The use of laboratory tests in the diagnosis of SLE

William Egner

Systemic lupus erythematosus (SLE) is a protean autoimmune disease where autoantibodies are frequently targeted against intracellular antigens of the cell nucleus (double and single stranded DNA (dsDNA and ssDNA, respectively), histones, and extractable nuclear antigens (ENAs). Most of these autoantibodies are not specific for SLE and might be produced non-specifically as a result of polyclonal B cell activation. This article will focus on the evidence base for the most commonly used laboratory assays for the detection of these autoantibodies. Updated American Rheumatism Association (ARA) criteria for the diagnosis of SLE include several autoantibodies (table 1).[1 2] SLE is likely if four of 11 criteria are met over any time period. Importantly, the methods for detecting these antibodies are not specified by the ARA, and this article aims to highlight the fact that the particular assay used will crucially influence the interpretation of the test (table 2). Autoantibodies are usually polyclonal—of mixed isotype, affinity, and avidity—and are often directed against multiple targets. Different assays detect particular antibody properties, which are often quite different, and the clinical importance of this for pathogenesis or diagnosis is rarely fully understood. The use of laboratory tests in SLE is a perfect example of this dilemma. The prevalence of autoantibodies varies widely in cross sectional studies, perhaps partly as a result of such differences (table 3). Immunodiffusion (ID) detects high affinity antibodies, immunofluorescence (IIF) moderate and high affinity antibodies, and enzyme linked immunosorbent assay (ELISA) low and high affinity antibodies. Purified antigens might have contaminants, or might not contain the full complement of native proteins. Recombinant antigens might lack certain epitopes, have altered glycosylation or tertiary structure, or contain contaminating bacterial antigens. All assays require careful validation to determine whether they perform adequately for detecting human autoantibodies. An ideal test

would be specific (detects only those with disease), sensitive (detects all those with disease), have a high positive predictive value (PPV)—where most positives have disease, and a high negative predictive value (NPV)—where most negatives do not have disease. In addition, assay results may reflect disease activity, correlate with organ involvement, or predict relapse, thus allowing pre-emptive treatment. No test or test panel can currently perform all these tasks because increases in specificity usually lead to reciprocal decreases in sensitivity, and because some of the clinical features of SLE are not antibody mediated. Therefore, the information obtained from any test will reflect the types of antibody detected, the prevalence of the disease in the population being tested, and the question being asked of the test.

Antinuclear antibodies (ANAs)

Any antibody to nuclear components is an ANA. Most patients with ANAs do not have SLE, but most people with SLE have ANAs. The most common screening test is IIF on rodent liver or human epithelial (HEp2) tissue,[3] although ELISA tests are available.[4 5] Lupus erythematous cells simply represent nuclei opsonised by ANAs and are no longer used in diagnosis. Although ANAs are very sensitive for SLE, positive ANAs are common, especially in unwell elderly individuals.[6–8] Therefore, ANAs have low PPV for SLE in unselected populations or when present in low titres,[6 9] and are not diagnostic. One in three healthy people have detectable ANAs on HEp-2 cells at a screening dilution of 1/40 and one in 20 will be positive at 1/160. HEp-2 cells produce more positive ANAs than rat tissue, and some ANAs (for example, anticentromere antibodies) can only be reliably detected on HEp-2 substrate. Although "ANA negative" SLE is reported,[10] it is not clear whether this is the result of a technical artifact or whether a subgroup of SLE exists. Most ANA negative patients are positive in DNA or ENA assays or when screened by IIF on a different substrate. ARA criteria refer to "abnormal" titres of autoantibodies, but there is no cut off value that will absolutely distinguish normality from autoimmune disease. In general, higher titres are more meaningful, particularly in young patients. ANA measurement is at best semi-quantitative, and is poorly standardised between laboratories owing to the lack of suitable reference preparations. The precision and accuracy of the technique depends on the assay configuration, the quality control procedures, and the experience of the reader (table 4). Patterns might suggest antibody specificities but are not diagnostic (table 5). Most clinically relevant ANAs are IgG antibodies and the detection of IgM antibodies usually reduces the

Department of Immunology and Protein Reference Unit, Northern General Hospital, Herries Road, Sheffield S5 7YT, UK
W Egner

Correspondence to:
Dr Egner
email: w.egner@shef.ac.uk

Accepted for publication
14 December 1999

Table 1   The American Rheumatology Association (ARA) criteria

ARA criteria 1982 (updated 1997)	Detail
Photosensitivity	Photosensitive skin rash
Malar rash	Flat or raised fixed erythema
Discoid rash	Raised with plugging/scarring/scaling
Oral ulcers	Usually painless
Arthritis	Non-erosive, 2+ peripheral joints
Serositis	Pleural or cardiac
Renal disorder	Proteinuria or cellular casts
Neurological disorder	Convulsions or psychosis without other cause
Haematological disorder	Haemolysis, cytopenia
"Immunological disorder" (modified 1997)	Anti-dsDNA, anti-Sm, antiphospholipid antibodies (ACAs, LA, or FP VDRL)
Antinuclear antibody (ANA)	"Abnormal titre" ANA at any time point by IIF or equivalent assay

ACA, anticardiolipin antibody; ANA, antinuclear antigen; dsDNA, double stranded DNA; FP VDRL, false positive venereal disease reference laboratory test; IIF, immunofluorescence; LA, lupus anticoagulant.

Table 2   Common attributes of individual assay technologies used for the diagnosis and monitoring of SLE (all assays produce some false positive results)

Assay	Problems	Advantages	Result
IIF rodent tissue	Subjective, Ro may be missed, semiquantitative, pattern not diagnostic, cannot detect cell cycle related patterns, not specific	Cheap, can be isotype specific	Semiquantitative end point titration or qualitative result at screening titre + pattern
IIF HEp-2	Subjective, Ro may be missed, semiquantitative (poor precision), pattern not diagnostic, not specific	Cheap, recombinant Ro60 expression to boost Ro sensitivity available, can be isotype specific	Semiquantitative end point titration or qualitative result at screening titre + pattern
Ouchterlony double diffusion (ID)	Slow, crude antigens, subjective, qualitative, requires experience, not isotype specific, some false negatives	Specific, cheap	Positive or negative + antigen specificity
Countercurrent immunoelectrophoresis (CIE)	Slow, crude antigens, semiquantitative, requires experience, not isotype specific, some false negatives	As ID, but more sensitive	Positive or negative + antigen specificity
Haemagglutination	Detects IgG and IgM, semiquantitative, subjective, detects low affinity antibodies	Cheap	Positive or negative + semiquantitative titre
Immunoblotting (IB)	Qualitative, may be insensitive for Ro, crude antigen, labour intensive	Sensitive, very specific for individual antigens	Positive or negative + antigen specificity
Immunoprecipitation (Farr)	Radioactive, labour intensive, expensive, technically difficult, no isotype specificity, false positivity	Quantitative, high specificity, detects high affinity antibodies	Quantitative result, potentially in standardised IU/l if reference preparation available
ELISA	Detects low affinity antibodies, needs high purity well defined antigens (native v recombinant), false positivity	Sensitive, variable, can be polyspecific or IgG specific	Qualitative or quantitative results, potentially in standardised IU/l if reference preparation available

ELISA, enzyme linked immunosorbent assay; IIF, immunofluorescence.

clinical usefulness of the test.[11][12] Antibody class switching to IgG usually occurs in established autoimmunity, and many low titre, low affinity IgM autoantibodies are found in healthy individuals. The absence of ANAs at titres of 1/160 or less makes SLE very unlikely. Approximately 10% of SLE like disease is drug induced and potentially reversible. However, drug induced ANAs are more common than disease, and careful interpretation of the possible clinical relevance of an ANA in this context is needed. Each laboratory should configure its protocol for an appropriate sensitivity/ specificity compromise, should perform adequately in local and national external quality assessment (EQA) schemes, and should not interpret results without reference to the clinical details. More specific and precise tests should be performed in ANA positive individuals to determine the autoantibody specificity (table 5).

