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A clinical overview of autoantibodies in general practice rheumatology

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INTRODUCTION

Rheumatic diseases encompass a vast spectrum of disorders that range from localised to complex systemic illnesses affecting joints and connective tissues. They are often associated with pain and can be extremely debilitating to the sufferer. Many non-specific presentations may make diagnosis problematic. Key tests used to facilitate this are the serological autoantibody tests that measure antibodies against self antigens.

AUTOANTIBODY TESTS

When should they be ordered?

Within the rheumatology framework, some clues in the history and examination that may prompt the GP to order autoantibody tests include: Raynaud's phenomenon; thinning of the skin; vasculitic-type rashes; keratoconjunctival sicca and xerostomia; uveitis; isolated photophobia; unexplainable myalgia, arthralgia, or fatigue; or evidence of unexplained acute kidney injury. Obviously, the suspected disease entity will dictate which autoantibody test to order, as will be reviewed below. There are also other times when other non-rheumatic clinical situations warrant autoantibody tests, for example, organ-specific autoimmune conditions, and these should be considered as well.

Healthy individuals, particularly older people, may also have positive autoantibodies in low concentrations. Although these are not perfect as markers for specific diseases, with reasonable pretest probability, they are useful for ruling in and out the possibility of certain diseases. As autoantibodies are rarely sufficient alone to do this, other appropriate tests to assist, including basic blood workups, should be ordered as well.

How are they reported?

Most autoantibodies are measured using immunoassays (for example enzyme-linked immunosorbent assay [ELISA]), and are read as a titre (for example 1:80) for the patient's serum to react to or display positive antibodies. Results are reported as a titre or in standardised international units (IU)/mL. Positivity of certain autoantibodies in low titres (for example 1:40) in the absence of clinical features of rheumatism have little consequence. In general, the higher the reported titre, the more likely that there is an associated rheumatic illness with definite positivity ≥1:640.

Anti-nuclear antibodies

The archetypal autoantibody, anti-nuclear antibodies (ANA), are targeted against conserved intranuclear antigens. The test is reported in titres (often performed by ELISA) as well as staining pattern when immunofluorescence microscopy is performed using the patient's serum. Four main staining patterns are recognised: homogeneous, speckled, nucleolar, and centromere. These are often associated with certain disease entities (Table 1). As there are overlapping disease entities and more specific autoantibodies available, the emphasis on staining pattern in diagnoses has diminished.

ANA testing and interpretation are quite complicated. As ANA may be positive in chronic infections, malignancy, other autoimmune conditions, and healthy people (up to 5%), it has only moderate specificity at best for diagnosing rheumatic conditions. Consequently, reasonable pretest probability for the latter is advised when ordering. The issue of false-positives and false-negatives should be considered as well, and ANA test results should not be taken as definitive. One factor that affects these rates is the testing method. Although the American College of Rheumatology asserts that the indirect fluorescent antibody (IFA) method of ANA detection is the gold standard and is more sensitive than ELISAs,[1] some laboratories still utilise the latter since it is cheaper and faster.

If ANA is positive and is reported as

Table 1. Sensitivities and specificities (%) of anti-nuclear antibody (ANA) for rheumatic conditions[5]

Disease	Sensitivity	Specificity	Staining pattern	Comments
SLE	93	57	Homogeneous Speckled Nucleolar	Anti-histone is an additional antibody that has very high sensitivity and specificity for drug-induced lupus A rim pattern may also be seen in SLE
Systemic sclerosis/ scleroderma	85	54	Centromere (limited form) Speckled (diffuse form) Nucleolar	
Polymyositis/ Dermatomyositis	61	63	Speckled	
Sjögren's syndrome	44	52	Speckled	
Juvenile chronic arthritis	57	39	Homogeneous	
Rheumatoid arthritis	41	56	Homogeneous	

The typical ANA staining patterns seen under immunofluorescence microscopy are also provided.
SLE = systemic lupus erythematosus.

homogeneous in staining pattern, then a possible follow-up test with anti-double-stranded DNA (dsDNA) is appropriate. This autoantibody to DNA is particularly useful for the diagnosis of systemic lupus erythematosus (SLE) since it has very high specificity for this disease (~99%) and low prevalence in healthy patients (1%). Thus, a patient who is positive for both ANA and anti-dsDNA has a high probability of SLE. Anti-dsDNA levels are also generally parallel with SLE disease activity so it is useful for monitoring progression and response to treatment.[2]

Table 2. Commonly tested antibodies against extractable nuclear antigens (ENA) and their respective sensitivities and specificities (%) for rheumatic diseases.[6]

Autoantibody	Disease	Sensitivity	Specificity	Comments
Anti-Ro (SS-A)	Sjögren's syndrome	87	49	
	SLE	29	49	
Anti-La (SS-B)	Sjögren's syndrome	83	60	
	SLE	23	48	
Anti-Sm	SLE	36	91	Anti-Sm may indicate less severe form of SLE without renal involvement
Anti-Jo-1	Polymyositis/ Dermatomyositis	51	96	
Anti-Mi-2	Polymyositis/ Dermatomyositis	13	99	
Anti-U1 RNP	Mixed CTD	91	64	
	SLE	38	60	
Anti-Scl-70	Systemic sclerosis	35	93	Anti-centromere is sometimes performed in parallel to help differentiate between limited and diffuse cutaneous forms of systemic sclerosis
	SLE	13	10	

CTD = connective tissue disorder. RNP = ribonucleoprotein. SLE = systemic lupus erythematosus.

