Autoantibodies in the autoimmune rheumatic diseases

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Abstract

The detection of autoantibodies to a variety of antigenic targets plays an important role in the diagnosis of autoimmune rheumatic disease. Rheumatoid factor (RF), antinuclear antibodies (ANA) and anti-neutrophil cytoplasmic antibodies (ANCA) have been key in the diagnosis of rheumatoid arthritis, systemic lupus erythematosus and systemic vasculitis for many years. Antibodies against cyclic citrullinated peptides (ACPA) are more specific than rheumatoid factor for rheumatoid arthritis. Dual positivity for ACPA and RF is strongly predictive of erosive rheumatoid arthritis. Screening for ANA and ANCA should not be undertaken routinely as the specificity of these tests is poor in this setting and low concentrations/titres of RF, ANA and ANCA are common in the well elderly. Repetition of a negative ANA is not likely to be helpful unless there has been a change in symptoms. Rising concentrations/titres of anti-dsDNA and ANCA (proteinase 3 and myeloperoxidase) indicate possible disease relapse, but should not be used as the sole reason for a change in therapy in the absence of clinical features of relapse.

Keywords ANA; ANCA; anti-ACPA antibodies; autoantibodies; ELISA; rheumatoid factor

What's new?

- Dual positivity for ACPA and RF is strongly predictive of erosive RA
- Therapeutic decisions should not be based solely on changes in concentrations of dsDNA, PR3 or MPO antibodies
- Increasing numbers of antigens can be detected using ELISA and these assays are becoming more widely available

The detection of autoantibodies in the serum of patients with suspected autoimmune rheumatic disease is an important part of the diagnostic process. The clinical utility of the results is dependent on the quality of the laboratory test. Many assays are now available commercially. Access to a reference laboratory providing assays for the less common autoantibodies is necessary for the comprehensive assessment of some patients.

An ideal diagnostic test has both high sensitivity and specificity, that is, it identifies all patients with the disease (high sensitivity) and is not present in those who do not have the disease (high diagnostic specificity). A number of different methods are used to detect autoantibodies with varying specificity and sensitivity. The most widely used methods in routine practice are enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF). ELISAs are readily automated, inexpensive and permit screening for a wide range of recombinant antigens. They are readily suited for use in a routine non-specialized laboratory. In general, they are more sensitive and less specific than other methods such as immunoprecipitation or immuno-electrophoresis.

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Anti-cyclic citrullinated peptide antibodies (ACPA)

ACPA are the most specific biomarkers in widespread use for detecting rheumatoid arthritis (RA). Citrullination of peptides occurs during cell apoptosis and is mediated by peptidyl-arginine deiminase (PAD). Disruption of the cell wall during apoptosis in an inflamed joint increases permeability to calcium and the resultant increase in intracellular calcium concentration activates PAD. PADs are present in high concentrations in neutrophils and monocytes, and their activation results in substitution of positively charged arginine by neutrally charged citrulline (citrullination). Increased citrullination of peptides in an inflamed joint may lead to the appearance of ACPA.[1]

ACPA are present in 80% of patients with established RA. The specificity is around 85–90%, with sensitivity of 50–60%. Furthermore, a positive anti-ACPA test predicts the development of erosive RA.[2,3]

Rheumatoid factor

Rheumatoid factors (RF) are primarily IgM antibodies directed against the Fc part of the patient's own IgG molecules.[4] Although 80% of patients with rheumatoid arthritis are RF positive, these antibodies are not specific for rheumatoid arthritis, occurring in a wide range of autoimmune rheumatic diseases and infections, usually at low concentration; 1–4% of Caucasians from northern Europe are positive for RF.

Dual positivity for RF and ACPA occurs in about 50% of RA patients and in healthy individuals is highly predictive for the subsequent development of RA.[5] ACPA and RF are not useful biomarkers of response to therapy.

Antinuclear antibodies

Antinuclear antibodies (ANA) are autoantibodies directed against cellular proteins or nucleic acids.[6,7] Despite the name, several of these antigens are partly or wholly confined to the cell cytoplasm. The most frequently occurring ANA react with DNA-protein or RNA-protein complexes. These autoantibodies, which are usually high-titre, high-affinity IgG antibodies, are generated by a T-cell dependent process driven by the autoantigen.

