

Indications and Interpretation of Serological Tests in the Context of Kidney Disease

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General Remarks

Immunological tests are of limited value in the diagnosis of kidney diseases, since

- clinical picture;
- other laboratory tests (e.g. proteinuria, serum creatinine, GFR etc);
- renal histology (kidney biopsy) have a more important role in establishing both the diagnosis and prognosis of kidney diseases, as well as in monitoring disease activity

Notice to Clinicians

- Be familiar with the limits and consequences of the test
- Majority of the tests are not sensitive enough to detect all cases
 - e.g. a test with 70% sensitivity will not detect 30% of cases
 - similarly, the specificity of the tests is well below 100%; e.g. a 90% specific test will result in 10% false-positive cases.

INFORMATIONAL INDICES

Sensitivity =
$$\frac{\text{No. of patients with a positive test}}{\text{Total No. of patients with the disease}}$$

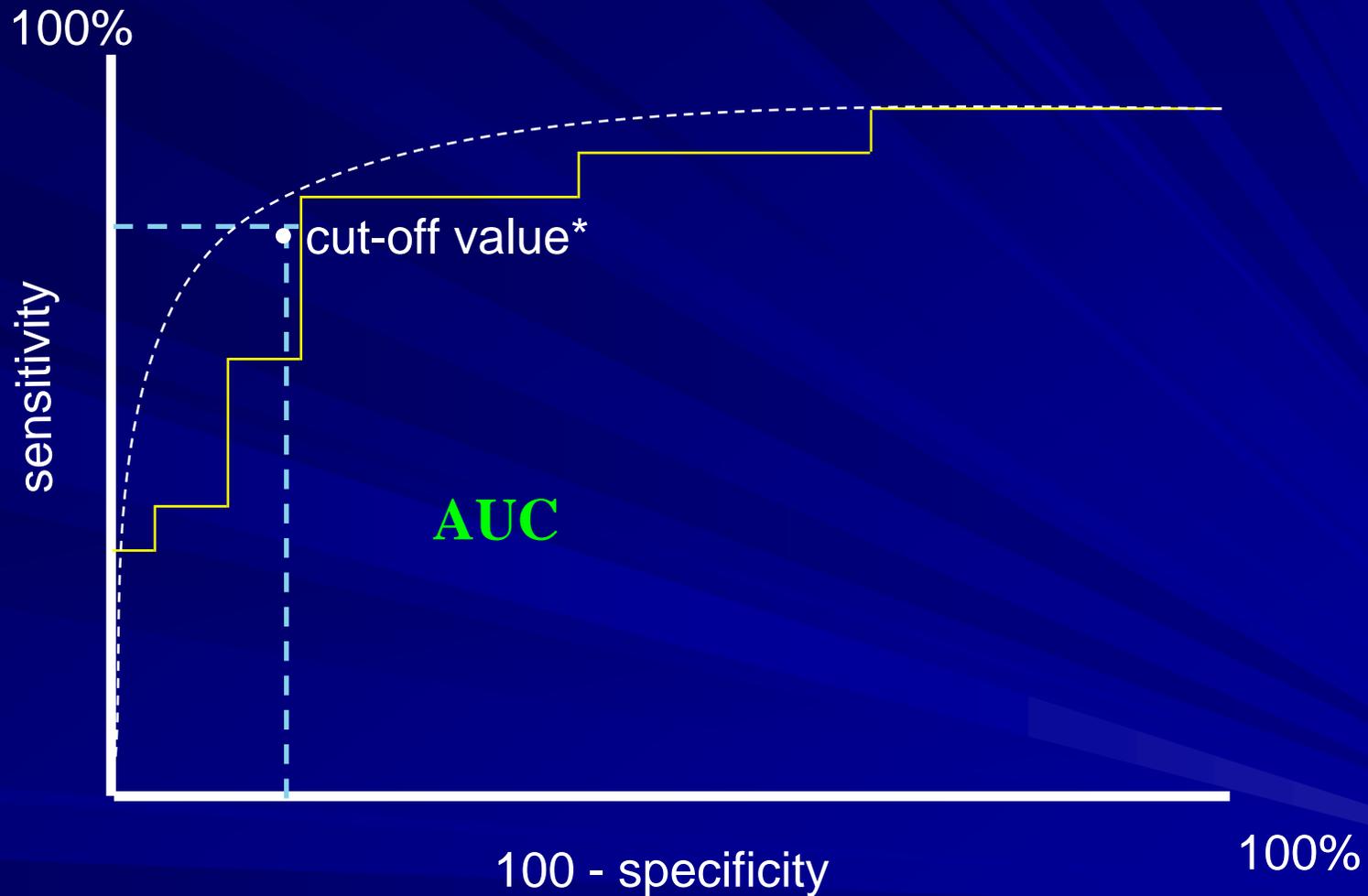
Specificity =
$$\frac{\text{No. of patients with a negative test}}{\text{Total No. of patients without the disease}}$$