Anti-DNA antibodies
dsDNA antibodies are associated with systemic lupus and nephritis, but not subacute cutaneous lupus or discoid lupus. The best method for detecting anti-dsDNA remains controversial[13–18] (table 4). The most common techniques in the UK are dsDNA ELISA, *Crithidia luciliae* IIF (CLIF), or Farr immunoprecipitation assays (table 2). Specific assays should be used for diagnosis, whereas sensitive assays might be more useful for monitoring.[4] There are several difficulties in the detection of

anti-dsDNA, which apply to other autoantibodies, including:

(1) Substrate differences: many sources of mammalian and non-mammalian DNA are used, but each might detect a different set of antibodies.
(2) The isotype of antibody detected: assays might detect different antibody isotypes (IgG, IgA, IgM, or any combination). All isotypes are detected by Farr assays, ELISA, or CLIF, which use polyspecific antisera. A positive polyspecific assay might have a different clinical relevance to that of an IgG specific assay.[19][20] IgM anti-dsDNA detected by ELISA might not be specific for SLE.[20][21]
(3) Antibody affinity: high affinity anti-dsDNA might be more relevant to SLE pathogenesis, particularly in nephritis. Low affinity antibodies are not detected by Farr assays, but are detected by ELISA.[22–29]
(4) Assay specific parameters: each assay has known causes of false positivity. For example, C reactive protein (CRP) or ssDNA contamination in the Farr; lipoprotein–IgG complexes in CLIF; antibodies to linkers in ELISA. Contamination with ssDNA leads to overestimation of anti-dsDNA titres, because anti-dsDNA antibodies regularly bind ssDNA, but ssDNA antibodies are not specific for SLE.[30] This might not be important for monitoring disease activity because ssDNA might reflect the overall antinucleosome immune responses,[31][32] but it might reduce the diagnostic usefulness of an assay because ssDNA antibodies are not specific for SLE.
(5) Problems with standardisation and calibration: an assay has to be precise (give the same result on the same serum every time) to enable successful monitoring of serial titres. An assay should be accurate and produce the same result as other assays on all serum samples to enable comparison between different centres. An international reference preparation (IRP) for anti-

Table 3   Frequency of serological positivity in SLE

Autoantibody target	% Positive at any stage of disease (any assay)	Possible clinical association (see text)
dsDNA	30–70	Nephritis, disease activity
Sm	20–40	Rarely seen outside SLE
RNP	40–60	MCTD/overlap features
Ro	10–15	Sjogren's/skin involvement/congenital heart block
Ribosomal P0, P1, P2	5–10	Neuropsychiatric SLE, disease activity
Histone	30	Drug induced SLE, idiopathic SLE, disease activity
ACA	40–50	Risk of thrombotic complications/fetal loss/ITP

ACA, anticardiolipin antibody; dsDNA, double stranded DNA; ITP; idiopathic thrombocytopenic purpura; MCTD, mixed connective tissue disease; RNP, ribonuclear protein.

Table 4 The clinical usefulness of commonly used autoantibody assays in SLE

Test (EQA scheme?)	Technique	Diagnostic specificity	Clinical usefulness	Use in monitoring activity	Evidence base
ANA (EQA+)	IIF rodent tissue	Moderate for homogenous or speckled pattern at ≥1/160 in selected patients, IgG only	Initial screen for further testing	Poor (semiquantitative)	Extensive
	IIF HEp-2	Moderate for homogenous or speckled pattern at ≥1/160 in selected patients, IgG only	Initial screen for further testing	Poor (semiquantitative)	Extensive
	IIF ELISA	Unknown	Unknown	Possible	Limited
dsDNA (EQA+)	Farr	Good, known causes of false positives	Good for diagnosis and monitoring	Useful in subset of patients	Extensive
	Crithidia IIF	Good, known causes of false positives	Good for diagnosis	Poor (semiquantitative)	Extensive
	ELISA	Variable, assay dependent, IgG specific good, known causes of false positives	Assay dependent, good for IgG specific	Assay dependent, good for IgG specific	Moderate
	Nitrocellulose	Moderate	Moderate	No	Limited
Complement C3, C4 (EQA+)	Nephelometry/ turbidometry	None	Moderate, low C3 in renal disease	Useful in subset of SLE, serial values essential	Extensive
CRP (EQA+)	Nephelometry/ turbidometry	None	CRP usually low in active SLE except in severe serositis	Useful to distinguish infection from SLE activity	Moderate
ENA Ro/La/ Sm/RNP (EQA+)	Immunodiffusion/ CIE	Good for Sm, moderate for Ro/La, may be poor for RNP	Good for defining ANA specificity	No, except where disease has evolved new features	Extensive
	IB	Good, but may be insensitive for Ro	Good	No	Moderate
	ELISA	As ID/CIE but more sensitive for Ro/RNP, less specific if low titre or IgM positive	Good for IgG antibody at appropriate cut off	No, except where disease has evolved new features	Extensive
Antiphospholipid antibodies (ACA EQA+)	Haemagglutination	Poor, less specific, detects IgM	Poor	No, semiquantitative	Limited
	ACA ELISA	Poor, even for IgG antibodies	Moderate, especially IgG specific	Possible	Extensive
	Anti-β2GP1 ELISA	Moderate for IgG antibodies	Moderate, especially IgG specific	Unknown	Moderate
	Lupus anticoagulant	Poor	Moderate	Unknown	Moderate
Antihistone antibodies	ELISA	Poor, even for drug induced SLE	Poor	Possible	Limited
	IB	Poor, even for drug induced SLE	Poor	No	Limited

EQA +, UK external quality assessment scheme available.
ACA, anticardiolipin antibody; ANA, antinuclear antigen; β2GP1, β2 glycoprotein 1; CIE, countercurrent immunoelectrophoresis; CRP, C reactive protein; dsDNA, double stranded DNA; ELISA, enzyme linked immunosorbent assay; ENA, extractable nuclear antigen; IB, immunoblotting; ID, immunodifussion; IIF, immunofluorescence; RNP, ribonuclear protein.

dsDNA (Wo80) is available to improve standardisation between assays (table 6), but there remains poor correlation between dsDNA assays in EQA schemes, even within a single technology.[20] All laboratories should participate in EQA schemes and maintain adequate internal quality control procedures.

DIAGNOSIS
Farr assays are quite specific and well documented, but also detect high affinity IgM anti-dsDNA. IgG specific ELISA or CLIF methods are commonly used in UK laboratories, and may produce similar results to Farr assays. Polyspecific ELISAs also detect low affinity IgM antibodies of dubious clinical relevance and are less useful. ELISA results (particularly those detecting IgM) should be confirmed by IgG specific CLIF or Farr assays.[33–36] CLIF is less likely than ELISA to detect low affinity anti-dsDNA of uncertain clinical relevance,[37–42] especially if IgG specific conjugates are used. Local validation of each assay is essential to ensure adequate diagnostic performance.

MONITORING OF DISEASE
Laboratory tests are no more effective than clinical review for detecting disease relapse,[43–50] but are helpful in confirming the activity of SLE. The rapidity of clinical relapse clouds the interpretation of the few prospective studies available, and regular sampling every six to eight weeks would be required to predict relapse reliably. dsDNA antibodies rise in active disease and in the evolution of lupus nephritis in most patients.[51–55] dsDNA antibody assays can be negative early in disease, after treatment, or when the patient is in clinical remission; therefore, not all patients with SLE are seropositive at any one time.[56] The absence of antibodies at any one time would not

Table 5 Common antinuclear antibody (ANA) HEp-2 patterns and their clinical use in SLE

Pattern	Autoantibody association	Clinical association
Homogenous	dsDNA, ssDNA, histones, nucleosomes, Ku	SLE, AICAH, and many non-pathological ANAs
Rim/peripheral	dsDNA, laminin, nuclear pore	SLE/AICAH
Speckled (coarse)	RNP/Sm	SLE/MCTD
Speckled (fine) may be missed on IIF owing to variable Ro expression on tissue	Ro/La (also cytoplasmic)	SLE/SCLE/scleroderma
Speckled (centromere)	Centromere A, B, C kinetechore proteins	Primary Raynauds, scleroderma, SLE

The main use of the ANA pattern on screening is to determine the need for further antibody specificity testing or to distinguish possible false positive ELISA.
Patterns are not diagnostic, most homogenous or speckled ANAs have no detectable dsDNA or ENA antibodies.
AICAH, autoimmune chronic active hepatitis; dsDNA, double stranded DNA; ENA, extractable nuclear antigen; IIF, immunofluorescence; MCTD, mixed connective tissue disease; RNP, ribonuclear protein; SCLE, subacute cutaneous lupus erythematosus; ssDNA, single stranded DNA.

