The clinical implications of autoantibody detection in rheumatology

ABSTRACT—The laboratory plays an increasing role in the diagnosis and clinical management of patients with rheumatic diseases. It is therefore essential that the results of laboratory tests are both accurate and reliable, and give clinicians correct information. It is also important to keep clinicians informed of the changes occurring in the rapidly evolving field of investigation of autoantibodies.

In rheumatology the detection of autoantibodies is useful in diagnosis, prognosis and monitoring disease activity. The first two are firmly established, while with certain exceptions, such as the quantitation of anti-double stranded DNA, the value of the third is still not fully assessed.

Specificity and sensitivity of the test

An ideal diagnostic assay is highly specific for the disease and occurs in the majority of patients with that disorder (high sensitivity). It must be borne in mind that diagnostic sensitivity may increase with frequent blood sampling since the level of autoantibody (eg rheumatoid factor) may correlate with the activity and stage of the disease, and may be affected by the drugs which are being used to treat the disease. Sensitivity may also reflect genetic heterogeneity and ethnic origins of populations under study, a notable example being the frequency of anti-Sm in SLE, which is 60% in Afro-Caribbean populations but only 5% in caucasoids [1]. Sensitivity and specificity may also depend on the method. Thus enzyme-linked immunosorbent assays (ELISA) are more sensitive than gel precipitation techniques [1]. However, increased sensitivity often goes hand in hand with a decrease in specificity as increasingly small amounts of antibody are detected; thus an assay with very high sensitivity may be of little use diagnostically assay due to very low specificity. The specificity of an assay may also be affected by the material used as the antigenic source, by its purity, and the nature and conformation of the peptides and epitopes presented in the assay.

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Specificity is usually measured in relation to a population of healthy subjects, but suitable age-matched control populations should be used, since some autoantibodies occur in healthy elderly people. It is often of greater relevance to compare the test population with others displaying clinical features which are likely to be confused (eg rheumatoid arthritis and SLE), or that are part of a differential diagnosis.

Antinuclear antibodies

Antinuclear antibodies (ANA) are directed against cellular components. The term 'ANA' is something of a misnomer as some of these antigens are actually cytoplasmic in location. Chromosomal antigens include single and double stranded deoxyribonucleic acid (DNA), deoxyribonucleoprotein (DNP) and histones. Antibodies have also been described against the centromeric proteins and various nucleolar proteins. Soluble nucleo/cytoplasmic antigens are readily extracted in phosphate-buffered saline (pH 7.2) and have been termed 'extractable nuclear antigens (ENA) or 'soluble cellular antigens'. Some of these antigens are named according to their biochemical nature (eg ribonucleoproteins: RNP) or according to the diseases in which they occur (eg Sjögren's syndrome: SS-A). ANA are usually tested by indirect immunofluorescence on Hep-2 cells or on cryostat sections of rodent tissues. In addition to the strength of reaction, either as intensity or titre, the pattern of staining should be recorded as this may indicate certain specificities, such as the centromeric antigen, and those located in the nucleolus. The specificity of the antibody is determined by techniques such as radioimmunoassay, gel diffusion, ELISA, or immunoblotting [2–5].

ANA with high specificity are termed 'disease markers' (Table 1). These antibodies are rarely found in other than their designated diseases. Certain specificities are also associated with specific clinical features (Table 2), and may point to future developments.

Anti-Ro (SS-A)

Antibodies to Ro are detected in about 60–80% of patients with primary Sjögren's syndrome and 40% of patients with SLE. They have also been detected in 1% of healthy adult controls, and in all these groups may be associated with fetal congenital heart block [6,7]. This condition is found in 5% of pregnant women who

Table 1. Diagnostic marker antibodies in rheumatic diseases

Antibody Specificity	Disease	Frequency	Disease Specificity
dsDNA	SLE	60%	High
Sm	SLE	10% (cauc)	High
		55% (Af-Car)	
La (SS-B)	Sjögren's syndrome	50%	High
SCL-70	Scleroderma	27%	High
Jo-1	Polymyositis	25%	High
Proteinase 3	Wegener's disease	80%	High
Centromere	CRST	70%	Moderate
nRNP	CRST	90%	Low
Ro (SS-A)	Sjögren's syndrome	80%	
	SLE	60%	Low

have circulating anti-Ro [6], and rises to 25% in women who have previously had one or more affected babies. These findings suggest that the damage may be caused by a subgroup of antibodies to Ro, probably directed against a particular epitope. Anti-Ro is also associated with a number of subsets of SLE, including the so-called ANA negative SLE syndrome [8], subcutaneous lupus erythematosus, and the lupus-like syndrome associated with homozygous complement C2 and C4 deficiency.

