a score equal or higher than 6 can be reached adding points from the sub-criteria on clinical data only (e.g. without serology). It is clear that the ACR/EULAR criteria [5, 6], defined with the goal of increasing sensitivity not to miss any patients, may generate a consistent number of False

Positive results. These results from Van Hoovels and colleagues[8] ascribed to the tests' poor harmonization and result interpretation the fact that RA classification may vary when different assays are used. To reduce both number and burden of False Positives, in clinical practice it becomes therefore pivotal to prefer the RF and especially CCP tests which are characterized by the highest specificity.

2. Laboratory testing in Rheumatoid Arthritis

Laboratory testing is of great value when evaluating a patient with suspected RA. Even if some of these tests may be non-specific, they can be useful in both diagnosis and management of patients, as well as to assess disease activity.

In clinical practice, interpreting the complexity of laboratory testing can be challenging, and even results interpretation from older tests can be confusing if not ordered in the right sequence or used with the correct clinical scenario.

Numerous paradigms exist in diagnostic testing; however, three key concepts should be considered when utilizing laboratory tests:
1) For most autoimmune diseases, including RA, a single laboratory test is not diagnostic, rather the diagnosis is made by a combination of clinical signs and symptoms with laboratory testing confirming the clinical symptomology.
2) No test is perfect (e.g., 100% sensitive and 100% specific), as all tests will either falsely label some individuals as positive when they do not have the disease and miss other patients when they do. Tests misclassification rates can be reduced by testing the correct population, i.e. those that are at risk, thereby increasing pre-test

probability and improving the effectiveness of testing.

3) It is essential that physicians are familiar with the platform and methodology utilized for the tests ordered, as the corresponding results are not always comparable since the performance varies between manufacturers.

2.1 Who should be tested?

Any patient with symmetric polyarthritis, morning stiffness or a family history of rheumatic disease should be considered a candidate for an RA work-up, especially if the arthritis symptoms have been present for more than 6 weeks.

2.2 When should testing be performed?

Early testing for RA is crucial since numerous studies have now demonstrated that early identification and consequently appropriate treatment of at risk patients prevents irreversible joint damage and the loss of function seen in chronic RA.

2.3 Which tests are appropriate for a diagnostic work-up in RA?

In contrast to other conditions such as diabetes, where single tests can label a patient pre-diabetic or diabetic, in RA laboratory testing is utilized to triage patients, to determine the need for additional or ancillary testing. Consequently, most patients will require a battery of tests that should be ordered simultaneously to prevent delay in the establishing the correct diagnosis and commencing therapy. These are discussed in the next Section.

2.4 Recommended serology testing in RA

a. Rheumatoid Factor is an antibody against the Fc portion of human IgG; it can be detected in the majority of patients with

RA (80%),[11] and has historically been used as the key diagnostic marker for RA. RF can be of any isotype of immunoglobulins (IgA, IgG, IgM and IgD), but, to date, only RF-IgM is included as a diagnostic criterion by the ACR and EULAR.[5, 6] A SLR and MA quantified the pooled sensitivity of RF IgM in 69% (95% CI 65-73%) and the pooled specificity in 85% (95% CI 82-88%).[12] Because of its low specificity, RF-IgM has limited clinical utility especially if tested in isolation, since it is also positive in numerous other diseases, as well as healthy controls. In fact, it can be found at low titre levels in other autoimmune conditions (such as systemic lupus erythematosus or Sjögren's syndrome), in some infective disorders (tuberculosis, hepatitis, bacterial endocarditis), non-autoimmune conditions (osteoarthritis), and malignancies.[13]

In clinical practice, many specialty physicians do utilize more individual RF isotypes (IgM and IgA in particular) in disease prognostication. In fact, emerging evidence showed that RF IgM, RF IgG and especially RF IgA are the first appearing antibodies in pre-symptomatic individuals, and can precede the onset of RA up to 20 years.[14] In particular, the presence of RF IgA is associated with disease activity, [15] and patient with raised RF IgA may develop more severe erosive disease with a greater number of erosions.[16, 17] Because of this, the presence of RF IgA could justify more aggressive treatment at an early stage.[16]

From a technical point of view, RF is usually measured by nephelometry, a technique which captures all classes of Igs returning an overall positivity without the possibility of documenting the individual Igs results. A very recent study[18] showed that nephelometry methods are characterized by lower sensitivity than ELISA tests, due to the higher number of

seronegative patients they are not able to correctly identify.

b. Anti-Citrullinated Peptide Antibodies (ANCA) are highly specific biomarkers for the diagnosis of RA. Citrullination is not specific for RA, with other rheumatologic diseases and even trauma showing the presence of citrullinated proteins (CCP). [19] Despite of this, the availability of ACPA testing represented a major breakthrough in the laboratory diagnosis of RA[20], as these autoantibodies are much more specific for RA than RF.[13] CCP can also be present in sera or plasma in pre-symptomatic patients years before disease onset.[14]

The recent SLR and MA by Mathsson Alm et al quantified CCP tests' overall pooled specificity as 91% to 97% and pooled sensitivity as 52% to 84%, depending on the test manufacturer and on the study design.[21] ACPA assays can be positive in 20% to 30% of RF-seronegative patients,[22] representing thus a complementary test. A recent prospective study[8] confirmed the excellent specificity of ACPA in diagnosing RA in clinical practice: specificities ranged from 97.1% to 99.1% across manufacturers.

