Standardization of autoantibody testing: a paradigm for serology in rheumatic diseases

Pier Luigi Meroni, Martina Biggiogero, Silvia S. Pierangeli, Joanna Sheldon, Ingrid Zegers and Maria Orietta Borghi

Abstract | Autoantibody measurement is an excellent tool to confirm the diagnosis of rheumatic autoimmune diseases. Hence, reliability and harmonization of autoantibody testing are essential, but these issues are still a matter of debate. Intrinsic variability in analytes and reagents as well as heterogeneity of the techniques are the main reasons for discrepancies in inter-laboratory variations and reporting of test results. This lack of reliability might be responsible for wrong or missed diagnoses, as well as additional costs due to assay repetition, unnecessary use of confirmatory tests and/or consequent diagnostic investigations. To overcome such issues, the standardization of autoantibody testing requires efforts on all aspects of the assays, including the definition of the analyte, the pre-analytical stages, the calibration method and the reporting of results. As part of such efforts, the availability of suitable reference materials for calibration and quality control would enable the development of a reliable reference system. Strong positive sera from patients have been used as reference materials in most of the autoantibody assays for rheumatic diseases; however, antigen-affinity-purified immunoglobulin fractions or in some cases reliable monoclonal antibody preparations offer more adequate tools for standardization. Systematic assessments of reference materials are currently underway, and preliminary results appear to be encouraging.


Introduction
Autoantibody testing in SARDs
Growing evidence indicates that early diagnosis followed by prompt and aggressive treatment increases the probability of inducing remission in the majority of systemic autoimmune rheumatic diseases (SARDs). Furthermore, treating the disease in the early phases can reduce the risk of irreversible tissue damage and improve the overall prognosis. As the most frequent biomarkers of many SARDs, autoantibodies form part of the classification criteria for most of these conditions (Table 1). Quantification of variations in serum titres of some autoantibodies can also offer information on disease activity and/or the effect of immunosuppressive treatment; a key example is levels of anti-double-stranded DNA (dsDNA) antibodies, which are commonly used for monitoring disease activity in patients with systemic lupus erythematosus (SLE). In addition to their defined role in the classification and diagnosis of SARDs, several autoantibodies can be used as biomarkers of the involvement of particular tissues or organs, making such assays important in defining relevant comorbidities and determining the risk of developing severe disease. Anti-citrullinated peptide antibodies (ACPA), for example, can be related to the prognosis of rheumatoid arthritis (RA), whereas anti-phospholipid antibodies (aPL) are associated with the presence of specific clinical manifestations of anti-phospholipid syndrome (APS).

Autoantibodies, therefore, are vital not only in evaluating the risk of developing specific complications of SARDs, but also in identifying subgroups of patients with different prognoses that require different clinical management strategies (Table 2). Autoantibody positivity may be the determining factor in starting primary prophylactic therapy, even in the absence of overt clinical signs, in order to reduce the risk to the patient. The analytical reproducibility and overall reliability of such measurements are, therefore, essential.

Assay development
Autoantibodies have historically been detected using manual or qualitative assays, or assays that allow only limited quantitative interpretation. Such tests are gradually being replaced by quantitative, automated, high-throughput solid-phase assays, which are moving from dedicated, specialized centres into routine service laboratories. The availability of these new assays has enabled laboratories to manage the increasing demand for rapid testing of samples, but the move from qualitative tests to reliable quantitative assays has uncovered several problems. These issues include quality control, quality assurance, standardization, analytical sensitivity and specificity, within-laboratory and between-laboratory reproducibility, and clinical sensitivity, all of which are relevant to the overall clinical interpretation and ‘value’ of the tests.

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Competing interests
The authors declare no competing interests.
Autoantibody measurement is required for the adequate diagnosis and management of systemic autoimmune rheumatic diseases.

The advent of new techniques, as well as the increasing number of autoimmune diagnostic laboratories, raises the issues of assay variability and reproducibility.

As a consequence, harmonization of the available assays is becoming increasingly urgent.