Table 6 International reference preparations for autoantibodies

Reference preparation	Specificity
WHO Wo80	dsDNA 100 IU/ampoule
WHO1064 or MRC research standard A 66/233	Homogeous ANA 100 IU/ampoule
WHO1063	nRNP
WHO1061	IgM ANA 100 IU/ampoule
CDC reference preparations	dsDNA, La, U1-RNP, Sm, Ro, centromere, Scl70, Jo-1

ANAs, antinuclear antibodies; CDC, Centers for Disease Control; dsDNA, double stranded DNA; nRNP, nuclear ribonuclear protein.

exclude a previous diagnosis of SLE, and ARA criteria include events that occur sequentially over time. Prophylactic treatment on the basis of rising titres of anti-dsDNA cannot be widely justified at present, but rises in titre merit closer clinical monitoring for relapse. IgM and low affinity anti-dsDNA antibodies might be less specific for SLE.[19 57 58] High avidity IgA antibodies are seen in active SLE,[59] but are no more helpful than IgG assays. Some IgG specific ELISAs have similar usefulness to the Farr assay,[23 60] but this requires validation on an assay by assay basis. It remains to be proved whether low affinity anti-dsDNA antibodies are specific for subclinical or mild SLE.[29 61]

Anti-ssDNA activity might be useful in monitoring activity,[62 63] but it is not clear that this offers any advantage over anti-dsDNA measurements and no EQA/IRP for ssDNA is available.[17 60]

Antihistone antibodies
Around 50–80% of patients with SLE have IgG and IgM antihistone antibodies detectable by immunoblotting (IB) or ELISA. ELISAs are described that detect antibodies to total histones or to subfractions (H1, H2a, H2b, H3, and H4),[64 65] but the clinical specificity is not well established for any subfraction. Titres of antihistone antibody might reflect disease activity, but are not specific for SLE and cannot distinguish drug induced SLE from idiopathic SLE.[52 66 67] Drug induced antibodies are often IgM and occur without any clinical manifestations.

Antibodies to ENAs
ANTI-Ro/La ANTIBODIES
Diagnosis
Antibodies to Ro(SS-A) and La(SS-B) are found in SLE and Sjogren's syndrome. Neither is specific for SLE, but both are very useful when anti-dsDNA is absent.[68] ID/CIE (countercurrent immunoelectrophoresis) assays were frequently used in the past, but are now superseded by more sensitive ELISA or IB assays (table 2). Some IB assays might be insensitive for anti-Ro. ELISAs are more sensitive for anti-Ro, anti-La, and anti-RNP, but are positive in other diseases also (table 4).[3 69 70] Disease specificity is improved by excluding weak positives. Unlike anti-dsDNA, anti-ENA antibody affinity does not appear to be important,[71] but IgG antibodies are of greater clinical relevance.[12] Little is known about the importance of IgA or IgM anti-Ro. Ro exists in two forms: Ro52 and Ro60. In SLE, anti-Ro60 antibodies predominate, whereas both are

present in Sjogren's syndrome.[72] Ro60 contains conformational epitopes that are absent in some assay substrates. Newer enzyme immunoassays and western blotting are capable of discriminating antibodies to Ro52 and Ro60 individually, but it is not clear how clinically useful this will be. Bovine spleen is less sensitive than human substrate in ID/CIE,[5 34] and can cause problems in detection.

Monitoring
There is little evidence that anti-ENA specificity or titres reflect SLE activity,[68 73] but anti-Ro is associated with cutaneous involvement in subacute cutaneous lupus erythematosus,[74] and with congenital heart block (CHB).[75] Anti-Ro52 in isolation is associated with CHB but is not detected by assays containing only Ro-60,[76] such as HEp-2000 cells.

Anti-La is rarely detected without anti-Ro[77 78] because both proteins associate with a common type of human RNA called hYRNA. There is little evidence that anti-La is associated with reduced renal disease. Titres of anti-Ro and anti-La increase more slowly than anti-dsDNA in relapse and quantitative reporting is unlikely to be useful.[79] Standardisation of results between laboratories is still problematic in EQA schemes. Centers for Disease Control anti-ENA reference preparations of defined specificity, but no IRP with defined units, are available.

Neonatal SLE and CHB
Anti-Ro52, anti-Ro60, anti-La, or anti-Sm IgG is transferred across the placenta in the last trimester and on rare occasions can lead to pathology in the child.[75 80–82] Serology can be performed on cord blood or the mother's blood antenatally, and intrauterine monitoring can be instituted in high risk seropositive pregnancies.

ANTIBODIES TO Sm/RNP
Diagnosis
High titre anti-Sm constitutes an ARA criterion for SLE and is highly SLE-specific, although low titre anti-Sm in ELISA/immunoprecipitation assays has been reported in other diseases.[5 83–85] Anti-Sm antibodies are rarely found without anti-RNP (ribonucleoprotein)[86] because both proteins associate with common snRNA species in the spliceosome. Anti-RNP is more common and less specific for SLE.[83 87] Anti-RNP ELISAs are more sensitive than ID, but ELISA and ID might be equivalent for anti-Sm[70 88 89] (table 2). Bovine thymus substrate has similar sensitivity to human thymus extract for Sm/RNP. The importance of antibody isotype or affinity is unknown.[90]

Monitoring
Anti-RNP or anti-Sm antibodies are not strongly associated with specific clinical features of SLE, outside mixed connective tissue disease (MCTD),[73 91–95] but anti-Sm might appear with disease evolution.[96] Titres can fluctuate with disease activity and

treatment,[97–99] but serial monitoring does not effectively predict relapse.[100 101]

There are insufficient data on the more rare ENA specificities to justify routine clinical use.

Ribosomal P antibodies

Anti-ribosomal antibodies detected by ELISA or IB are associated with neuropsychiatric SLE,[102–106] but their predictive value is uncertain and controversial.[107 108] Titres rise in active SLE.[109] Data associating lymphocytotoxic antibodies with cognitive dysfunction are very limited.[110]

Antiphospholipid antibodies

Anticardiolipin antibodies (ACAs) of all isotypes are seen in 16–60% of patients with SLE. IgG ACAs[111–115] are a risk factor for thrombosis and the antiphospholipid syndrome, but are controversial risk factors for renovascular events.[116 117] Not all ACA positive patients with SLE have an antiphospholipid syndrome and ACA negative patients can have thrombotic complications. ACAs might be an additional risk factor for pregnancy outcome in SLE.[118 119] Titres vary with disease activity, perhaps explaining an association with severe renal disease.[120 121] EQA schemes reveal poor standardisation, despite the availability of reference materials for calibration.

IgG anti-β2 glycoprotein 1 antibodies are more closely associated with thrombosis in the primary antiphospholipid syndrome and SLE,[122] and approximately 25% of SLE patients may be positive. Other antiphospholipid antibodies are of uncertain importance including antiphosphatidyl serine, anti-annexin V, and antithromboplastin.

