Autoantibodies in autoimmune rheumatic disease

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Abstract

Initial characterization of autoimmune rheumatic disease through clinical assessment and investigation including serological measurements offers a vital opportunity to personalize treatment regimens, contributes to accurate prognosis and therefore optimizes outcomes. Disease monitoring using serological tests as biomarkers also contributes to earlier recognition of disease relapse or remission, and allows appropriate titration of medication. All rheumatic diseases have typical antibody associations, and understanding how to request tests appropriately and thereafter analyse them forms an important part of disease management. In this article, we introduce the standard tests available in the UK and a pragmatic approach to testing in inpatient and outpatient environments.

Keywords Antinuclear antibodies; autoantibodies; autoimmune rheumatic disease; MRCP; myositis; rheumatoid arthritis; rheumatoid factor; scleroderma; SLE

Key points

- Autoantibody testing contributes to an accurate diagnosis and treatment strategy in autoimmune rheumatic disease
- Inappropriate tests are costly and can lead to unnecessary investigations
- Most autoimmune rheumatic diseases have typical patterns of serology that help direct further investigation or intervention

Introduction

This review aims to help in deciding what tests to request and why for the more common inpatient and outpatient situations in which autoimmune rheumatic disease (ARD) is a possibility. When faced with an empty immunology form and a request to exclude a rheumatological cause or to check autoantibodies or a vasculitis screen, it can be difficult to know where to start. We discuss access to more specialized testing and what to do when tests are negative. Serology of course forms only part of any clinical evaluation, and the additional biochemical and other testing required in these circumstances is beyond the scope of this article.

In the following articles, common patterns of reactivity for each particular ARD are described, and this pattern recognition may help to determine prognosis or focus investigations and management approaches. One common example is the presence of high-affinity double-stranded (ds) DNA antibodies, which are often associated with the development of renal lupus (reviewed in Hahn[1]). However, patients in whom a new diagnosis of ARD is made may not present with a clear diagnosis, so here we try to offer a pragmatic approach to testing when the diagnosis is unknown.

Serology requests

Immunology tests are expensive and labour-intensive, and unnecessary testing or repeat testing of autoantibodies or serological tests often occurs. The only reasons to repeat most immunology tests are because either a clinical change has occurred that suggests a new condition, or the test in question is a helpful disease biomarker. Examples of tests that may be repeated regularly include dsDNA antibody tests, or proteinase 3 (PR3) and myeloperoxidase (MPO) antibodies when positive initially. A recent audit of all-user requests in a large regional hospital found that, within a 2-year period, repeat extractable nuclear antigen (ENA) tests formed approximately 10% of all ENA requests.[2] Your laboratory may refuse some serology requests on this basis (e.g. repeat antinuclear antibody (ANA) testing within a narrow timeframe).

A further problem presents when a test is requested outside a clinical context and then found to be positive. Very few data exist on the prevalence of the less common autoantibodies in the absence of clinical disease states, and this commonly leads to unnecessary additional investigation and management. Before ordering an autoantibody test, it is worth asking whether you are requesting the test to aid confirmation of disease activity or to aid prognosis when the clinical condition is already apparent. If not these, are you looking for either a positive result to guide you in making a diagnosis, or excluding a diagnosis based on a negative result? All are appropriate in the correct clinical context, but the application of pre-test probability is essential.

Most rheumatic diseases remain clinical diagnoses, as discussed in the following reviews, and other diagnoses are based on mixed clinical and laboratory parameters. The presence of some autoantibodies are strongly predictive of disease in some cases despite the (relative) absence of clinical features. Polyclonal B cell activation caused by viral infection (HIV, hepatitis C virus), malignancy or drug reactions can also lead to false-positive results.

The immunology laboratory may use the clinical information you provide to inform their reporting in some cases or to direct

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additional tests; it is therefore helpful to provide a clear clinical indication. Modern laboratory testing is not simply a binary positive or negative result, so an understanding of borderline or indeterminate results and testing methods used locally is also required to inform interpretation.

Understanding laboratory techniques

In the UK, all accredited immunology laboratories provide reliable methods of basic serological testing and autoantibody measurement, and, usually on request, can send serum to reference laboratories for more specialized testing. Reference laboratories are usually able to employ 'gold standard' testing procedures that may not be available locally, or can offer a wider range of tests for rarer autoantibodies. It is not financially viable for every laboratory to maintain the expertise, techniques and equipment for very rarely requested tests.

