Abnormal antibodies: what do you do?

Systemic autoimmune rheumatic diseases encompass a vast array of autoantibodies which are very useful to confirm a suspected diagnosis. This article gives an overview of the most common autoantibodies, how they are tested and the significance of a positive test in a clinical context.

Autoantibodies are frequently used to confirm a diagnosis of a systemic autoimmune rheumatic disease. The challenge to the rheumatologist and the general physician, in both hospital and non-hospital medicine, is how to recognize the significance of these autoantibodies with regard to a clinical diagnosis. Some autoantibodies also play a role in establishing prognosis, monitoring disease activity or as biomarkers of involvement of particular organs or tissues. Some autoantibodies are more specific for one disease while others can be found in different diseases.

Table 1. Autoantibodies associated with systemic autoimmune rheumatic diseases used in clinical practice

Autoantibodies	Subspecificities	
Antinuclear antibodies	Anti-dsDNA	
	Anti-centromere	
	Antibodies to extractable nuclear antigens (ENA)	Anti-Sm
		Anti-RNP
		Anti-Ro
		Anti-La
		Anti-SCL70
		Anti-PM/SCL70
		Anti-Jo1
		Anti-Mi 2
		Anti-PL12
		Anti-PL7
Rheumatoid factor		
Antibodies to citrullinated peptides (anti-CCP)		
Anti-neutrophil cytoplasmic antibodies (ANCA)	Anti-PR3	
	Anti-MPO	
Anti-phospholipid antibodies	Lupus anticoagulant	
	Anti-cardiolipin antibodies	
	Anti-B2GP1 antibodies	

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Understanding autoantibodies in both rheumatic and non-rheumatic conditions can be quite daunting especially for junior doctors and non-specialists. This article reviews current understanding of the relevance of various autoantibodies in rheumatic diseases, and highlights other associations and causes of false positive antibody testing.

What are autoantibodies?

The immune system comprises innate and adaptive components and pathways. The adaptive immune system allows antigen recognition and helps differentiate foreign from self antigens.

An antibody is a protein also known as immunoglobulin, synthesized by B lymphocytes and plasma cells, which consists of two light chains and two heavy chains of polypeptides, linked by disulfide bonds. The light and heavy chains both have regions on them known as domains, and both chains have varying quantities of variable and constant domains. The combination of a light chain variable domain with a heavy chain variable domain is called the antigen binding region of the immunoglobulin molecule. The interaction between the antibody and the antigen activates the classical pathway of the immune system resulting in complement activation and an immune response. It also activates phagocytic cells and other immune cells through specific cell receptors. On occasion antibodies produced by the immune system recognize native proteins as being foreign resulting in the immune system reacting against the body itself. These autoantibodies are commonly found in autoimmune conditions.

Autoantibodies found in systemic autoimmune rheumatic diseases

This article discusses relevant autoantibodies, both antinuclear antibodies (ANA) and extractable nuclear antibodies (ENA) (*Table 1*). Other antibodies discussed include anti-neutrophil cytoplasmic antibodies (ANCA), rheumatoid factor, antibodies against citrullinated peptides (anti-CCP antibodies) and antiphospholipid antibodies. Each section explains what the antibodies are, how they are usually tested and conditions in which a positive test may be found. An overview of this is given in *Table 2*.

Antinuclear antibodies

ANAs are autoantibodies directed against the nucleus of native cells. They are traditionally detected by indirect immunofluorescence, using HEp-2 cells (from human laryngeal carcinoma cell line). A fluorescent-labelled anti-

body binds to the antigen–antibody complex and a titre of positivity is obtained. A dilution less than 1:40 is negative (Bhagat et al, 2014). A titre of more than 1:40 is positive but higher titres are more clinically significant (Kourilovitch et al, 2014). The titre alone is not sufficient for a diagnosis – clinical correlation is needed.

The pattern of ANA staining, diffuse or homogeneous, speckled, nucleolar or anti-centromere, may also be helpful in the clinical setting. A diffuse pattern is very characteristic of systemic lupus erythematosus, a speckled pattern can be seen in systemic lupus erythematosus but is often associated with other autoimmune rheumatic diseases, and a centromere pattern is associated with the limited form of systemic sclerosis. Recently, several laboratories have started using solid-phase techniques, more frequently ELISA (enzyme-linked immunosorbent assay), to screen for the presence of ANAs. ELISA uses the interaction of antibodies present in the serum sample with a prepared antigen. An antibody coupled with an enzyme is then added to the serum sample followed by the enzyme substrate. The binding of antigen and antibody causes a reactive colour change which is then measured. There is controversy to what extent ELISA can replace indirect immunofluorescence in ANA detection and uncertainty about whether the clinical