Positive predictive value (PPV) = The probability that a disease is present if a certain test is positive

Negative predictive value (NPV) = The probability that a disease is not present if a certain test is negative

Receiver Operating Characteristic (ROC) curve

(relation between specificity and sensitivity)



* upper (lower) limit of normal (= mean of „healthy individuals” +/- 2SD)

The effect of cut-off value on sensitivity and specificity

<u>Test</u>	<u>Cut-off</u>	<u>Specificity</u>	<u>Sensitivity</u>
Anti-DNA	56 U/ml	85%	57%
	35	79%	70%
	26	50%	77%

Immunological Tests („immunoserology“)

- Antibodies – autoantibodies
- Complement system (complement components, activation products)
- Serum/plasma proteins (M component(s), cryoglobulins, immunoglobulins)
- Cells of the immune system (CD markers)
- Inflammatory mediators (CRP, soluble IL-2 receptor, neopterin, etc)

The Role of Autoantibody Tests

Antibody tests help differentiate

1. Secondary nephritis due to systemic autoimmune diseases:
 - Systemic lupus erythematosus (SLE)
 - Sjögren's syndrome (acute/chronic interstitial nephritis)
 - Rheumatoid arthritis (RA) – most common is secondary amyloidosis
 - Scleroderma
 - Mixed connective tissue disease (MCTD)
 - Systemic vasculitis (e.g. Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, Behçet's disease, Schönlein Henoch purpura)
2. Secondary nephritis due to virus-induced cryoglobulinaemia
3. Glomerular basement membrane (GBM) antibody-induced nephritis
4. Vascular kidney disease due to antiphospholipid syndrome (APS)

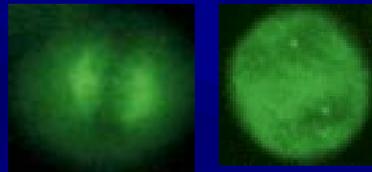
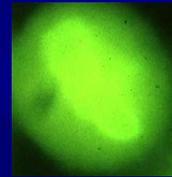
Antinuclear antibody (ANA)

Target: nuclear antigens - collective name of many antibodies of various specificity

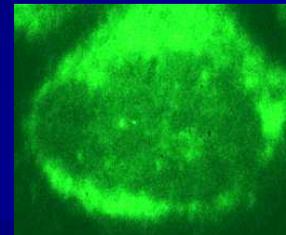
Determination: by indirect immunofluorescence (IIF) or enzyme immunoassay (EIA/ELISA)

Why IIF?

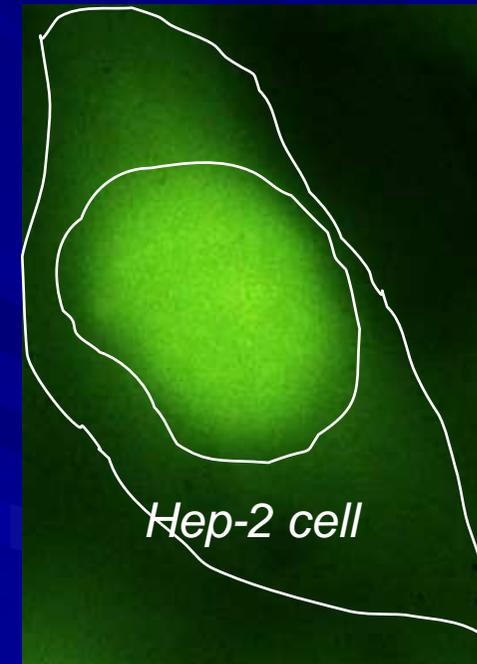
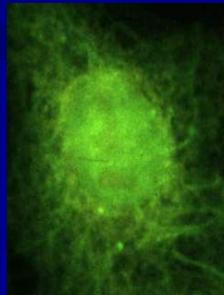
- ANA titre ($\geq 1:160$)
- chromatin staining (e.g. anti-centromere)
- staining of mitotic proteins