Anti-La (SS-B)

Antibodies to La are found in approximately 50% of patients with primary Sjögren's syndrome. In the absence of SLE associated antibodies, anti-La is a diagnostic marker for primary Sjögren's syndrome, particularly in patients with extraglandular features of the syndrome [9]. In general, patients with lupus and anti-La have a later age of onset and a lower incidence of nephritis than other SLE patients [10].

Anti-snRNP (U1–U6) antigens

Antibodies to the protein antigens found on the uridine-rich (U_1, U_2, $U_{4,5,6}$)ribonucleoproteins include anti-nRNP and anti-Sm, which are often found in combination. The epitopes for these antibodies are well characterised [11–13].

The nRNP antigens recognised by patients' sera are located exclusively on the U1 particle, whereas Sm antigens are located on the U2, 4, 5 and 6 particles. Antibodies to nRNP react with the 71kd, A and C peptides, whereas anti-Sm react with the B, B' and D peptides. The E, F, and G proteins are rarely targets for antibodies in the autoimmune diseases. Anti-nRNP is found in 80% of patients with mixed connective tissue disease (MCTD), in 10% of patients with SLE, 10% with scleroderma, and in some patients with overlapping or undifferentiated connective tissue disease; it is also associated with Raynaud's phenomenon and fibrosing lung disease.

Anti-Sm is found in SLE. In caucasoid populations it occurs in only 5% of patients, but its prevalence rises to 60% in Afro-Caribbean populations [1].

Anti-ribosomal RNP

Antibodies to ribosomal RNP (rRNP) or ribosomal P protein are present in approximately 15% of patients with SLE. They were originally described as a marker for neuropsychiatric SLE [14], but recent studies suggest that they are more likely to be a marker for active generalised disease [15].

Anti-double stranded DNA (anti-dsDNA)

These antibodies occur almost exclusively in SLE and are present in 60–70% of patients with active disease [4,16]. Increasing levels predict increasing disease activity; the rise in antibody levels is often coupled with a decrease in complement C3 and C4 levels, indicating complement consumption [17].

Table 2. Association of ANA with clinico-pathological features

Feature	ANA specificity	Comment
Diffuse glomerulonephritis	dsDNA	SLE with nephritis
Membraneous glomerulonephritis	Ro, Sm	SLE with nephritis
Neonatal heart block	Ro	Maternal antibody
Sjögren's syndrome	La	
Raynaud's phenomenon	nRNP	As part of overlap syndrome
Fibrosing alveolitis	nRNP	Overlap, especially Raynaud's phenomenon
	Jo-1	With fibrosing alveolitis
Polymyositis	nRNP	
	Jo-1	With fibrosing alveolitis
SLE-vasculitis	Ro	

Anti Scl-70

Antibodies to DNA topoisomerase 1 are found in 30% of patients with diffuse scleroderma, and less frequently in patients with limited scleroderma. Their presence in patients with Raynaud's phenomenon or limited scleroderma may indicate that the disease is becoming more aggressive [18].

Anti-centromere (ACA)

This antibody gives a characteristic pattern visualised in an immunofluorescent test using cells in mitosis. Antibodies are directed against the A, B and C proteins of the centromeric complex [19,20]. The antibody is found in approximately 30% of patients with scleroderma, and in 80–90% of patients with the CRST syndrome (calcinosis, Raynaud's, sclerodactyly, and telangiectasia) [21]. Occasionally, ACA may be present in other connective tissue diseases such as primary biliary cirrhosis, where it usually identifies patients who may develop features of scleroderma [22].

Anti-nucleolar antibodies

Anti-nucleolar antibodies react with diverse antigens located within the nucleolar region of the cell. These include anti 6–7S RNA, RNA polymerase–1, fibrillarin, and U3 RNA, which are all present in low frequencies in scleroderma. Another nucleolar specific antibody, PM–1 or PM/Scl, is found in patients with features of polymyositis and scleroderma. Anti-nucleolar antibodies in low titre are occasionally seen in other connective tissue diseases.

Anti Jo–1

Antibodies to Jo–1 (histidyl tRNA synthetase) are present in 20% of patients with primary polymyositis, especially in those with pulmonary fibrosis and arthritis. Antibodies to five other tRNA synthetases—threonyl (PL7), alanyl (PL12), isoleucyl (OJ), glycyl (EJ), and lysyl—have been described. All appear to be associated with a clinical syndrome similar to Jo–1, but are rare, each occurring in less than 4% of polymyositis patients.