Additional lupus anticoagulant testing (for example, dilute Russell's viper venom test or activated partial thromboplastin time) is essential because lupus anticoagulant might predispose to thrombosis, and might occur without ACAs.[112] The reproducibility of the lupus anticoagulant test is variable and it cannot be used to monitor disease activity.[123]

Acute phase proteins/cytokines

The erythrocyte sedimentation rate is a sensitive but non-specific indicator of activity in SLE, and is slow to reflect changes in disease activity. CRP has a short half life and rapidly reflects acute inflammation. A high CRP can distinguish bacterial infection from active SLE, where the CRP is usually low,[124–126] but CRP might be raised in severe lupus serositis.[127] Soluble interleukin 2 receptor or tumour necrosis factor receptor values might reflect disease activity, but are not specific for SLE, and are of uncertain clinical relevance.[128 129]

Complement

Although immune complexes are seen in SLE, immune complex assays are poorly reproducible, non-specific, and rarely useful,[130] except for cryoglobulins. Complement assays are occasionally useful.[18 28 131–136] A single C4 is not informative and serial monitoring is necessary because C4 null alleles are common in SLE, so that the baseline C4 may be chronically low.[137]

SLE can also be active without causing changes in C3 and C4.[48] Persistently low C3 is associated with chronic renal disease.[138 139] Classical pathway assays (CH100/CH50) cannot distinguish deficiency from severe consumption, but can exclude early pathway complement deficiencies (C1, C2, or C4), which are associated with SLE. In contrast, C3 and C4 values are precise and economical, even if useful in some patients only.

Complement activation products (C3d, C3a, C4a, C5a, iC3, C4d, Bb, C5b–9, and erythrocyte CR1) are raised in active disease,[140–143] but assays to measure these molecules are not widely available and require special sample handling to a degree that makes routine clinical use impracticable.

Anti-C1q antibodies

Anti-C1q antibodies are detected by ELISA in 90% of patients with SLE,[144] but are also found in membranoproliferative glomerulonephritis and rheumatoid vasculitis. High titres are associated with proliferative glomerulonephritis,[145 146] but these antibodies are of limited clinical use.

Anti-endothelial cell antibodies

Anti-endothelial cell antibodies may reflect disease activity,[147] but are poorly characterised.

Antineutrophil cytoplasmic antibodies

Antineutrophil cytoplasmic antibodies of all types are found in SLE and are not clinically relevant.[148]

Summary

ANA IIF is an effective screening assay in patients with clinical features of SLE and will detect most anti-ssDNA, anti-dsDNA, ENAs, and other autoantibodies. False positives are common. The clinical importance cannot be extrapolated from the ANA titre or pattern, although higher titres (> 1/160) are more likely to be important. HEp-2 cells are the most sensitive substrate for ANA detection, but this must be balanced against an increased incidence of insignificant positivity.

ANA positive samples should be subjected to more specific assays for the diagnosis of SLE. A combination of ENA (Ro/La/Sm/RNP) and dsDNA assays will detect most patients with SLE[5 18 149] as long as the characteristics of the assays used are well understood. ESR and CRP measurements provide useful additional information. Sjogren's syndrome and MCTD will produce overlapping serology with SLE, and anti-dsDNA titres are sometimes seen in autoimmune hepatitis and rheumatoid arthritis. All results should be reported in the light of the clinical details, by an experienced immunologist. A suggested diagnostic protocol is outlined in fig 1. The type of assay used crucially influences the predictive value of the tests. ELISA technology dominates routine laboratory practice, but tends to produce more false positive and true weak positive results, which may reduce the PPV of the test. This can be minimised by using IgG specific conjugates and careful assay validation. The NPV for SLE
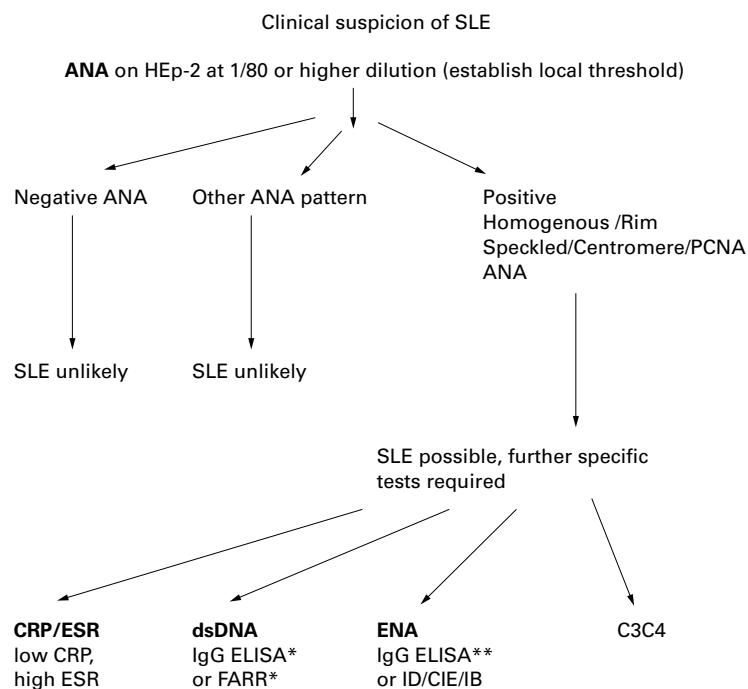
Clinical suspicion of SLE

ANA on HEp-2 at 1/80 or higher dilution (establish local threshold)

Negative ANA   Other ANA pattern   Positive
Homogenous /Rim
Speckled/Centromere/PCNA
ANA

SLE unlikely   SLE unlikely

SLE possible, further specific
tests required

CRP/ESR   **dsDNA**   **ENA**   C3C4
low CRP,   IgG ELISA*   IgG ELISA**
high ESR   or FARR*   or ID/CIE/IB

*Figure 1   Suggested diagnostic protocol for investigation of suspected SLE. *Confirm
weak positives or possible false positives by IgG CLIF; **confirm weak positives or possible
false positives by ID/CIE/IB. Antiphospholipid/lupus anticoagulant assays might be
necessary if relevant clinical features of an antiphospholipid syndrome are present. ANA,
antinuclear antibody; CIE, countercurrent immunoelectrophoresis; CLIF, Crithidia luciliae
immunofluorescence; CRP, C reactive protein; dsDNA, double stranded DNA; ELISA,
enzyme linked immunosorbent assay; ENA, extractable nuclear antigen; ESR, erythrocyte
sedimentation rate; ID, immunodiffusion; IB, immunoblotting; PCNA, proliferating cell
nuclear antigen.*

is high for most assays but the PPV varies. Where necessary, laboratories should use crithidia or Farr dsDNA assays to confirm dubious ELISA dsDNA results, and ID/IB to confirm dubious ENA results.

For monitoring, a precise, quantitative assay is required. It is unclear whether the detection of IgM or low affinity antibodies has a role here. A combination of anti-dsDNA, C3, C4, CRP, and ESR assays provides the most useful clinical information. Anti-ssDNA assays are likely to be useful, and are potentially more robust than anti-dsDNA assays, but require more validation.

Local validation of individual assays and EQA participation is essential. Not all assays that apparently measure the same antibody specificities have equal clinical relevance, even within a single technology. Insufficient international or national reference preparations are currently available for many antibody specificities to enable effective standardisation. Quality assurance schemes reveal large differences in units reported by different assays for some analytes, even when calibrated against an IRP or equivalent reference preparation. Serial results can therefore only be compared from the same laboratory at present. Most autoantibodies increase during active disease, but few prospective data are currently available to justify treatment on the basis of rising titres. Further randomised prospective studies are required to examine the importance of antibody isotype and affinity in the monitoring of SLE by individual assay methods. The most

important aspect of the appropriate use of laboratory assays is to become familiar with the limitations of the technology currently in use in your local laboratory, and to consult with your clinical immunologist in cases of doubt, preferably before commencing serological screening.