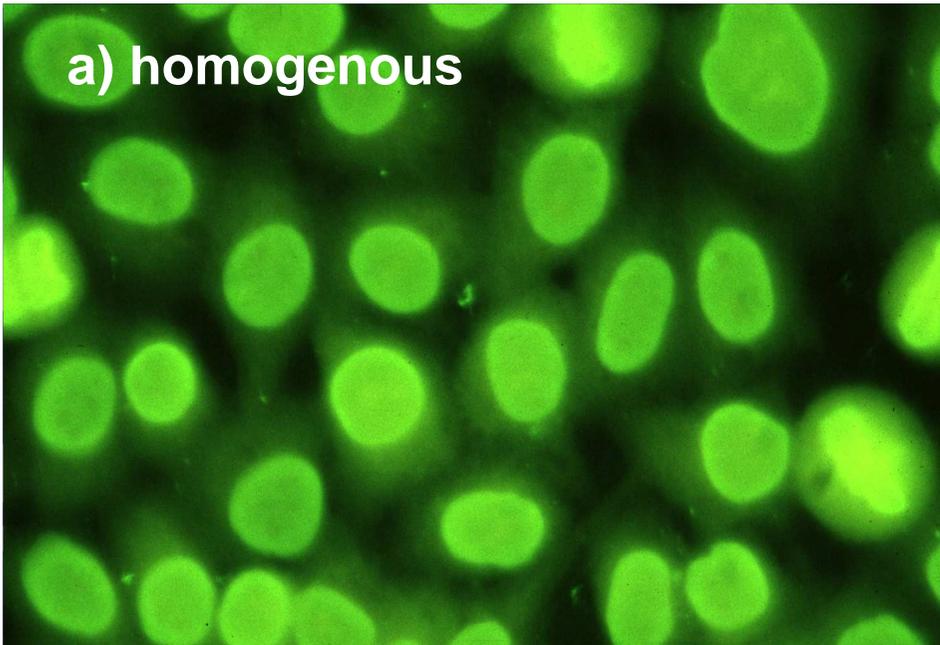
- ANA pattern
- special ANA types (e.g. PCNA)
- cytoplasm staining (e.g. ribosomal)