Anti-Ku

Antibodies to Ku are found in patients with SLE, MCTD, Sjögren's syndrome, scleroderma, and myositis [23]. Further studies will be required to elucidate the clinical usefulness of this specificity.

Anti-proliferating cell nuclear antigen (PCNA)

Antibodies to PCNA (cyclin) are a specific marker for SLE, found in 5% of patients. There is no known association with clinical features [24].

Anti RA-33

Antibodies to RA–33 are a recently described specificity found in 30% of patients with rheumatoid artritis (RA) and 1% of other connective tissue diseases [25]. These may occur earlier than other autoantibodies, and in patients who are persistently rheumatoid factor negative [26].

Rare ANA

Many rare ANA have been described, as shown below.

Autoantibody	Disease association
Anti-Mi-1	Polymyositis/dermatomyositis overlap
Anti-Mi-2	Polymyositis/dermatomyositis overlap
Anti-Ma-1	Active SLE
Anti-SL	SLE/Sjögren's overlap
Anti-Ki	SLE
Anti-Su	SLE
Anti-NuMa	RA, CRST, SLE, MCTD
Anti-MSA	RA, CRST, SLE, MCTD
Anti-centriole	Scleroderma
Anti-Nsp 1	Chronic active hepatitis
Anti-Nsp 2	Sjögren's syndrome
Anti-dsRNA	SLE
Anti-nuclear matrix	MCTD
Anti-nuclear lamina	SLE

Anti-phospholipid antibodies

Antibodies directed against negatively charged phospholipids occur in SLE and other connective tissue diseases. They are responsible for the false positive syphilis serology and the lupus anticoagulant. In patients with SLE, anti-phospholipid antibodies (APL) have been associated with vascular thrombosis, recurrent fetal loss, and thrombocytopaenia [27,28]. In some patients with a similar clinical syndrome, the only serological abnormalities are the presence of anti-phospholipid antibodies and occasionally a weak ANA. This syndrome has been termed 'anti-phospholipid syndrome'. Anti-phospholipid antibodies have also been detected in infections [29,30], and other diseases. However, the association with thrombosis, fetal loss, and thrombocytopaenia has not always been found in these groups. It has been suggested that the lupus anticoagulant is a more reliable predictor of risk for fetal loss, thrombosis, and thrombocytopaenia [31].

Some assays for APL give variable results and may give false positive reactions due to non-specific binding [32]. Co-factors may be required for the binding of anti-cardiolipin to its antigen [33]. Due to these variations it is difficult to compare the many studies of APL in different diseases. At present there are few data

on the clinical associations of the many commercial assays on the market.

Anti-neutrophil cytoplasmic antibodies (ANCA)

Antibodies to myeloid lysosomal enzymes have recently been the subject of much study [34]. The two main specificities are anti-29kd serine protease, which is found in 80% of patients with active Wegener's granulomatosis, and myeloperoxidase, found in polyarteritis nodosa, Churg-Strauss syndrome, and renal vasculitis. Antibodies to myeloperoxidase and lactoferrin may be associated with vasculitis in patients with RA [35,36]. A number of other related specificities have been described, but their clinical usefulness is as yet unknown.

Rheumatoid factors

The association of IgM rheumatoid factor and the diagnosis of RA is well known, but rheumatoid factors are also found in other immunoglobulin classes. IgA rheumatoid factor levels correlate with cartilage loss and bone erosion in RA [37], and correlate well with disease activity [38]. IgG rheumatoid factors have been associated with vasculitis, but have not, so far, found a useful role in diagnosis and management [39].

Perinuclear antibodies

Antibodies reactive with the perinuclear granules of human buccal mucosal cells occur in 78% of patients with classical RA and in 40% of patients with seronegative RA. In seronegative RA they are said to be associated with a poor prognosis [40].

Autoantibodies as a measure of disease activity

Despite the rapid expansion in the number of autoantibody specificities for diagnosis, there has been little work on the relationship between the amount of antibody and disease activity. There are few studies correlating antibody levels with disease activity similar to those that show that increasing levels of anti-ds DNA indicate increasing disease activity [41,42]. In patients with SLE but without anti-ds DNA, disease activity may be assessed by sequentially measuring antibodies to the soluble cellular antigens (ANCA) [43–45]. ANCA antibodies persisting after treatment are associated with a greater risk of relapse; increasing levels of ANCA are associated with an increase in disease activity [34].

Quality assurance

To ensure the reliability of the results of autoantibody investigations, it is necessary to assess their quality independently of the laboratory [46]. Within the UK this is the responsibility of the national external quality assurance scheme (NEQAS).