Table 2. An overview of autoantibodies in rheumatic diseases

Antibody	Target	Test most commonly used	Systemic rheumatic diseases associations	Other clinical associations	Sensitivity
Antinuclear antibody	Multiple nuclear antigens	Indirect immunofluorescence, ELISA	Systemic lupus erythematosus, drug-induced lupus, undifferentiated autoimmune rheumatic disease (also called mixed connective tissue disease), Sjögren's syndrome, systemic sclerosis, polymyositis, dermatomyositis, juvenile idiopathic arthritis	Viral infection, medication, age, malignancy, 0–30% healthy population	90–100%
Rheumatoid factor	FC fragment of IgG	Agglutination test, ELISA	Rheumatoid arthritis	Tuberculosis, syphilis, Epstein–Barr virus, influenza, viral hepatitis, 10–15% healthy population, primary biliary cirrhosis, autoimmune hepatitis, chronic lymphocytic leukaemia	60–80%
Anti-CCP antibody	Citrullinated peptides	ELISA	Rheumatoid arthritis	Psoriasis, tuberculosis, chronic hepatitis C, smoking, malignancy, psoriatic arthritis	90%
Anti-dsDNA	dsDNA	ELISA, indirect immunofluorescence	Systemic lupus erythematosus	Epstein–Barr virus, drug-induced (e.g. anti-tumour necrosis factor)	60–80%
Anti-Ro	Nuclear ribonucleoproteins	ELISA, indirect immunofluorescence	Sjögren's syndrome, congenital heart block, systemic lupus erythematosus, neonatal lupus	Rheumatoid arthritis, cryoglobulinaemia, polyclonal hypergammaglobulinaemia	90%
Anti-La	Nuclear ribonucleoproteins	ELISA, indirect immunofluorescence	Sjögren's syndrome, systemic lupus erythematosus	Rheumatoid arthritis, cryoglobulinaemia, polyclonal hypergammaglobulinaemia	80%
Anti-centromere	Centromere	Indirect immunofluorescence	Limited systemic sclerosis (including CREST syndrome)	Chronic autoimmune active hepatitis, primary biliary cirrhosis	60%
Anti-Scl70	Topoisomerase 1	Indirect immunofluorescence, ELISA	Diffuse systemic sclerosis		97%
Anti-RNP	Small nuclear ribonucleoproteins	ELISA, indirect immunofluorescence	Undifferentiated autoimmune rheumatic disease (including mixed connective tissue disease), systemic lupus erythematosus, systemic sclerosis	Rheumatoid arthritis	50%
Anti-smooth muscle	Small nuclear ribonucleoproteins	ELISA	Systemic lupus erythematosus		20%
C-ANCA	Myeloperoxidase	Immunofluorescence, ELISA	Eosinophilic granulomatosis with polyangiitis	Tuberculosis, HIV, Hodgkin's lymphoma, multiple myeloma, autoimmune hepatitis	80–90%
P-ANCA	Proteinase 3	Immunofluorescence, ELISA	Eosinophilic granulomatosis with polyangiitis, microscopic polyangiitis, polyarteritis nodosa	Ulcerative colitis, rheumatoid arthritis, primary sclerosing cholangitis, focal necrotizing glomerulonephritis, autoimmune hepatitis	80–90%
Anti Jo1 antibody	Histidyl-tRNA synthetase	ELISA	Myositis	Interstitial lung disease, anti-synthetase syndromes	50%
Lupus anticoagulant	B2GP1, cardiolipin		Antiphospholipid syndrome, systemic lupus erythematosus		80%

B2GP = beta 2 glycoprotein; C-ANCA = cytoplasmic anti-neutrophil cytoplasmic antibodies; CCP = cyclic citrulinated peptide; dsDNA = double-stranded DNA; ELISA = enzyme-linked immunosorbent assay; FC IgG = fragment crystallisable region of immunoglobulin gamma; P-ANCA = proteinase 3 anti-neutrophil cytoplasmic antibodies; RNP = ribonucleotide protein.

associations previously described with indirect immunofluorescence remain the same (Meroni et al, 2014).