1 Tan E, Cohen A, Fries J, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosis. *Arthritis Rheum* 1982;**25**:1271–7.
2 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;**40**:1725.
3 van Venrooij WJ, Charles P, Maini, RN. The consensus work-shops for the detection of autoantibodies to intracellular antigens in rheumatic diseases. *J Immunol Methods* 1991;**140**:181–9.
4 Emlen W, O'Neill L. Clinical significance of antinuclear antibodies: comparison of detection with immunofluorescence and enzyme-linked immunosorbent assays. *Arthritis Rheum* 1997;**40**:1612–18.
5 Froelich CJ, Wallman J, Skosey JL, *et al*. Clinical value of an integrated ELISA system for the detection of 6 autoantibodies (ssDNA, dsDNA, Sm, RNP/Sm, SSA, and SSB). *J Rheumatol* 1990;**17**:192–200.
6 Tan EM, Feltkamp TE, Smolen JS, *et al*. Range of antinuclear antibodies in "healthy" individuals [see comments]. *Arthritis Rheum* 1997;**40**:1601–11.
7 Ruffatti A, Calligaro A, Del Ross T, *et al*. Anti-double-stranded DNA antibodies in the healthy elderly: prevalence and characteristics. *J Clin Immunol* 1990;**10**:300–3.
8 Juby AG, Davis P. Prevalence and disease associations of certain autoantibodies in elderly patients. *Clin Invest Med* 1998;**21**:4–11.
9 Sheil WC, Jason M. Diagnostic associations of patients with antinuclear antibodies referred to community rheumatologists. *J Rheumatol* 1989;**16**:782–5.
10 Bohan A. Seronegative systemic lupus erythematosus. *J Rheumatol* 1979;**6**:534–40.
11 de Vlam K, De Keyser F, Verbruggen G, *et al*. Detection and identification of antinuclear autoantibodies in the serum of normal blood donors. *Clin Exp Rheumatol* 1993;**11**:393–7.
12 Wahren M, Ringertz NR, Petterson I. IgM and IgG subclass distribution of human anti-Ro/SSA 60 kDa autoantibodies. *Scand J Immunol* 1994;**39**:179–83.
13 Eilat D. The measurement of anti-DNA reactivity in the sera of patients with SLE: theoretical and practical considerations. *Autoimmunity* 1989;**3**:299–6.
14 Isenberg DA, Dudeney C, Williams E, *et al*. Measurement of anti-DNA antibodies: a reappraisal using five different methods. *Ann Rheum Dis* 1987;**46**:448–56.
15 Kadlubowski M, Jackson M, Yap PL, *et al*. Lack of specificity for antibodies to double stranded DNA found in four commercial kits. *J Clin Pathol* 1991;**44**:246–50.
16 Chuan MT, Wu YC, Ang ET, *et al*. Clinical significance of anti-nDNA antibodies in ANA-positive systemic lupus erythematosus: comparison of the Farr radioimmunoassay and the Crithidia luciliae immunofluorescent technique. *Chinese Journal of Microbiology and Immunology* 1985;**18**:15–24.
17 Okamura M, Kanayama Y, Amastu K, *et al*. Sigificance of enzyme linked immunosorbent assay (ELISA) for antibodies to double-stranded and single-stranded DNA in patients with lupus nephritis: correlation with severity of renal histology. *Ann Rheum Dis* 1993;**52**:14–20.
18 Clough JD, Chang RK. Effectiveness of testing for anti-DNA and the complement components iC3b, Bb, and C4 in the assessment of activity of systemic lupus erythematosus. *J Clin Lab Anal* 1990;**4**:268–73.
19 Gripenberg M, Helve T. Anti-DNA antibodies of IgA class in patients with systemic lupus erythematosus. *Rheumatol Int* 1986;**6**:53–5.
20 Avina-Zubieta JA, Galindo-Rodriguez G, Kwan-Yeung L, *et al*. Clinical evaluation of various selected ELISA kits for the detection of anti-DNA antibodies. *Lupus* 1995;**4**:370–4.
21 Ehrenstein MR, Swana M, Keeling D, *et al*. Anti-DNA antibodies in the primary antiphospholipid syndrome (PAPS) [see comments]. *Br J Rheumatol* 1993;**32**:362–5.
22 Preud'homme JI, Rochard E, Gouet D, *et al*. Isotypic distribution of anti-dsDNA antibodies: a diagnostic evaluation by ELISA. *Diagn Clin Immunol* 1988;**5**:256–61.
23 Halliday GM, Salaman MR, Seifert MH, *et al*. Evaluation of an ELISA system for determination of class-specific antibodies to native and denatured DNA in man. *Ann Rheum Dis* 1985;**44**:507–13.
24 Punsar S, Lee SR, LePage S, *et al*. Serial studies of IgG subclass and functional affinity of anti-dsDNA antibodies in systemic lupus erythematosis. *J Autoimmun* 1988;**1**:483–94.
25 Sabbaga J, Pankewycz OG, Lufft V, *et al*. Cross-reactivity distinguishes serum and nephritogenic anti-DNA antibodies in human lupus from their natural counterparts in normal serum. *J Autoimmun* 1990;**3**:215–35.
26 Winfield J, Faiferman I, Koffler D. Avidity of anti-DNA antibodies in serum and glomerular eluates from patients with SLE. *J Clin Invest* 1977;**59**:90–6.
27 Leon S, Green A, Ehrlich G, *et al*. Avidity of antibodies in SLE: relation to severity of renal involvement. *Arthritis Rheum* 1977;**20**:23–9.

28 Swaak AJG, Aarden LA, Statius van Eps LW, *et al*. Anti-dsDNA and complement profiles as prognostic guides in systemic lupus erythematosus. *Arthritis Rheum* 1979;**22**: 226–35.

29 Smeenk R, van der Lelij G, Swaak T, *et al*. Specificity in systemic lupus erythematosus of antibodies to double-stranded DNA measured with the polyethylene glycol precipitation assay. *Arthritis Rheum* 1982;**25**:631–8.

30 Albani S, Massa M, Vioila S, *et al*. Antibody reactivity against single stranded DNA of various species in normal children and in children with diffuse connective tissue diseases. *Autoimmunity* 1990;**8**:77–80.

31 Misra R, Malaviya AN, Kumar R, *et al*. Clinical relevance of the estimation of antibodies to single stranded DNA in SLE. *Indian J Med Res* 1988;**87**:463–7.

32 Koffler D, Agnello V, Winchester R. Occurrence of single-stranded DNA in serum of patients with SLE and other diseases. *J Clin Invest* 1973;**52**:198–4.

33 Werle E, Blazek M, Fiehn W. The clinical significance of measuring different anti-dsDNA antibodies by using the Farr assay, an enzyme immunoassay and a Crithidia luciliae immunofluorescence test. *Lupus* 1992;**1**:369–77.

34 James K, Meek G. Evaluation of commercial enzyme immunoassays compared to immunofluorescence and double diffusion for autoantibodies associated with autoimmune diseases. *Am J Clin Pathol* 1992;**97**:559–65.

35 Fisher-Smikle M, James OB. Diagnosis of systemic lupus erythematosus in Jamaica by Crithidia luciliae indirect immunofluorescence test. *Trans R Soc Trop Med Hyg* 1987; **81**:255–6.

36 Hodinka L, Meretey K, Jancso A, *et al*. Antibodies to native DNA in connective tissue disease. A comparison of radioimmunoassay, counterimmunoelectrophoresis and indirect immunofluorescence on Crithidia luciliae substrate. *Arch Immunol Ther Exp* 1979;**27**:641–6.

37 Monier JC, Sault C, Bringuier JP. Discrepancies between 2 procedures for dsDNA antibody detection: Farr test and indirect immunofluorescence on Crithidia luciliae. *J Clin Lab Immunol* 1988;**25**:149–52.

38 Tipping PG, Buchanon RRC, Riglar AG, *et al*. Detection of anti-DNA antibodies: a comparison between two Farr assays, Crithidia luciliae and a human chromosomal substrate assay. *Br J Rheumatol* 1988;**27**:206–10.

39 Huber O, Greenberg ML, Huber J. Complement-fixing anti-double-stranded DNA with the Crithidia method: a better indicator of active SLE than anti-DNA with the Farr method. *J Lab Clin Med* 1979;**93**:32–9.