Clearly, a large number of CCP tests are nowadays available on the market, and considerable variations can be found in their diagnostic performance. Despite there is no evidence that a single test proved to be statistically superior to another in accuracy, the literature revealed some manufacturer-related differences in the pooled estimates of sensitivity and specificity between the commercially available CCP assays.[8, 21]

Moreover, several proprietary versions of CCP antigens exist, and they are labeled CCP2, CCP3.0, CCP3.1. However, despite the sequential numbering of these antigens, higher numbers do not signify improved clinical performance with CCP2 actually

demonstrating the greatest combination of sensitivity and specificity for RA.[21]

Approximately one third of RA patients are both RF and ACPA seronegative.[18] **14-3-3η** is a novel marker which has been associated with inflammatory changes and prediction of joint damage.[23] However, a recent review failed to demonstrate the incremental benefit with 14-3-3η and further study is needed before implementation into clinical practice is recommended.[24]

Elevations in acute phase reactants, such as **ESR and CRP** are consistent with an inflammatory state and used as a diagnostic criteria for the diagnosis and monitoring of RA. Studies so far have not demonstrated superiority of one reactant versus the other or their combined use for this purpose.[25, 26]

Antinuclear Antibody (**ANA**) screening should be performed to exclude other rheumatic diseases, since early RA may be difficult to distinguish from other rheumatic conditions, including Systemic Lupus Erythematosus (SLE).[27]

A Complete Blood Count (**CBC**) may simply show persistent anemia and thrombocytosis consistent with inflammation and may be the first clue as to the ideology.[28]

Additional ancillary tests to consider: multiple biomarker disease activity (**MBDA**) tests are panel tests that combine multiple markers linked to a proprietary algorithm to predict the disease activity, though these are not utilized for the initial diagnosis of RA. Proponents of panel testing state theoretical advantages with this methodology compared with conventional tests.[29, 30] However, the role of these panels in routine clinical practice still remains to be established.

3. Clinical Utility of Multi-Analyte Testing in RA

In clinical practice, it is considered beneficial to test both for RF and CCP rather than testing for each antibody alone. To minimize time-to-diagnosis, both RF and ACPA markers should be measured in tandem, since many times one marker may be positive while the other is negative or vice versa, especially in early stages of the disease.

Combined testing is known to improve the sensitivity or the specificity of establishing a diagnosis of RA. In fact, when interpreting laboratory results together, the clinician decides whether test sensitivity or specificity is preferable. This is usually implicitly determined by the individual patient characteristic (gender, age, smoking status, etc) together with the presence / absence of appropriate signs and symptoms. By defining positivity as "positivity to at least one test", the pool of positive individuals becomes bigger, as it includes the ones positive to both tests as well as individuals positive to one test only; from the mathematical point of view, this is known to increase overall sensitivity compared to single-analyte testing, but to reduce overall specificity. On the other hand, the definition "positivity to all tests" reduces the number of "patients", thus reducing overall sensitivity compared to the single-analyte tests performance, but it increases overall specificity.

The 2010 ACR/EULAR criteria[5, 6] suggest to test for RF IgM and CCP together, and positivity should be interpreted as "positivity to at least one test" (Table 1), scores being assigned depending on the level of positivity. The overall diagnostic accuracy of this testing combination was recently documented[23] as 68.4% [61.3%-75.0%] overall sensitivity and 86.3% [80.7%-90.8%] overall specificity. Comparing with the overall ACR/EULAR 2010 criteria's

performance[9], serology tests are characterized by compatible sensitivity but much higher specificity. The criteria in fact work well in identifying autoantibody positive for RA, but autoantibody-negative individuals are still missed with these criteria.[31]

An important increase of the likelihood ratio from 51.3 for both RF IgM and CCP when resulting individually high positive, to 86.7 when they both are high positive was documented in.[8]

The scientific literature offers also some scarce examples of the benefits of testing with more than two analytes. Brink et al [14] showed that adding one, two or three RFs to CCP increased specificity to 99.4% [98.1%-99.9%], 99.6% [98.4%-100.0%], and 99.8% [98.7%-100.0%] respectively. An algorithm including RF IgM, RF IgA, RF IgG and CCP was proposed in the attempt of maximizing the efficacy of RF testing;[32] according to the authors, positivity to RF IgM, RF IgA and CCP makes RA almost certain.

4. Discussion

The present review is intended to provide a guidance to physicians in appreciating nuances of laboratory testing in Rheumatoid Arthritis diagnosis, with the ultimate goal of taking informed decisions about who should be tested, when these tests should be utilized, and which tests are most appropriate for a diagnostic work-up. The role of serology in the clinical scenario is to assist in confirming the clinical diagnosis. A certain number of markers are available on the market, and some of them are recommended in the 2010 ACR/EULAR criteria.[5, 6] These criteria were conceived to maximize overall sensitivity not to miss any patients. In practice, the direct application of these criteria may lead to an important number of False Positive results, and it becomes therefore pivotal to prefer the RF and

especially CCP tests which are characterized by the highest specificity. In fact, when pre-test probability is low, a non-specific test is a false economy from both patient's and payer's perspectives.

It emerges clearly from the scientific literature that no single tests should be used in isolation to make neither a RA diagnosis nor a treatment plan. However, if the diagnosis or RA can be confidently made, there should be no delay in referring the patient appropriately or commencing patients on definitive therapy, since early and rapid control of inflammation with DMARDs prevents irreversible joint destruction and loss of function.

5. Ethical Approvals

NA

6. Conflict of Interest

Barbara Mascialino and Anagh Vora are employees of Thermo Fisher Scientific.

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