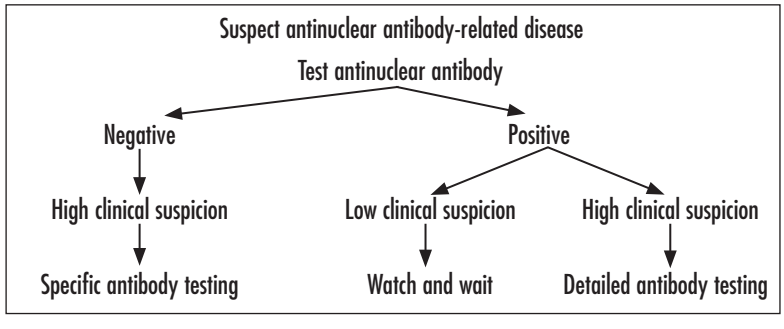
ANAs can be found in systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, polymyositis and dermatomyositis, antisynthetase syndrome, undifferentiated autoimmune rheumatic disease, oligoarticular juvenile idiopathic arthritis and others. They can also be present in patients with non-rheumatic diseases, e.g. autoimmune thyroid disease and autoimmune hepatitis. However, ANAs can also be found in up to 30% of healthy individuals, more often relatives of patients with autoimmune systemic rheumatic diseases, elderly, women or in patients taking certain drugs (Bhagat et al, 2014), most of whom will never develop systemic lupus erythematosus or another disease associated with ANAs. Viral infections may cause false positive results, usually temporarily. It is imperative that results are interpreted within a clinical context. If the patient has a positive ANA but is completely asymptomatic it is unlikely to be significant. If the patient tests positive for ANA with symptoms suggestive of lupus or another autoimmune disease then the significance of positivity needs to be taken into account. *Table 3* lists conditions associated with positive ANA and the sensitivity of ANA testing. In clinical practise the ANA should be performed if an autoimmune condition is suspected based on the individual patient history (*Figure 1*).

Table 3. Conditions associated with ANA and rheumatoid factor sensitivity

Condition associated with ANAs	ANA sensitivity (%)	Rheumatoid factor (%)
Drug-induced lupus	100	N/A
Systemic lupus erythematosus	99	30
Scleroderma	60	60
Sjögren's syndrome	40–70	70
Mixed connective tissue disease	93	25
Polymyositis and dermatomyositis	78	20
Rheumatoid arthritis	40	60–80
Systemic vasculitis	15	N/A
Oligoarticular juvenile idiopathic arthritis	57	15
Healthy old age	5–30	10–15

ANA = antinuclear antibody. From Kavanaugh et al (2000), Lane and Gravel (2002), Kourilovitch et al (2014)

Figure 1. Schematic approach to antinuclear antibody positive testing.



Antibodies to double-stranded DNA

Antibodies to double-stranded DNA (anti-dsDNA) are very specific for systemic lupus erythematosus and are present in up to 70% of patients. Anti-dsDNA titres are routinely used to monitor disease activity in systemic lupus erythematosus, especially in the context of lupus nephritis (Kavanaugh et al, 2000). Antibodies to single-stranded DNA can be found in systemic lupus erythematosus but also in several other diseases. They are not specific to a particular condition and do not correlate with disease activity.

Anti-dsDNA antibodies are usually detected by ELISA or indirect immunofluorescence. Immunofluorescence testing uses HEp-2 cells or *Crithidia luciliae*. *C. luciliae* is a haemoflagellate protozoan with a large amount of dsDNA and therefore used to detect dsDNA (Slater et al, 1976). Using *C. luciliae* to test for anti-dsDNA is less sensitive than ELISA but more specific for systemic lupus erythematosus.

Molecular mimicry is thought to be the basis of false positive anti-dsDNA antibodies with Epstein–Barr virus infection or post pneumococcal vaccine (Poole et al, 2006).

Antibodies to extractable nuclear proteins

Antibodies to extractable nuclear antigens (anti-ENA) are antibodies to various non-DNA proteins and nucleic acids. They are frequently associated with a speckled pattern on ANA indirect immunofluorescence testing. Most laboratories use solid phase immunoassays, in particular ELISA, to detect anti-ENA. Anti-ENA include anti-Ro, anti-La, anti-RNP, anti-Sm, anti-Scl70, anti-PM/Scl70 and antisynthetase antibodies, most commonly anti-Jo1.