40 Henderson T, Medsger TA, Jr, Sontheimer RD, *et al*. Specificity of the hydrochloric-acid-modified Crithidia luciliae immunofluorescence assay for detection of antibody to native DNA. *Diagn Clin Immunol* 1987;**5**:20–4.

41 Aarden L, de Groot E, Feltkamp T. Immunology of DNA: III. Crithidia luciliae, a simple substrate for the determination of anti-dsDNA with immunofluorescent technique. *Ann N Y Acad Sci* 1975;**254**:505–8.

42 Kumar V, Krasny S, Beutner EH. Specificity of the Crithidia luciliae method for detecting anti-DNA antibodies. Effect of absorption for lipoproteins. *Immunol Invest* 1985;**14**: 199–210.

43 Fortin PR, Abrahamowicz M, Danoff D. Small changes in outpatiens' lupus activity is better detected by clinical instruments than by laboratory tests. *J Rheumatol* 1995;**22**: 2078–83.

44 Petri M, Genovese M, Engle E, *et al*. Definition, incidence and clinical description of flare in systemic lupus erythematosus: a prospective cohort study. *Arthritis Rheum* 1991;**34**:937–44.

45 Bootsma H, Spronk P, Derksen R, *et al*. Prevention of relapses in systemic lupus erythematosus [published erratum appears in *Lancet* 1995;**346**:516]. *Lancet* 1995; **345**:1595–9.

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

47 ter Borg EJ, Horst G, Hummel EJ, *et al*. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. *Arthritis Rheum* 1990;**33**:634–43.

48 Esdaile JM, Abrahamowicz M, Joseph L, *et al*. Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus. Why some tests fail. *Arthritis Rheum* 1996;**39**:370–8.

49 Kallenberg CG, Bootsma H, Spronk PE, *et al*. Laboratory tests as predictors of flares in systemic lupus erythematosus: comment on the article by Esdaile *et al* [letter]. *Arthritis Rheum* 1997;**40**:393–4.

50 Jonsson H, Nived O, Strufelt G. Outcome in systemic lupus erythematosus: a prospective study of patients from a defined population. *Medicine* 1989;**68**:141–50.

51 Smeenk R, Brinkman K, van den Brink H, *et al*. Antibodies to DNA in patients with systemic lupus erythematosus. Their role in the diagnosis, the follow-up and the pathogenesis of the disease. *Clin Rheumatol* 1990;**9**(suppl 1):100–10.

52 Massa M, De Benedetti F, Pignatti P, *et al*. Anti-double stranded DNA, anti-histone, and anti-nucleosome IgG reactivities in children with systemic lupus erythematosus. *Clin Exp Rheumatol* 1994;**12**:219–25.

53 Swaak AJ, Groenwold J, Aarden LA, *et al*. Prognostic value of anti-dsDNA in SLE. *Ann Rheum Dis* 1982;**41**:388–95.

54 Clough JD, Barna BP, Danao-Camara TC, *et al*. Serological detection of disease activity in SLE. *Clin Biochem* 1992;**25**: 201–18.

55 Spronk PE, Limburg PC, Kallenberg CG. Serological markers of disease activity in systemic lupus erythematosus. *Lupus* 1995;**4**:86–94.

56 Gladman DD, Urowitz MB, Keystone EC. Serologically active clinically quiescent systemic lupus erythematosus: a discordance between clinical and serological features. *Am J Med* 1979;**34**:937–44.

57 Nossent JC, Huysen V, Smeenk RJ, *et al*. Low avidity antibodies to dsDNA as a diagnostic tool. *Ann Rheum Dis* 1989;**48**:748–52.

58 Smeenk R, Hylkema M. Detection of antibodies to DNA: a technical assessment. *Mol Biol Rep* 1992;**17**:71–9.

59 Miltenburg AMM, Roos A, Slegtenhorst L, *et al*. IgA anti-dsDNA antibodies in systemic lupus erythematosus: occurence, incidence and association with clinical and laboratory variables of disease activity. *J Rheumatol* 1993;**20**:53–8.

60 Sarvas H, Gripenberg M, Leirisalo-Repo M. Anti-DNA antibodies: the choice of assays for routine diagnostic work. *Acta Pathol Microbiol Immunol Scand* 1985;**93**:13–18.

61 Hahn B. Antibodies to DNA. *N Engl J Med* 1998;**338**:1359–68.

62 Heinzerling RH, Dziuba DS, Federyszyn HM, *et al*. Significance of levels of specific immunoglobulins to DNA in SLE patients' sera detected by solid phase radioimmunoassay. *J Invest Dermatol* 1979;**72**:55–8.

63 Yu CL, Huang MH, Tsai CY, *et al*. The reactivity of sera from patients with systemic lupus erythematosus to seven different species of single and double stranded deoxyribonucleic acids. *Clin Exp Rheumatol* 1996;**14**:137–44.

64 Rubin R, Josli F, Tan E. A solid-phase radioimmunoassay for anti-histone antibodies in human sera: comparison with immunofluorescence. *Scand J Immunol* 1982;**15**:63–70.

65 Aitkaci A, Monier J, Mamelle N. Enzyme-linked immunosorbent assay for anti-histone antibodies and their presence in SLE sera. *J Immunol Methods* 1981;**44**:311–22.

66 Rubin W, Waga S. Anti-histone antibodies in systemic lupus erythematosus. *J Rheumatol* 1997;**14**(suppl 3):118–26.

67 Molden D, Klipple G, Peebles C, *et al*. IgM anti-histone H3 antibodies associated with undifferentiated connective tissue disease. *Arthritis Rheum* 1986;**29**:39–46.

68 Sanchez-Guerrero J, Lew RA, Fossel AH, *et al*. Utility of anti-Sm, anti-RNP, anti-Ro/SS-A, and anti-La/SS-B (extractable nuclear antigens) detected by enzyme-linked immunosorbent assay for the diagnosis of systemic lupus erythematosus. *Arthritis Rheum* 1996;**39**:1055–61.

69 Gordon T, Mavrangelos C, McCluskey J. Restricted epitope recognition by precipitin-negative anti-La/SS-B positive sera. *Arthritis Rheum* 1992;**35**:663–6.

70 Garcia Lerma JG, Mendoza AZ, Ramos MJ, *et al*. Evaluation of recombinant Ro/SSA, La/SSB, Sm, and U1 RNP autoantigens in clinical diagnosis [see comments]. *J Clin Lab Anal* 1995;**9**:52–8.

71 Gaither KK, Fox OF, Yamagata H, *et al*. Implications of anti-Ro/Sjogren's syndrome antigen autoantibody in normal sera for autoimmunity. *J Clin Invest* 1987;**79**:841–6.

72 Slobbe RL, Pruijn GJM, Damen WGM, *et al*. Detection and occurence of the 60- and 52-kD Ro (SS-A) antigens and of autoantibodies against these proteins. *Clin Exp Immunol* 1991;**86**:99–105.

73 Clotet B, Guardia J, Pigrau C, *et al*. Incidence and clinical significance of anti-ENA antibodies in systemic lupus erythematosus. Estimation by counterimmunoelectrophoresis. *Scand J Rheumatol* 1984;**13**:15–20.

74 Lopez-Longo FJ, Monteagudo I, Gonzalez CM, *et al*. Systemic lupus erythematosus: clinical expression and anti-Ro/SSA response in patients with and without lesions of subacute cutaneous lupus erythematosus. *Lupus* 1997;**6**: 32–9.

75 Buyon JP, Winchester R. Congenital complete heart block. A human model of passively acquired autoimmune injury. *Arthritis Rheum* 1990;**33**:609–14.

76 Oshiro AC, Derbes SJ, Stopa AR, *et al*. Anti-Ro/SSA and anti-La/SSB antibodies associated with cardiac involvement in childhood systemic lupus erythematosus. *Ann Rheum Dis* 1997;**56**:272–4.