References

1 Field M, Williams DG, Charles P, Maini RN. Specificity of anti-Sm antibodies by ELISA for systemic lupus erythematosus: increased sensitivity of detection using purified peptide antigens. *Ann Rheum Dis* 1988;**47:**820–5.

2 Isenberg DA, Madisson PJ. Detection of antibodies to double stranded DNA and extractable nuclear antigen. *J Clin Pathol* 1987;**40:**1374-81.

3 Venables PJW, Charles PJ, Buchanan RC, *et al.* Quantitation and detection of isotypes of anti-SS-B antibodies by ELISA and Farr assays using affinity purified antigens. *Arthritis Rheum* 1983;**26:**146–55.

4 Isenberg DA, Dudeney C, Williams W. Measurement of anti-DNA antibodies: A reappraisal using five different methods. *Ann Rheum Dis* 1987;**46:**448–56.

5 van Venrooij WJ, Charles PJ, Maini RN. Report on European consensus finding study for autoantibodies 1989–1990. *J Immunol Meth* 1991;**140:**181-9.

6 Ramsey-Goldman R, Hom D, Deng J-S. Anti-SS-A antibodies and fetal outcome in maternal systemic lupus erythematosus. *Arthritis Rheum* 1986;**29:**1269–73.

7 Lane AT, Watson RM. Neonatal lupus erythematosus. *Am J Dis Child* 1984;**138:**663–6.

8 Maddison PJ, Provost TT, Reichlein M. Serological findings in patients with 'ANA-negative' systemic lupus erythematosus. *Medicine* 1981;**60:**87–94.

9 Provost TT. Anti-Ro (SS-A) and anti-La (SS-B) antibodies in lupus erythematosus and Sjögren's syndrome. *Keio J Med* 1991:**40:**72.

10 Maddison PJ, Isenberg DA, Goulding NJ, *et al.* Anti-La (SS–B) identifies a distinct subgroup of systemic lupus erythematosus. *Br J Rheum* 1988;**27:**27-31.

11 Tan EM. Antinuclear antibodies: diagnostic marker for autoimmine diseases and probes for cell biology. *Adv Immunol* 1989;**44:**93-152.

12 van Venrooij WJ, Sillekens PTG. Small nuclear RNA associated proteins: autoantigens in connective tissue diseases. *Clin Exp Rheum* 1989;**7;**635-45.

13 Sperling R. Autoantibodies against nuclear ribonucleoprotein complexes. *Is J Med Sci* 1988;**24:**358–62.

14 Bonfa E, Golombek SJ, Kaufmann LD. Association between lupus psychosis and anti-ribosomal P protein antibodies. *New Engl J Med* 1987;**317:**265–71.

15 Teh LS, Bedwell AE, Isenberg DA, *et al.* Antibodies to P protein in systemic lupus erythematosus. *Ann Rheum Dis* 1992;**51:**489– 94.

16 Maini RN, Charles PJ, Venables PJW. Antinuclear antibodies in the immunotaxonomy of connective tissue disease. *Scan J Rheumatol* 1984;**56;**(supp):49–57.

17 Swaak AJG, Groenwold J, Bronsveld W. Predictive value of complement profiles and anti-dsDNA in systemic lupus erythematosus. *Ann Rheum Dis* 1986;**45:**359–66.

18 Kallenberg CGM, Wouda AA, Hoet MH, van Venrooij WJ. Development of connective tissue disease in patients presenting with Raynaud's phenomenon: a six-year follow-up with emphasis on the predictive value of antinuclear antibodies as detected by immunoblotting. *Ann Rheum Dis* 1988:**44:**634–71.

19 Kingwell B, Rattner JB. Mammalian kinetochore/centromere composition: a 50 kDa antigen is present in the mammalian kinetochore/centromere. *Chromosoma* 1987;**95:**403–7.

20 McHugh NJ, James TI, Maddison PJ. Differential isotype recognition of two centromere associated polypeptides by immunoblotting in connective tissue disease. *Clin Exp Immunol* 1988;**72:**457–64.

21 Steen VD, Powell DL, Medsger TA. Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. *Arthritis Rheum* 1988;**31:**196-203.