Anti-Ro and anti-La antibody

Anti-Ro and anti-La are directed against ribonucleoproteins. Anti-Ro (formerly anti-SSA) antibodies are associated with Sjögren's syndrome as well as other connective tissues disease such as systemic lupus erythematosus, rheumatoid arthritis and congenital heart block, and neonatal lupus in the neonates of affected pregnant women. In patients who complain of sicca symptoms (dry eyes and dry mouth) and fall pregnant it is important to check for the anti-Ro antibody. Anti-Ro can be present in up to 90% of patients with Sjögren's syndrome. In systemic lupus erythematosus, the presence of anti-Ro is associated with cutaneous involvement disease, neonatal lupus and congenital heart block (Sibilia, 1998). Anti-Ro are usually present in around 40% of patients with systemic lupus erythematosus.

Anti-La (formerly anti-SSB) antibody is also associated with Sjögren's syndrome and can be present in up to 90% of patients. In systemic lupus erythematosus, anti-La is less often present – around 10–15% of patients.

Anti-RNP and anti-Sm antibodies

Anti-RNP and anti-Sm are directed against small nuclear ribonucleoproteins which are part of the spliceosome, a complex of ribonucleoprotein particles involved in pre-

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messenger RNA splicing. Anti-Sm antibodies are thought to be specific for systemic lupus erythematosus (present in 20–30% of cases), while anti-RNP antibodies are positive in up to 40% of systemic lupus erythematosus cases but not specific. Anti-RNP are often seen in patients with undifferentiated autoimmune rheumatic disease or syndromes classified by some as mixed connective tissue disease (Tsai et al, 2010). They may be found in myositis/scleroderma overlap conditions along with anti-PM-Scl antibodies.

Anti-PM-Scl antibodies

Anti-PM-Scl antibodies are directed to components of the exosome, a complex that regulates ribosomal RNA. They are associated with diffuse systemic sclerosis and overlap syndromes with myositis.

Anti-centromere and anti-topoisomerase I (anti-Scl-70) antibodies

Anti-centromere antibodies are targeted against centromere antigens – part of the mitotic spindle which promotes chromosome separation during mitosis. They are associated with a typical indirect immunofluorescence pattern in ANA testing (centromere staining). Anti-centromere antibodies are present in around 60% of patients with the limited form of systemic sclerosis, so should be tested in patients who have signs and symptoms suggestive of sclerodactyly, Raynaud's phenomenon, oesophageal dysmotility and telangectasia (Reveille and Solomon, 2003) or pulmonary hypertension.

Anti-topoisomerase I antibodies (also called anti-Scl-70) target topoisomerase I. They are found in around 30% of patients with diffuse systemic sclerosis and are associated with diffuse skin involvement, pulmonary fibrosis, cardiac disease and a poor prognosis (Hanke et al, 2009).

Anti-Jo1 and other anti-tRNA synthetase antibodies

Antisynthetase antibodies target aminoacyl-tRNA synthetases. They include anti-Jo-1 (anti-histidyl tRNA synthetase), anti-PL7 (anti-threonyl) and anti-PL-12 (anti-alanyl), among others. These antibodies are characteristic of antisynthetase syndrome which includes myositis, interstitial lung disease, arthritis, Raynaud's phenomenon, mechanic's hands, calcinosis (Mahler et al, 2014). Anti-Jo-1 antibody is seen in both polymyositis and dermatomyositis and associated with lung involvement.

Anti-neutrophil cytoplasmic antibodies

ANCAs are directed to proteins in granules of neutrophils and monocytes. These antibodies are found in various vasculitic conditions such as granulomatous polyangiitis (formerly Wegener's granulomatosis), granulomatous polyangiitis with eosinophilia (formerly Churg–Strauss syndrome) and microscopic polyangiitis. ANCA in immunofluorescent assays can produce two main staining patterns: cytoplasmic staining (cANCA) and perinuclear staining (pANCA). Broadly speaking, cANCA are usually directed against proteinase-3 and pANCA against myeloperoxidase although overlap may occur. Anti-proteinase-3 and anti-myeloperoxidase antibodies are usually detected by ELISA or other solid base immunoassays (Cohen Tervaert and Damoiseaux, 2012). cANCA and anti-proteinase-3 are more often associated with granulomatosis with polyangiitis while pANCA and anti-myeloperoxidase are more often associated with microscopic polyangiitis but again overlap can occur.

It is important to note in clinical practice that a positive ANCA may occur with a negative ELISA for myeloperoxidase and proteinase-3 antibodies. Such a result would be more suggestive of a false positive antibody test than a primary vasculitic process. Perinuclear staining can be seen in patients who are positive for ANA. It is also important to remember that patients with inflammatory bowel disease may have a false positive proteinase-3-ANCA (Blockmans et al, 1998). A positive ANCA is only diagnostic when combined with clinical evidence of vasculitis or granulomatosis. Clinical indications for testing for ANCA include glomerulonephritis, pulmonary haemorrhage, subglottic tracheal stenosis, longstanding sinusitis or otitis and skin rashes suggestive of cutaneous vasculitis. It is controversial whether ANCA titres reflect disease activity but patients who are persistently positive or who become positive again should be followed up carefully (Finkielman et al, 2007).