77 Harley J, Yamagata H, Reichlin M. Anti-La (SSB) antibody is present in some normal sera and is co-incident with Ro (SSA) precipitins in SLE. *J Rheumatol* 1984;**11**:309–14.

78 Meilof JF, Veldhoven CH, Swaak AJ, *et al*. Production of anti-Ro/SSA and anti-La/SSB autoantibodies is closely coordinated in systemic lupus erythematosus and independent of anti-dsDNA production. *J Autoimmun* 1997;**10**: 67–75.

79 Schofield RH, Zhang F, Kurien BT, *et al*. Development of the anti-Ro autoantibody response in a patient with systemic lupus erythematosus. *Arthritis Rheum* 1996;**39**:1664–8.

80 Pilkington C, Taylor PV, Silverman E, *et al*. Agalactosyl IgG and materno–fetal transmission of autoimmune neonatal lupus. *Rheumatol Int* 1996;**16**:89–94.

81 Franco H, Weston W, Peebles C, *et al*. Autoantibodies directed against Sicca syndrome antigens in the neonatal lupus syndrome. *J Am Acad Dermatol* 1981;**4**:67–72.

82 Sheth AP, Esterly NB, Ratoosh SL, *et al*. U1RNP positive neonatal lupus erythematosus: association with anti-La antibodies? *Br J Dermatol* 1995;**132**:520–6.

83 Maddison PJ, Skinner RP, Vlachoyiannopolous P, *et al*. Antibodies to nRNP, Sm, Ro(SSA) and La(SSB) detected by ELISA: their specificity and interrelations in connective tissue disease sera. *Clin Exp Immunol* 1985;**62**:337–45.

84 Pan LT, Tin SK, Boey ML, *et al*. The sensitivity and specificity of autoantibodies to the Sm antigen in the diagnosis of systemic lupus erythematosus. *Ann Acad Med Singapore* 1998;**27**:21–3.

85 Sirota P, Firer M, Schild K, *et al*. Increased anti-Sm antibodies in schizophrenic patients and their families. *Progress in Neuro-psychopharmacology and Biological Psychiatry* 1993;**17**:793–800.

86 Habets WJA, Hoet MH, Sillekens PTG, *et al*. Detection of autoantibodies in a quantitative ELISA using recombinant ribonucleoprotein antigens. *Clin Exp Immunol* 1989;**76**:172–7.

87 Guldner HH, Lakomek HJ, Bautz F. Anti-(U1)RNP and anti-Sm autoantibody profiles in patients with systemic rheumatic disease: differential detection of imunoglobulin G and M by immunoblotting. *Clin Immunol Immunopathol* 1986;**40**:532–8.

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

89 Molden DP, Suzuki H, Nakamura RM. Assays for Sm and RNP antibodies: pitfalls and technical considerations. *Diagn Immunol* 1985;**3**:24–8.

90 Vlachoyiannopoulos PG, Guialis A, Tzioufas G, *et al*. Predominance of IgM anti-U1RNP antibodies in patients with systemic lupus erythematosus. *Br J Rheumatol* 1996;**35**:534–41.

91 Snowden N, Hay E, Holt PJL, *et al*. Clinical course of patients with anti-RNP antibodies. *J Rheumatol* 1993;**20**:1256–8.

92 Calderon J, Rodriguez-Valverde V, Sanchez Andrade S, *et al*. Clinical profiles of patients with antibodies to nuclear ribonucleoprotein. *Clin Rheumatol* 1984;**3**:483–92.

93 Tikly M, Burgin S, Mohanal P, *et al*. Autoantibodies in black South Africans with systemic lupus erythematosus: spectrum and clinical associations. *Clin Rheumatol* 1996;**15**:261–5.

94 Hirohata S, Kosaka M. Association of anti-Sm antibodies with organic brain syndrome secondary to systemic lupus erythematosus [letter]. *Lancet* 1994;**343**:796.

95 Swaak AJ, Nossent JC, Bronsveld W, *et al*. Systemic lupus erythematosus. II. Observations on the occurrence of exacerbations in the disease course: Dutch experience with 110 patients studied prospectively. *Ann Rheum Dis* 1989;**48**:455–60.

96 Satoh M, Yamagata H, Watanabe F, *et al*. Development of anti-Sm and anti-DNA antibodies followed by clinical manifestation of systemic lupus erythematosus in an elderly woman with long-standing Sjogren's syndrome. *Lupus* 1995;**4**:63–5.

97 Hoet RM, Koornneef I, de Rooij DJ, *et al*. Changes in anti-U1 RNA antibody levels correlate with disease activity in patients with systemic lupus erythematosus overlap syndrome. *Arthritis Rheum* 1992;**35**:1202–10.

98 ter Borg E, Horst G, Limburg P, *et al*. Shifts of anti-Sm specific antibodies in patients with systemic lupus erythematosus: analysis by countercurrent-immunoelectrophoresis, immunoblotting and RNA-immunoprecipitation. *J Autoimmun* 1991;**4**:155–64.

99 Yashuma M, Takasaki Y, Matsumoto K, *et al*. Clinical significance of IgG anti-Sm antibodies in patients with SLE. *J Rheumatol* 1990;**17**:469–75.

100 ter Borg EJ, Horst G, Limburg PC, *et al*. Changes in levels of antibodies against the 70 kDa and a polypeptides of the U1RNP complex in relation to exacerbations of systemic lupus erythematosus. *J Rheumatol* 1991;**18**:363–7.

101 Habets WJ, de Rooij DJ, Holt MH, *et al*. Quantitation of anti-RNP and anti-Sm antibodies in MCTD and SLE by immunoblotting. *Clin Exp Immunol* 1985;**59**:457–66.

102 Arnett FC, Reveille JD, Moutsopolous HM, *et al*. Ribosomal P autoantibodies in systemic lupus erythematosus. Frequencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum* 1996;**39**:1833–9.

103 Isshi K, Hirohata S. Association of anti-ribosomal P protein antibodies with neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum* 1996;**39**:1483–90.

104 Watanabe T, Sato T, Uchiumi T, *et al*. Neuropsychiatric manifestations in patients with systemic lupus erythematosus: diagnostic and predictive value of longitudinal examination of anti-ribosomal P antibody [see comments]. *Lupus* 1996;**5**:178–83.

105 Agius MA, Chan JW, Chung S, *et al*. Role of antiribosomal P protein antibodies in the diagnosis of lupus isolated to the central nervous system. *Arch Neurol* 1997;**54**:862–4.

106 West SG, Emlen W, Wener MH, *et al*. Neuropsychiatric lupus erythematosus: a 10-year prospective study on the value of diagnostic tests. *Am J Med* 1995;**99**:153–63.

107 Iverson GL. Are antibodies to ribosomal P proteins a clinically useful predictor of neuropsychiatric manifestations in patients with systemic lupus erythematosus. *Lupus* 1996;**5**:634–5.

108 Press J, Palayew K, Laxer RM, *et al*. Antiribosomal P antibodies in pediatric patients with systemic lupus erythematosus and psychosis. *Arthritis Rheum* 1996;**39**:671–6.

109 Sato T, Uchiumi T, Ozawa T, *et al*. Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. *J Rheumatol* 1991;**18**:1681–4.

110 Long AA, Denburg SD, Carbotte RM, *et al*. Serum lymphocytotoxic antibodies and neurocognitive function in systemic lupus erythematosus. *Ann Rheum Dis* 1990;**49**:249–53.

111 Escalante A, Brey RL, Mitchell BDJ, *et al*. Accuracy of anticardiolipin antibodies in identifying a history of thrombosis among patients with systemic lupus erythematosus. *Am J Med* 1995;**98**:559–65.

112 Ghirardello A, Doria A, Ruffatti A, *et al*. Antiphospholipid antibodies (aPL) in systemic lupus erythematosus. Are they specific tools for the diagnosis of aPL syndrome [see comments]? *Ann Rheum Dis* 1994;**53**:140–2.

113 Gulko PS, Reveille JD, Koopman WJ, *et al*. Anticardiolipin antibodies in systemic lupus erythematosus: clinical correlates, HLA associations, and impact on survival. *J Rheumatol* 1993;**20**:1684–93.