22 Powell FC, Winkelmann RK, Venencie-Lemarchand F, *et al.* The

anti-centromere antibody: disease specificity and clinical significance. *Mayo Clin Proc* 1984;**27:**125–31.
23 Yaneva M, Arnett FC. Antibodies against Ku protein in sera from patients with autoimmune diseases. *Clin Exp Immunol* 1989;**76:**255–60.
24 Fritzler MJ, McCarty GA, Ryan JP, Kinsella TD. Clinical features of patients with antibodies directed against proliferating cell nuclear antigen. *Arthritis Rheum* 1983;**26:**140–5.
25 Hassfield W, Steiner G, Hartmuth K, Smolen JS. Demonstration of a new antinuclear antibody (anti RA33) that is highly specific for rheumatoid arthritis. *Arthritis Rheum* 1989;**32**: 1515–20.
26 Smolen JS, Hassfield W, Graininger W, Steiner G. Antibodies to antinuclear antibody subsets in systemic lupus erythematosus and rheumatoid arthritis. *Clin Exp Rheum* 1990;**8**(suppl):41–4.
27 Harris EN, Ghavari AE, Boey ML, Hughes GRV. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983;**2:**1211–4.
28 Love PE, Santaro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann Intern Med* 1990;**112:**682–98.
29 Vaarala O, Palosuo T, Kleemola M, Aho K. Anticardiolipin response in acute infections. *Clin Immunol Immunopathol* 1986;**41:**8–15.
30 Lawrence RA, Brookes SM, Fox J, *et al.* Antibodies to phospholipids in chronic fatigue syndrome: Implications for a viral aetiology. *Br J Rheum* 1991;**30**(suppl 1):17.
31 Derkson RHWM, Hasselaar P, Blokziji L, *et al.* Coagulation screen is more specific than the anticardiolipin ELISA in defining a thrombotic subset of lupus patients. *Ann Rheum Dis* 1988;**47:**364–71.
32 Hughes TP, Jones P, Rozenberg MC. Hypergammaglobulinaemia and the anticardiolipin antibody test. *Arthritis Rheum* 1989;**32:**813.
33 Matsura E, Igarashi Y, Fujimoto M, *et al.* Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. *Lancet* 1990;**336**:177–8.
34 Hagen EC, Ballieux BEPB, Daha MR, *et al.* Fundamental and clinical aspects of anti-neutrophil cytoplasmic antibodies (ANCA). *Autoimmunity* 1992;**11:**199–207.
35 Charles PJ, Maini RN. ANCA in rheumatoid arthritis. *Br J Rheum* 1992;**31**(suppl 2):206.
36 Savige JA, Gallicchio MC, Stockman A, *et al.* Anti-neutrophil cytoplasm antibodies in rheumatoid arthritis. *Clin Exp Immunol* 1991;**86:**92–8.
37 Withrington RH, Teitsson I, Valdimarsson H, Seifert MH. Prospective study of early rheumatoid arthritis: II—Association of rheumatoid factor isotypes with fluctuations in disease activity. *Ann Rheum Dis* 1984;**43:**679–85.
38 Eberhardt KB, Truedsson L, Pettersson H. Disease activity and joint damage progression in early rheumatoid arthritis: relation to IgG, IgA, and IgM rheumatoid factor. *Ann Rheum Dis* 1990;**49:**906–9.
39 Robbins DL, Feigal DW, Leek JC. Relationship of serum IgG rheumatoid factor to IgM rheumatoid factor and disease activity in rheumatoid arthritis. *J Rheum* 1986;**13:**259–62.
40 Weestgeest AAA, Boerbooms AMTH, Jongmans M. Antiperinuclear factor: indicator of more severe disease in seronegative rheumatoid arthritis. *J Rheum* 1987;**14:**893–7.
41 Davis P, Percy JS, Russell AS. Correlation between levels of DNA antibodies and clinical disease activity in SLE. *Ann Rheum Dis* 1977:**36**:157.
42 Pincus T, Schur PH, Rose JA, *et al.* Measurement of serum DNA-binding activity in systemic lupus erythematosus. *New Engl J Med* 1969;**281:**701.
43 Barada FA, Andrews BS, Davis JS, Taylor RP. Antibodies to Sm in patients with systemic lupus erythematosus. Correlations of Sm antibody titres with disease activity and other laboratory parameters. *Arthritis Rheum* 1981;**24:**1236.
44 De Rooij DJ, Habets WJ, van de Putte LBA, *et al.* Use of recombinant RNP peptides 70K and A in an ELISA for measurement of antibodies in mixed connective tissue disease: a longitudinal follow-up of 18 patients. *Ann Rheum Dis* 1990;**49:**391.
45 Scopelitis E, Biundo JJ, Alspaugh MA. Anti-SS-A antibody and other nuclear antibodies in systemic lupus erythematosus. *Arthritis Rheum* 1980;**23:**287.
46 Charles PJ, van Venrooij WJ, Maini RN. The consensus workshops for the detection of autoantibodies to intracellular antigens in rheumatic diseases: 1989–1992. *Clin Exp Rheum* 1992;**10:**507–11.

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