The availability of suitable reference material for calibration and quality control is emerging as a valuable tool for increasing assay reliability.

Initiatives for harmonization of testing have been started by several international committees and organizations.

**Challenges of autoantibody testing**

The clinical information offered by autoantibody testing is invaluable for the diagnosis and management of SARDs; however, substantial limitations result from the complex analytical specificity of the tests and the need for high sensitivity. Methodological issues, such as source and preparation of antigen, coating systems and the use of different calibrators and calibration protocols, are critical variables that can affect autoantibody test results.

In this Review, we discuss efforts to standardize serology in SARDs, including the requirements for establishing suitable reference materials. We consider illustrative examples from various diseases, and draw on the particular expertise of the authors to focus particularly on aPL. Several issues that influence autoantibody assays at pre-analytical and post-analytical phases, such as sample preparation methods, setting cutoffs and reporting of methodology (Box 1), are not explored, owing to space limitations.

**Methodological issues in SARD serology**

**Antigen characteristics**

The determined specificity of a given autoantibody is closely related to the characteristics of the antigen(s) used in the analytical system. A good example is the solid-phase assay for the detection of IgG antibodies to dsDNA. Preparation of dsDNA is complex, with the product frequently contaminated by single-stranded DNA. Such contamination renders the assay nonspecific for antibodies against dsDNA and thus not useful for the diagnosis or monitoring of SLE. In fact, antibodies to single-stranded DNA, although present in patients with SLE, are not specific for the disease and their levels do not correlate with SLE disease activity.

The analytical and clinical sensitivity and specificity of tests for ACPA can also be affected by the antigens used in the reagents. Although a substantial number of the ACPA assays now commercially available use citrullinated antigen mixtures from the same or similar sources, some tests detect slightly different subpopulations of ACPA. The majority of patients with ACPA-positive RA express autoantibodies that crossreact with all the various citrullinated antigens; however, the spectrum of antigen–antibody reactivity (in terms of affinity, avidity and titre) varies between patients. In a small number of patients, ACPA have restricted reactivity and might not produce positive results in all analytical systems.

**Immobilization of antigen**

Besides the biochemical characteristics of the antigen, the manner in which it is fixed into the solid phase—how it is coated onto an ELISA plate, for example—can also affect the assay specificity. The orientation of the antigen molecules is important, as the relevant epitopes need to be readily available for autoantibody binding rather than hindered by close proximity to the solid surface. For example, the process of coating polystyrene plates with proteinase 3 (PR3)—the main autoantigen associated with anti-neutrophil cytoplasmic antibodies (ANCA)—can bind parts of the molecule that are adjacent to the important epitopes, masking those epitopes and generating the potential for false-negative test results. Use of new capture ELISA systems in which the plates are coated with monoclonal antibodies that bind unimportant parts of the PR3 molecule and thus orient it better for autoantibody binding might increase both the specificity and sensitivity of the assay.

The coating process used in assays for anti-prothrombin (anti-PT) autoantibodies has also been shown to affect the antigen characteristics. Anti-PT antibodies have been detected in sera from patients with APS, and correlations have been reported between their presence and clinical manifestations of the syndrome, particularly thrombotic events. Only weak clinical correlation could be detected...
with the use of plates coated with prothrombin alone, which has been attributed to prothrombin adopting a nonphysiological conformation. This issue has been overcome by precoating plates with phosphatidylserine and then adding prothrombin in the presence of Ca\(^{2+}\) to reproduce the physiological conditions. The resultant assay has been reported to offer the best correlation between the presence of anti-PT antibodies and the clinical events in patients with APS.\(^6,7\)

**Nonlinear epitopes**

The finding that changes in conformation disrupt the prothrombin–anti-PT antibody interaction is illustrative of the notion that some autoantibodies are directed against conformational rather than linear epitopes.\(^8\) Other known examples include some anti-β₂-glycoprotein I (β₂GPI) autoantibodies that react with a conformational epitope that becomes exposed when the molecule opens in response to binding anionic phospholipids or lipopolysaccharide.\(^9\) Similarly, major epitopes targeted by sera from many patients with autoimmune disease positive for anti-Ro60 autoantibodies are also reportedly discontinuous or conformational.\(^10\)