114 Merkel PA, Chang Y, Pierangeli SS, *et al*. The prevalence and clinical associations of anticardiolipin antibodies in a large inception cohort of patients with connective tissue diseases. *Am J Med* 1996;**101**:576–83.

115 Day HM, Thiagarajan P, Ahn C, *et al*. Autoantibodies to beta2-glycoprotein I in systemic lupus erythematosus and primary antiphospholipid antibody syndrome: clinical correlations in comparison with other antiphospholipid antibody tests. *J Rheumatol* 1998;**25**:667–74.

116 Abu-Shakra M, Urowitz MB, Gladman DD, *et al*. The significance of anticardiolipin antibodies in patients with lupus nephritis. *Lupus* 1996;**5**:70–3.

117 Bhandari S, Harnden P, Brownjohn AM, *et al*. Association of anticardiolipin antibodies with intraglomerular thrombi and renal dysfunction in lupus nephritis. *Q J Med* 1998;**91**:401–9.

118 Ogasawara M, Aoki K, Matsuura E, *et al*. Anti-beta 2 glycoprotein I antibodies and lupus anticoagulant in patients with recurrent pregnancy loss: prevalence and clinical significance. *Lupus* 1996;**5**:587–92.

119 Tomer Y, Viegas OA, Swissa M, *et al*. Levels of lupus autoantibodies in pregnant SLE patients: correlations with disease activity and pregnancy outcome. *Clin Exp Rheumatol* 1996;**14**:275–80.

120 Buttgereit F, Grunewald T, Schuler-Maue W, *et al*. Value of anticardiolipin antibodies for monitoring disease activity in systemic lupus erythematosus and other rheumatic diseases. *Clin Rheumatol* 1997;**16**:562–9.

121 Cooper RC, Klemp P, Stipp CJ, *et al*. The relationship of anticardiolipin antibodies to disease activity in systemic lupus erythematosus. *Br J Rheumatol* 1989;**28**:379–82.

122 Tsutsumi A, Matsuura E, Ichikawa K, *et al*. Antibodies to beta 2-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus [see comments]. *Arthritis Rheum* 1996;**39**:1466–74.

123 Jennings I, Kitchen S, Woods TA, *et al*. Potentially clinically important inaccuracies in testing for the lupus anticoagulant: an analysis of results from three surveys of the UK national external quality assessment scheme (NEQAS) for blood coagulation. *Thromb Haemost* 1997;**77**:934–7.

124 Becker GJ, Waldburger M, Hughes GR, *et al*. Value of serum C-reactive protein measurement in the investigation of fever in systemic lupus erythematosus. *Ann Rheum Dis* 1980;**39**:50–2.

125 Hind C, Ng SC, Feng PH. Serum C-reactive protein measurements in the detection of intercurrent infection in oriental pateints with SLE. *Ann Rheum Dis* 1985;**44**:260–1.

126 ter Borg E, Horst G, Limburg P, *et al*. C-reactive protein levels during exacerbations and infections in SLE: a prospective longitudinal study. *J Rheumatol* 1991;**17**:1642–8.

127 Zein N, Ganuza C, Kushner I. Significance of C-reactive protein elevation in patients with SLE. *Arthritis Rheum* 1979;**22**:7–12.

128 Aderka D, Wysenbeek A, Engelmann H, *et al*. Correlation between serum levels of soluble tumor necrosis factor receptor and disease activity in systemic lupus erythematosus. *Arthritis Rheum* 1993;**36**:1111–20.

129 Wolf RE, Brelsford WG. Soluble interleukin-2 receptors in systemic lupus erythematosus. *Arthritis Rheum* 1988;**31**:729–35.

130 Inman R, Fong J, Pussel B, *et al*. The C1q binding assay in SLE: discordance with disease activity. *Arthritis Rheum* 1980;**23**:1282–6.

131 Abrass CK, Nies KM, Louie JS, *et al*. Correlation and predictive accuracy of circulating immune complexes with disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 1980;**23**:273–82.

132 Buyon JP, Tamerius J, Belmont HM, *et al*. Assessment of disease activity and impending flare in patients with systemic lupus erythematosus. Comparison of the use of complement split products and conventional measurements of complement. *Arthritis Rheum* 1992;**35**:1028–37.

133 Valentijn RM, van Overhagen H, Hazevoet HM, *et al*. The value of complement and immune complex determinations in monitoring disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 1985;**28**:904–13.

134 Esdaile JM, Joseph L, Abrahamowicz M, *et al*. Routine immunologic tests in systemic lupus erythematosus: is there a need for more studies? *J Rheumatol* 1996;**23**:1891–6.

135 Weinstein A, Bordwell B, Stone B, *et al*. Antibodies to native DNA and serum complement (C3) levels. Application to diagnosis and classification of systemic lupus erythematosus. *Am J Med* 1983;**74**:206–16.

136 Harkiss GD, Hazleman BL, Brown DL. A longitudinal study of circulating immune complexes, DNA antibodies and complement in patients with systemic lupus erythematosus: an analysis of their relationship to disease activity. *J Clin Lab Immunol* 1979;**2**:275–83.

137 Uko G, Christiansen FT, Dawkins RL. Serum C4 concentration in the monitoring of systemic lupus erythematosus: requirement for C4 allotyping. *Rheumatol Int* 1986;**6**:111–14.

138 Baqi N, Moazami S, Singh A, *et al*. Lupus nephritis in children: a longitudinal study of prognostic factors and therapy. *J Am Soc Nephrol* 1996;**7**:924–9.

139 Sullivan KE, Wisnieski JJ, Winkelstein JA, *et al*. Serum complement determinations in patients with quiescent systemic lupus erythematosus. *J Rheumatol* 1996;**23**:2063–7.

140 Wild G, Watkins J, Ward AM, *et al*. C4a anaphylatoxin levels as an indicator of disease activity in systemic lupus erythematosus. *Clin Exp Immunol* 1990;**80**:167–70.

141 Negoro N, Okamura M, Takada T, *et al*. Clinical significance of iC3b neoantigen expression in plasma of patients with systemic lupus erythematosus. *Arthritis Rheum* 1989;**32**:1233–42.

142 Manzi S, Rairie JE, Carpenter AB, *et al*. Sensitivity and specificity of plasma and urine complement split products as indicators of lupus disease activity. *Arthritis Rheum* 1996;**39**:1178–88.

143 Gawryl MS, Chudwin DS, Langlois PF, *et al*. The terminal complement complex, C5b–9, a marker of disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum* 1988;**31**:188–95.

144 Sjoholm AG, Martensson U, Sturfelt G. Serial analysis of autoantibody responses to the collagen-like region of C1q, collagen type II, and double-stranded DNA in patients with systemic lupus erythematosus. *J Rheumatol* 1997;**24**:871–8.

145 Gunnarsson I, Ronnelid J, Huang YH, *et al*. Association between ongoing anti-C1q antibody production in peripheral blood and proliferative nephritis in patients with active systemic lupus erythematosus. *Br J Rheumatol* 1997;**36**:32–7.

146 Siegret C, Daha M, Westedt ML, *et al*. IgG autoantibodies against C1q are correlated with nephritis, hypocomplementaemia, and dsDNA antibodies in SLE. *J Rheumatol* 1991;**18**:230–34

147 Chan TM, Cheng IKP. A prospective study on anti-endothelial cell antibodies in patients with systemic lupus erythematosus. *Clin Immunol Immunopathol* 1996;**78**:41–6.

148 Scnabel A, Csernok E, Isenberg DA, *et al*. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Prevalence, specificities, and clinical significance. *Arthritis Rheum* 1995;**38**:633–7.

149 Isenberg DA, Garton M, Reichlin MW, *et al*. Long-term follow-up of autoantibody profiles in black female lupus patients and clinical comparison with Caucasian and Asian patients. *Br J Rheumatol* 1997;**36**:229–33.