Rheumatoid factor

Rheumatoid factors are autoantibodies directed against the Fc part of the human IgG molecule. A range of methods can be used to test for rheumatoid factor. Agglutination tests using sheep erythrocytes sensitized with rabbit IgG were first used in the early 1940s. This test was modified to use polystyrene latex particles coated with human IgG. Newer tests include solid-phase immunoassays such as ELISA. Agglutination tests mainly detect rheumatoid factor of the IgM isotype. Rheumatoid factors are found in around 70% of patients with rheumatoid arthritis. They can also be found frequently in patients with Sjögren's syndrome, systemic lupus erythematosus and other autoimmune rheumatic diseases (*Table 3*).

As with ANA a positive rheumatoid factor does not conclude that a patient has rheumatoid arthritis. Patients with positive rheumatoid factor can be part of the healthy population (10–15% of patients) or have another underlying condition such as infection (tuberculosis, syphilis, viral hepatitis, Epstein–Barr virus, influenza) (Kourilovitch et al, 2014)(*Table 3*).

Anti-CCP antibodies

Antibodies to citrullinated-protein antigens include not only anti-CCP but also other antibodies against citrullinated proteins. Anti-CCP antibodies derive their name from the most common method used for its detection, an ELISA test where the antigen used is a combination of

cyclic citrullinated peptides produced in the laboratory. Antibodies to citrullinated-protein antigens can also be detected by several other tests including an immunofluorescence test using filaggrin (Suzuki et al, 2003).

Anti-CCP antibodies are directed against citrullinated peptides or proteins. Citrullination is a physiological process, controlled by peptidyl arginine-deiminases, whereby arginine is converted to citrulline. Inflammatory states and apoptosis promote citrullination (Vossenaar et al, 2003). Testing of anti-CCP is important in the context of suspected rheumatoid arthritis. These antibodies have similar sensitivity to rheumatoid factor but higher specificity. Anti-CCP antibodies are associated with worse prognosis and erosive disease (Van der Helm-van Mil et al, 2005). Anti-CCP antibodies can be found years before the onset of clinical symptoms (Van Gaalen et al, 2004).

Antiphospholipid antibodies

Antiphospholipid antibodies are antibodies directed against complexes of proteins and phospholipids. They include anticardiolipin antibodies, anti-beta2 glycoprotein I antibodies and lupus anticoagulant. The first two are detected by ELISA and the latter by functional in-vitro coagulation tests. Demonstration of a lupus anticoagulant requires different steps to demonstrate the presence of an inhibitor that prolongs clotting time in a phospholipid-dependent assay such as activated partial thromboplastin time or dilute Russell viper venom time.

Antiphospholipid antibodies are characteristic of the antiphospholipid syndrome, associated with arterial and venous thrombosis and pregnancy morbidity. They are often seen in patients with systemic lupus erythematosus and less often in patients with other autoimmune diseases and not necessarily associated with specific manifestations (Ruiz-Irastorza et al, 2010). They can occur transiently after certain infections and in this case are not associated with increased risk of thrombosis. They should be tested twice 12 weeks apart to confirm a truly positive result.

Conclusions

Autoimmunity and the production of autoantibodies is not limited to rheumatological conditions – improved methods of testing are seeing more positive antibody tests across a spectrum of clinical medicine. In systemic autoimmune rheumatic diseases, different autoantibodies are frequently used to confirm a clinical diagnosis. It is important to interpret results within a clinical context and not to rely on the antibody test only for diagnostic purposes. **BJHM**

KEY POINTS

- Autoantibodies are useful tools to confirm the diagnosis of a systemic autoimmune rheumatic disease but the presence of a positive test is not always clinically relevant.
- Positive antinuclear antibodies are not limited to rheumatic diseases and may be seen in autoimmune thyroiditis and hepatitis as well as other autoimmune conditions.
- Infections may cause false positive autoantibodies, including antinuclear antibodies and rheumatoid factor.
- In the context of inflammatory arthritis it is important to test anti-cyclic citrullinated peptide antibodies as they are a predictor of erosive disease.
- Tests should only be requested if there is a clinical suspicion.

Conflict of interest: none.

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