**Harmonization issues and initiatives**

**External quality assurance initiatives**

The increasing number of available techniques for autoantibody testing, as well as their wide distribution, complicates inter-laboratory comparisons of data. Participation in external quality assurance (EQA) schemes is a requirement of many diagnostic service laboratories around the world. Autoimmune disease serology presents particular challenges for the development of EQA schemes (Box 1).**

**International standardization efforts**

The first initiative on autoantibody standardization began in the early 1980s under the umbrella of a joint committee, the Autoantibody Standardization Committee,\(^11\) which brought together the Arthritis Foundation, World Health Organization, US Centre for Disease Control and Prevention (CDC), and the International Union of Immunological Societies in a group chaired by Dr Eng M. Tan. The group selected sera with monospecific reactivity to a given autoantigen from patients with SARDs; these samples are stored at the CDC and are available upon request as reference materials to evaluate the reliability of assays. In 2002, a group called the European Autoimmune Standardization Initiative\(^12\) started a similar initiative but with additional objectives. Besides aiming to harmonize technical assays for autoantibodies, the group worked with clinicians and researchers working in autoimmunity with the purpose of improving reporting, sharing and interpretation of data.

More recently, in 2009, the Working Group on Harmonization of Autoantibody Tests (WG-HAT) was started in the framework of the International Federation of Clinical Chemistry and Laboratory Medicine.\(^13\) The task of the new initiative is to prepare new reference materials according to the guidelines used for other analytes, such as plasma proteins in human serum, and in close cooperation with the Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre of the European Commission. The Autoantibody Standardization Committee, European Autoimmune Standardization Initiative and WG-HAT are now formally working together with common projects and initiatives.

**Reference sample requirements**

The need for initiatives such as those introduced in the previous paragraph, and the relevance of the variables listed in Box 1, can be clearly seen in the results generated from EQA samples. For example, a sample distributed by the UK National External Quality Assessment Service...
Box 1 | Analytical issues in autoantibody standardization

Pre-analytical issues
- Patient identification
- Sample collection parameters
- Sample storage and handling
- Transport conditions
- Patient factors (physiological variables, pathological states)

Analytical issues
- Assay variability
- Antigen characteristics
- Immobilization of antigen
- Calibration and standardization systems
- Detection systems

Post-analytical issues
- Reporting of the results
- Units of measurement
- Setting of the cutoff: analysis of distributions among healthy subjects matched for sex and age, and among pathological controls (including infectious diseases, samples with paraproteins or hypergammaglobulinemia)
- Interpretation and categorization; for example, “negative”, “borderline”, “positive”, “strong positive”
- Interpretation of new assays in comparison with older ones (for example, controimmunoelectrophoresis versus ELISA, chemiluminescence versus ELISA, determination of the range of sensitivity)

(UKNEQAS)\(^{14}\) and known to be negative for IgG antibodies to PR3, was recorded as negative by each of the tests applied, but with value ranges that varied considerably between methods (for example, 0–4 U/ml for one method but 0.3–26.2 U/ml for another method [UKNEQAS for ANCA distribution 132]). A subsequent distribution of a sample known to be positive for IgG antibodies to PR3, and reported as positive, gave values in the range of 6–1,256 U/ml (UKNEQAS for ANCA distribution 134). The worrying consequence of this overlap between the values for negative and positive samples, using assays that claim to measure the same analyte, is the potential for misinterpretation when results for a patient are generated in more than one laboratory by different assays.

Generally, reference materials can serve as an anchor point for keeping values equivalent over time, and between different methods. The use of reference materials as common calibrators can lead to harmonization if the reference materials meet certain requirements, which include homogeneity, stability and commutability.\(^{15}\) In practice, the establishment of a reference material requires careful feasibility studies and validation.