Screening for ANA is an important initial stage in the assessment of autoimmune rheumatic disease. A high-titre of ANA (>1:160) is more likely to be clinically significant in the presence of autoimmune rheumatic disease. ANA is a useful

screening investigation for systemic lupus erythematosus (SLE) with sensitivity of 90–95%.

Although many ANA are disease specific, around 3–5% of healthy northern Europeans will be positive, and the prevalence of positivity increases with age. The clinical associations of these autoantibodies are given in Table 1.

ANA staining on Hep-2 cells may be broadly grouped into three categories: nucleoplasmic, nucleolar and cytoplasmic.

Nucleoplasmic-staining antibodies can be further subdivided into homogenous, which includes specificities for dsDNA, histone, chromatin and topoisomerase 1; speckled (Sm, nRNP, Ro, La, PCA, Ku RNA polymerase 1); peripheral (Lamin A/B/C) nuclear pores); and centromere. Nucleolar-staining antibodies are commonly found in SSc fibrillarin (U3-RNP), RNAP I/III, hUBF (NOR-90), Pm-SCl and Th(1–7). Cytoplasmic-staining antibodies comprise the myositis-related autoantibodies (aminoacyl tRNA synthetases,

Disease associations of commonly detected autoantibodies

Autoantibody	Disease	Prevalence	Disease specificity	Associated clinical features
RA-associated antibodies				
RF	RA	80%	Moderate	Erosive RA
ACPA	RA	80%	High	Erosive RA
Antinuclear antibodies				
dsDNA	SLE	70%	High	Lupus nephritis
Sm	SLE	5% (Caucasian); 30–50% (African–Caribbean)	High	Vasculitis, CNS lupus
Ro (SS-A)	SLE	40%	Low	Photosensitivity, subacute cutaneous LE, congenital heart block, neonatal LE
	Sjögren's syndrome	80%	High	Extra-glandular disease, vasculitis, lymphoma
La (SS-B)	SLE	15%	Low	As for Ro
	Sjögren's syndrome	50%	High	As for Ro
U1RNP	MCTD, IM, SSc	30%	Low	Raynaud's phenomenon, swollen fingers, arthritis, myositis (overlap syndrome – MCTD)
rRNP	SLE	15%	High	CNS lupus (psychosis, depression)
PCNA (cyclin)	SLE	5%	High	
Topoisomerase 1 (SCl 70)	SSc	30%	High	Diffuse cutaneous variant of scleroderma, pulmonary fibrosis
Centromere	SSc	30%	Moderate	Limited cutaneous variant of scleroderma, absence of lung disease, pulmonary hypertension
RNA-polymerases	SSc	20%	High	Diffuse cutaneous variant of scleroderma, visceral involvement
PM-Scl	SSc	5%	High	Scleroderma/myositis overlap
Jo-1	IM	30%	High	Polymyositis with fibrosing alveolitis (anti-synthetase syndrome)
SRP	IM	4%	High	Severe myositis
Mi-2	IM	10%	High	Necrotizing myopathy
Antiphospholipid antibodies				
Phospholipid	APS	50–60%	High	Thrombosis, fetal loss, thrombocytopenia
β2-glycoprotein I	APS	~45%	High	Thrombosis, fetal loss, thrombocytopenia
Anti-neutrophil cytoplasmic antibodies				
PR3	Vasculitis	90%	High	Granulomatosis with polyangiitis (Wegener's)
MPO	Vasculitis	50%	Moderate	Microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis (Churg–Strauss)
Miscellaneous				
GBM	Anti-GBM disease	>95%	High	Anti-GBM disease (Goodpasture's syndrome)
C1q	SLE	40%	Moderate	Lupus nephritis

ACPA, cyclic citrullinated peptide; APS antiphospholipid syndrome; GBM glomerular basement membrane; IM, inflammatory myopathy; MCTD, mixed connective tissue disease; MPO, myeloperoxidase; PCNA, proliferating cell nuclear antigen; PR3, proteinase 3; RA, rheumatoid arthritis; RF, rheumatoid factor; RNP, ribonucleoprotein; SLE, systemic lupus erythamatosus; SRP, signal recognition particle; SSc systemic sclerosis.

Table 1