Extractable nuclear antigen antibodies

Extractable nuclear antigens (ENA) are so-named because they were originally isolatable from the soluble saline fraction of disrupted cells. ENA consists of a large number of antigens, but only autoantibodies to selected antigens are tested for (Table 2). If ANA is 'positive', it should be followed with ENA testing to ascertain the precise antigen ANA is targeting, as this will assist with a diagnosis. A speckled ANA pattern will usually produce positive anti-ENAs. However, it is possible for ENA to be positive in light of a negative ANA result, and anti-ENA as a group may be seen in 1% of apparently healthy patients. If clinical suspicion is strong for a particular disease entity detectable by ENA testing then this test is warranted. A suggested clinical workup using ANA and ENA is depicted in Figure 1.

Rheumatoid factor and anti-CCP

Rheumatoid factor (RF) is an autoantibody directed to the Fc region of self immunoglobin G (IgG). The most commonly measured autoantibody is the IgM isotype. RF complexes activate complement, promote inflammation, and therefore create local tissue damage. When combined with the anti-cyclic citrullinated peptide antibody (anti-CCP/ACPA), the two become particularly useful in the laboratory diagnosis of rheumatoid arthritis (RA). IgM RF and anti-CCP have sensitivities of 69% and 67%; and specificities of 85% and 95% for RA respectively.[3] RF, especially anti-CCP, has prognostic value, as high titres tend to correlate with a more severe form of RA. It should be noted, nonetheless, that RF can be also found in other rheumatic conditions such as Sjögren's syndrome and SLE. Positivity may also be found in other conditions including hepatitis C, sarcoidosis, and infective endocarditis. Again, healthy individuals can display positivity to these autoantibodies (5% for RF and 2% for anti-CCP); hence, interpretation within the clinical context is vital for these to be of value.

Anti-neutrophil cytoplasmic antibodies

Anti-neutrophil cytoplasmic antibodies (ANCA) are antibodies directed against cytoplasmic components of neutrophils. The primary component of this test involves

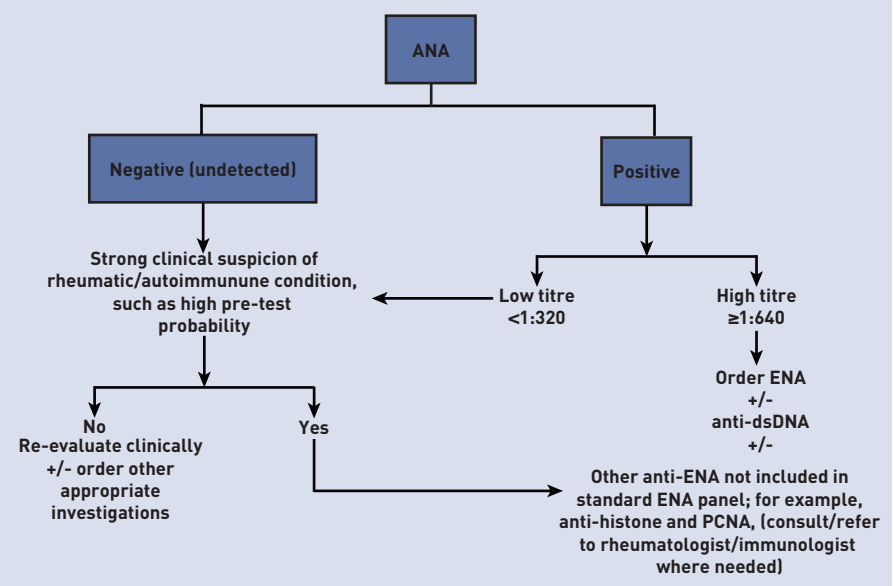

Figure 1. A flowchart of the suggested clinical utilisation of anti-nuclear antibody (ANA) and extractable nuclear antigens (ENA).

subjecting the patient's serum to ethanol-fixed neutrophils and observing the staining pattern of these antibodies under indirect immunofluorescence microscopy. Two staining patterns can be appreciated: first, cytoplasmic (c)-ANCA (diffuse staining in cytoplasm); and, second, perinuclear (p)-ANCA (peripheral staining around nuclei). ELISA is used to ascertain the antigen that the autoantibodies are directed against (commonly PR3 for c-ANCA, and MPO for p-ANCA). ANCA are useful as markers for when small-vessel vasculitides (for example Churg-Strauss syndrome) are suspected. Positive p-ANCA (and the closely related 'atypical p-ANCA') titres may be seen also in drug-induced vasculitis, glomerulonephritis, ulcerative colitis, and autoimmune hepatitis.[4] ANCA levels generally do not reflect disease activity and can fluctuate.

CONCLUSION

The sheer variety and amount of rheumatological autoantibody tests available can be somewhat confusing; however, with a basic understanding for the indications and limitations of each test, the diagnostic workup and monitoring for suspected rheumatological diseases can be strategically performed. These tests are not screening tests, and therefore, clinical acumen and reasonable pretest probability are still required before they are ordered by the clinician.

Provenance

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Competing interests

The authors have declared no competing interests.

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