Commutability, a critical feature of a reference material, indicates that the standard behaves in the same way as patient samples across a number of assays. The commutability of antibodies depends not only on the characteristics of the antibodies, but also on those of the substrate. Therefore, developing a suitable reference material requires the use of appropriate matrix materials, with the autoantibodies present in serum or a serum-like matrix. The material, produced in bulk, must withstand long-term storage periods. Different lots, consisting of pooled material, might differ in the production methods used (such as via plasmapheresis versus use of serum), in the presence of additives such as sodium azide, or in freeze-drying or frozen-storage methods. The material is only commutable if its behaviour remains equivalent to that of a fresh patient sample with respect to two or more of the assays concerned, despite these differences. Commutability is verified by measuring the reference material in the same run as a set of representative patient samples, using at least two different assays. The reference material results should fall on the regression line of the patient samples.

**Applicability of reference samples**

Before testing commutability, the assays, or analytes, for which a candidate reference material is valid must be verified; results should correlate across relevant tests, such as any that measure antibodies with a particular target.\(^{16}\) The example of PR3, as we have discussed, indicates that such correlation cannot always be found in autoantibody testing; results from two different anti-PR3 antibody assays can differ not only in magnitude, but also in being positive or negative. Samples that test ‘high’ in one assay may give low results in the second assay, whereas other samples differ in the opposite direction. This situation causes results to scatter around the correlation line, and harmonization of samples from individual patients is impossible. For other combinations of assays, results may be different in magnitude, but follow the same trend. When results for individual patient samples correlate in this manner, it should be possible to use a single scale to harmonize the results. Verification of the correlation of assay results can be performed by measuring the same representative set of patient samples with all the assays, and performing regression analysis.

The clinical significance of assays for which results cannot be harmonized should be re-examined. Standardization efforts should focus on those with definite clinical applicability. In the event that two non-correlating assays both have good, but different, clinical significance, efforts should focus on better definition of the analytes. The results of assays that measure different analytes can still be harmonized if the analytes are present at a constant ratio in all patient samples. Where analyte ratios differ, it might be possible to relate the different information obtained with the assays to the clinical status of patients, enabling better definition of subgroups and/or stages of disease, for example. Evaluating clinical significance in detail, however, requires large numbers of patient samples with complete clinical information, which can be difficult to obtain.

**Monoclonal versus polyclonal reference material**

The ideal reference material for autoantibody measurements would be a polyclonal immunoglobulin preparation representative of the spontaneously occurring autoantibody subpopulations in patients with a given autoimmune rheumatic disease. IgG, IgM and in some cases IgA preparations should display the same antigen specificity and ideally the same epitope specificity and avidity as the
spontaneous autoantibodies. Preparations should be available in quantities suitable for large-scale distribution and standardization initiatives. Ideally, samples should be pooled from several patients to reduce the risk of selecting limited subpopulations of autoantibodies.

Purified immunoglobulin fractions specific to a relevant epitope can be used, and their characterization enables quantitative values to be assigned to them (see section below on transferability and scales of measurement). Antigen-affinity purification of immunoglobulin fractions from sera can offer polyclonal (IgG, IgM and IgA) preparations with specific reactivity for a given autoantigen. Unfortunately, purified autoantigens are unavailable in many SARDs, owing to reasons that include the autoantigen being insufficiently characterized or of a chemical nature that does not permit affinity purification. Furthermore, affinity-purified immunoglobulin fractions might artificially contain only some autoantibody subpopulations, for example excluding those with low avidity.