Immunology laboratories have agreed pathways for investigation and reporting of autoimmune serology. This depends to some extent on local laboratory expertise, which varies widely between laboratories. Reliable rheumatoid factor (RhF) and cyclic citrullinated peptide (CCP, ACPA) antibody results will be available. Investigation of other ARDs commonly begins with ANA testing. Several techniques can be employed; the gold standard is an experienced observer examining the nuclear (and sometimes cytoplasmic) staining pattern of serum at increasing dilution on Hep-2 cells using immunofluorescence (IF). If an observer is not available, ANA reactivity, titre and the more common patterns can be reliably identified using automated machines. A strongly positive ANA (ANA 1:1000 or higher) usually requires further evaluation, and lower or mid-titres need to be considered in the clinical context; these can be associated with both severe ARD or no illness at all.

Different patterns of ANA are described when initially tested. These occur because specific antibodies (usually termed ENAs) react to specific components of the nucleus and hence show different patterns depending on where those components lie within the nucleus. The pattern of positive ANA staining may help sufficiently in diagnosis that no other 'secondary' testing to determine that antibody is required; for instance, staining of the centromere is strongly associated with systemic sclerosis (scleroderma; SSc) in the correct clinical context and is usually obvious. In some cases, additional tests to determine those specific antibodies are not readily available and the described pattern suffices (e.g. U3-RNP staining).

The more usual scenario is a request for additional or secondary tests. The usual aim is to identify individual ENAs, although ENA pre-screening enzyme-linked immunosorbent assay (ELISA) is widely available and avoids costly individual ENA characterization. ELISA is a very reliable and readily automated plate-based technique where antigens are attached to the plate base and specific antibodies applied in liquid form over the surface. The antibody is linked to an enzyme following which, the enzyme substrate is applied. The reaction produces a quantitative signal (usually a colour change) for antibody detection. Standard ELISA techniques are reliable for many disease-specific autoantibodies, but an understanding of departmental technique is necessary when examining borderline results. Reference laboratories offering testing for rarer autoantibodies have their own validated techniques and pathways, again depending on expertise. The gold standard test for most ENAs is counter-immunoelectrophoresis.

In some cases, ENA testing should be requested irrespective of the ANA result; for instance, Ro antibodies can be positive when ANA is negative, and the antibodies associated with inflammatory muscle disease or autoimmune liver disease stain the cytoplasm but not the nucleus, which is not picked up on some automated ANA tests. Table 1 summarizes the six ENAs that are most commonly tested, and the patterns seen on ANA staining. Over 100 ENAs have been described, many of which can be requested in addition to the six described in Table 1.

There are two patterns of IF for antineutrophil cytoplasmic antibody (ANCA): cytoplasmic and perinuclear. Although IF has historically been the test used to evaluate ANCA, it has more recently been replaced by a reliable ELISA that allows the identification of two ANCAs: anti-PR3 and anti-MPO. The presence of a low-titre ANCA on IF but not confirmed by ELISA may be of clinical significance, but carries little significance for diagnosis of an ARD. ELISA testing, if available, following a positive IF result is usually automatically requested by the laboratory; the quantitative results of the ELISA should then be used as well as the IF pattern.

Negative test results should not deter a clinical diagnosis, although they may alter the prognosis or prompt additional investigation or extended serological tests for rarer autoantibodies, for instance specialized testing for myositis-specific antibodies in the correct clinical context. Paraneoplastic autoimmune disease is more common if a condition is seronegative.

Appropriate serology tests for common clinical presentations

In this section, we describe the appropriate use of autoimmune serology for some of the common presentations of ARDs. These should be requested alongside other laboratory and imaging investigations; full descriptions of the recommended investigations are outside the scope of this article.

Inflammatory arthritis: an inflammatory arthritis can be found as an isolated or prominent feature in rheumatoid arthritis (RA) and psoriatic arthritis, both of which are systemic diseases, or can be present with manifestations of other ARDs. The symmetry of the arthritis, the age of onset, the presence of other manifestations (rashes, respiratory or neurological symptoms) can aid in defining the diagnosis. Gastrointestinal symptoms, ocular inflammation, axial pain/stiffness and recent urinary tract or gastrointestinal infections should also be carefully evaluated as they are features of seronegative spondyloarthritis.

What to request? — ANA, RhF and anti-CCP antibodies/ACPA are the most important antibodies to check initially in the absence of clinical features that would point to a specific ARD.

What if the tests are negative? — the only diagnosis that can be formally excluded is seropositive RA. Up to 20% of patients with RA are seronegative for RhF and ACPA.[3] However, RhF and ACPA have strong prognostic importance in RA, and their presence should be kept in mind when optimizing treatment for the patient. Other inflammatory arthritis presentations are

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