Over the past 20 years, monoclonal antibody technology has started to offer new possibilities. Monoclonal antibodies raised in animals and directed against putative autoantigens involved in SARDs potentially represent good reference materials because they are monospecific and available in unlimited amounts, and the cell clones that produce them are quite stable over time. In addition, the pathogenic effects of several such monoclonal preparations have been demonstrated in experimental models of SARDs. One of several limitations to this approach is that xenogeneic monoclonal antibodies are usually high-avidity antibodies directed against linear epitopes, which contrasts with spontaneously occurring human autoantibodies that are often directed against conformational epitopes and display low avidity. The use of human monoclonal antibodies obtained using cells cloned from patients could solve this problem, as they are theoretically representative of the disease autoantibodies. Although these monoclonal antibodies are the final product of one cell clone and are thus not necessarily representative of the whole panel of antigen specificities, their use has been validated in preliminary studies. The use of human monoclonal anti-β2GPI antibodies in aPL standardization, for example, is discussed later in this manuscript. Most of these monoclonal antibodies are produced through Epstein–Barr virus infection of B cells from patients, but the resultant cell clones are quite unstable over time. The use of monoclonal antibodies obtained using phage display libraries derived from patient samples might be a promising solution, as they can be selected on the basis of their antigen specificity and avidity and are potentially available in unlimited amounts. The use of a pool of several monoclonal antibodies might produce a reference material that combines advantageous characteristics of both monoclonal and polyclonal immunoglobulin preparations.

Transferability and scales of measurement

Once a commutable matrix reference material has been identified, it becomes possible to set a common scale for a particular analyte. Arbitrary values (such as international units) or values derived from SI units might be assigned to the reference material, or a system of measurement can be defined by a reference method. The second option has the advantage of being reproducible over time; by contrast, value-assigning each new generation of reference material by comparison with the previous one carries a risk of drift in the values. Finally, the use of reference materials for calibration requires careful validation of the manner in which they are used. For example, if values are transferred from a certified reference material to a manufacturer’s in-house calibrator, a well-controlled procedure should be developed. Such a procedure should cover the reconstitution of the reference material, preparation of dilutions, the need for multiple measurements and intermediate precision, and the manner in which uncertainties are treated.

Progress in SARD serology

The example of aPL

The diagnosis of APS relies on the persistent presence of aPL; the laboratory criteria in the APS classification criteria (which, in the absence of specific diagnostic criteria, are frequently used in diagnosis) include positivity on two or more occasions at least 12 weeks apart of one or more of three different assays: anti-β2GPI, anti-cardiolipin (aCL) and lupus anticoagulant (LA). Although aPL were traditionally thought to be a heterogeneous family of autoantibodies, antibodies directed against either β2GPI or prothrombin are now considered to be the main diagnostic and pathogenic aPL. The major diagnostic aPL can be detected by three formal solid-phase assays (anti-β2GPI, aCL and anti-PT assays), and additional tests do not increase the diagnostic power in a significant manner (Table 3). In addition, the LA functional assay can detect both anti-β2GPI and anti-PT antibodies.

The need for standardization of aPL detection is clear, given the diagnostic value of these antibodies as well as their roles as risk factors for the clinical manifestations of APS. Although agreement between some kits is good, as the European Forum on Antiphospholipid Antibodies Standardization Group concluded in 2005, discrepant results between some laboratories indicate the need for greater standardization efforts. Several explanations have been suggested to underlie the variability in laboratory aPL reports. The hypothesis that some aPL subpopulations cannot be detected in the conventional assays is questionable, because antibodies directed against phospholipid-binding proteins other than β2GPI or prothrombin have been reported in only a few isolated patients with APS. Transient seronegativity was also suggested as a possible explanation, but has only been observed during acute, and particularly systemic, thrombotic manifestations (that is, in catastrophic APS). Patients with the classical clinical features of the syndrome but with repeated negativity in aPL assays have been suggested to represent ‘seronegative APS’, but whether this group has a variant of the syndrome or another disease entirely remains a matter of debate.

In contrast to these debatable explanations, methodological issues related to pre-analytical, analytical and
Post-analytical phases of APS serology (Box 1) seem to be major causes for inter-laboratory variability in results. The identification of minimal requirements and the development of strict guidelines for correct performance of aPL assays has been shown to improve the reproducibility of test results, including those of modern, automated and high-throughput assays for aCL and anti-β2GPI antibodies (Table 3). Consensus guidelines for aCL and anti-β2GPI antibody testing were consequently published in 2012 by an international aPL task force, and should help to eliminate these issues. Now, the development of international standards for calibration of aPL serology is one of the most pressing requirements. Polyclonal, affinity-purified IgG and IgM aPL and human (or chimeric) monoclonal IgG and IgM aPL that react with human β2GPI have been selected as potential candidates for reference materials. Preliminary analysis has shown that pooled, affinity-purified polyclonal anti-β2GPI IgG from patients with APS produced stable and reproducible results when distributed to several commercial and academic laboratories. The investigators established standardized international units and demonstrated their equivalency with the arbitrary units used in the various kits. These standardized units helped to show the high degree of commutability between the reference material and patient samples in the various anti-β2GPI IgG assays tested (which included the QUANTA Lite® β2GPI IgG ELISA and QUANTA Flash® β2GPI IgG chemiluminescent assay from INOVA Diagnostics; the BioPlex®2200 APLS IgG kit and anti-β2 glycoprotein I IgG EIA test kit from Bio-Rad Laboratories; the EL-β2GPI IgG kit from TheraTest; the IgG anti-β2 glycoprotein I test kit from Corgenix; the anti-β2 glycoprotein I IgG on Phadia® 250 from Phadia; and HaemosIL® AcuStar anti-β2 glycoprotein-I IgG from Instrumentation Laboratory). A preliminary evaluation of the first candidate reference serum sample for ACPA testing was published in 2012; it tested positive in all 12 of the commercially-available ACPA assays used, and seems suitable as a reference material for establishing calibration curves to facilitate inter-assay and inter-laboratory comparisons of results. Mean coefficient of variation values were reduced from 94.6% to 19.5% when using the reference material rather than kit calibrators. Nevertheless, the residual differences are fairly substantial, and are probably attributable to different assay reagents and procedures and to the intrinsic analytical variability of each method.

### Troubles of new technology: ANA example

Techniques for autoantibody detection have quickly evolved in the past few years. Immunodiffusion, agglutination and immunofluorescence procedures are being substituted by new solid-phase techniques with higher analytical sensitivities and with the possibility, in many instances, of automation and the use of multiparametric analytical systems. These developments have raised the question of whether the results of new studies are comparable in terms of clinical specificity and sensitivity with data from historical assays. Actually, as we discuss in this section, some of these new and more sensitive techniques have produced different and/or weaker associations of the autoantibodies with clinical disease manifestations than their predecessors.

The methodology for detection of antinuclear antibodies (ANA) in patient blood samples has changed over the years from the LE cell prep (lupus erythematosus cell preparation), to immunodiffusion and indirect immunofluorescence (IF) using sections of various rodent organs (such as rat or mouse liver or kidney, among others), and then to cell lines, in particular HEP-2 cells, which are used for ANA screening by IIF. Both pattern and titre of IIF can be described, and IIF ANA is considered the

### Table 3 | PL-binding proteins recognized by aPL in solid-phase assays* for APS

<table>
<thead>
<tr>
<th>Assay</th>
<th>Recognized antigen</th>
<th>Plate coating</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main proteins detectable by aPL solid-phase assays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL assay</td>
<td>Bovine or human β2GPI24</td>
<td>Cardiolipin plus β2GPI</td>
</tr>
<tr>
<td>Anti-β2GPI assay</td>
<td>Modified human β2GPI on oxidized solid surfaces24</td>
<td>γ-Irradiated plates plus β2GPI</td>
</tr>
<tr>
<td>Anti-PT assay</td>
<td>Human prothrombin25</td>
<td>Anionic phospholipid (for example, phosphatidylserine) plus prothrombin</td>
</tr>
<tr>
<td><strong>Additional proteins that might be detectable by aPL solid-phase assays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PE assay</td>
<td>High molecular weight kinogen-17</td>
<td>Neutral phospholipid (for example, phosphatidylethanolamine)</td>
</tr>
<tr>
<td>Thrombomodulin27</td>
<td></td>
<td>Anionic phospholipid plus bovine serum</td>
</tr>
<tr>
<td>Annexin A576</td>
<td></td>
<td>Anionic phospholipid plus bovine serum</td>
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</tbody>
</table>

*The lupus anticoagulant assay is not included as it is not a solid phase assay; this functional assay measures the time taken for phospholipid-dependent clotting mediated by anti-β2GPI and/or anti-PT antibodies. Abbreviations: aCL, anti-cardiolipin antibodies; anti-PE, anti-phosphatidyl ethanolamine; anti-PT, anti-prothrombin; aPL, anti-phospholipid antibodies; APS, anti-phospholipid syndrome; β2GPI, β2 glycoprotein I.
gold standard of ANA testing. When combined with a clinical history and physical examination of patients, the test identifies almost all patients with SLE (sensitivity over 95%), although the specificity of this assay is only 57% for SLE when compared with patients with related rheumatic and autoimmune disorders. In addition, the IIF ANA is important for the screening and diagnosis of several other SARDs.

Several attempts have been made over the past few years to develop solid-phase immunoassays for the detection of general and specific ANA species, as they are easier and cheaper to perform than IIF. Few of these new assays have been standardized and compared to IIF assays using fixed HEp-2 cells as a substrate. Many laboratories have adopted these techniques without proper post-market validation or inter-laboratory validation, which has resulted in a substantial increase in false-negative test results; many patients with SLE and other connective tissue diseases might express autoantibodies that are detectable by IIF but not by solid-phase substrates that use a limited number of autoantigens. A specific ACR task force reviewed the relevant literature on ANA and reported in 2010 that solid-phase immunoassays might not yet be appropriate for replacing IIF as a screening test for the detection of ANA. Up to 35% of patients with SLE are, according to the study authors, failing to be diagnosed because of a negative result using ANA ELISA or multiplex assay, although no studies that specifically address this point are available in the literature.

Troubles of new technology: sensitivity

Whereas the new solid-phase immunoassays for ANA discussed in the previous paragraph lack the sensitivity of the established IIF ANA assay, new serology technologies in other diseases present the opposite problem. Greater sensitivity using some new assays makes it possible to differentiate more defined subgroups of patients with SARDs in terms of autoantibody positivity and titres than could be achieved with traditional tests. This increased precision can facilitate assessment of associations between autoantibodies and clinical disease parameters. In particular, fluctuations in autoantibody levels can be more easily monitored; better understanding and evaluation of such changes should help in monitoring disease activity and response to therapy. Increased sensitivity, however, might also challenge established clinical associations with autoantibodies that were obtained using older techniques, as well as potentially revealing new associations. All these aspects should be validated in new prospective studies.

Conclusions

Measurements of autoantibodies are often useful for the confirmation of diagnosis of SARDs, and are required in the classification of many of them. Nevertheless, the intrinsic variability of the available assays causes discrepancies between reported results, which limit their usefulness. As the use of autoantibody assays has widened, and new techniques have been introduced, their standardization has become more important. The development of technical guidelines and the availability of suitable reference materials for calibration and quality control is emerging as a potential solution for the harmonization of these tests. Strongly positive sera from patients with SARDs were initially selected as reference materials. The use of antigen-affinity-purified immunoglobulin fractions or fully characterized monoclonal antibody preparations may offer new reliable tools for standardization. Validation of new reference materials for aPL (anti-β2 GPI), anti-MPO and anti-PR3 antibodies is almost completed, and these materials should be available in the next year.

Review criteria

Original articles on SARD autoantibody detection in the PubMed database were identified using the search terms “autoantibody”, “systemic autoimmune rheumatic diseases”, “standardization” and “reference material” in various combinations. Only English-language, full-text papers were selected, with a focus on studies published after 2000. In addition, relevant publications were selected from other reviews on similar topics and from the authors’ own bibliographic files.


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Author contributions
All authors made substantial contributions to writing the manuscript and reviewing/editing it before submission. In addition, P. L. Meroni, M. Biggioggero, J. Sheldon, I. Zegers and M. O. Borghi researched data for the article, and P. L. Meroni, S. S. Pierangeli, J. Sheldon, I. Zegers and M. O. Borghi made substantial contributions to discussions of article content.