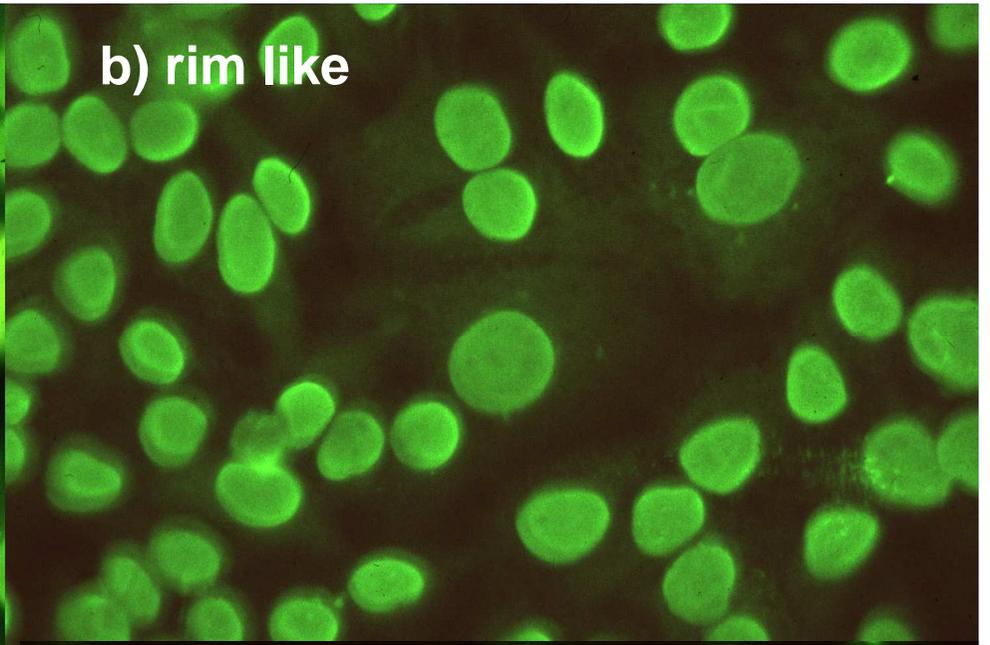
- cytoskeleton staining



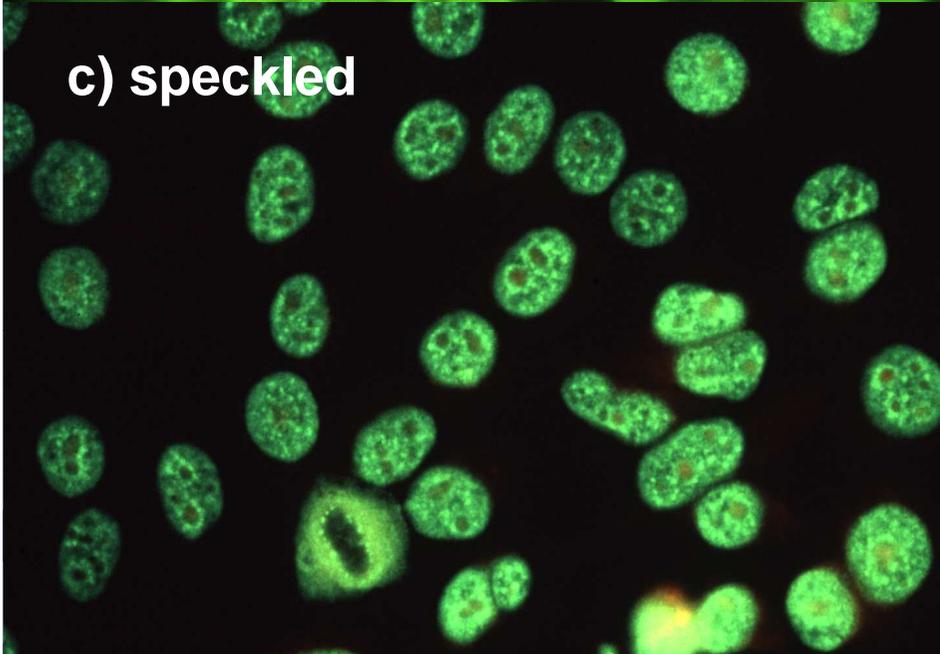
a) homogenous



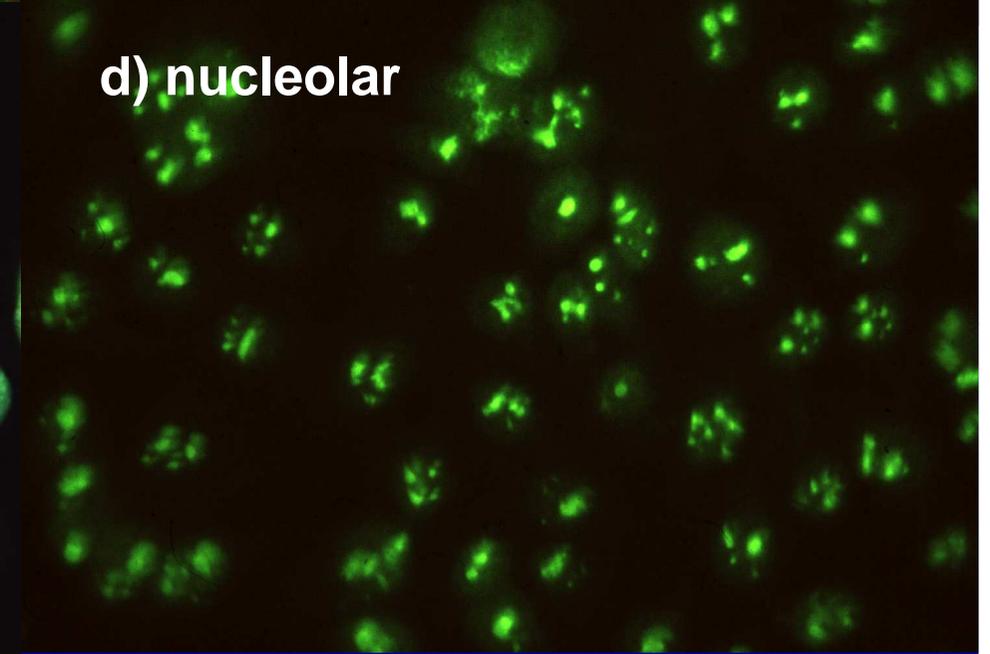
b) rim like



c) speckled



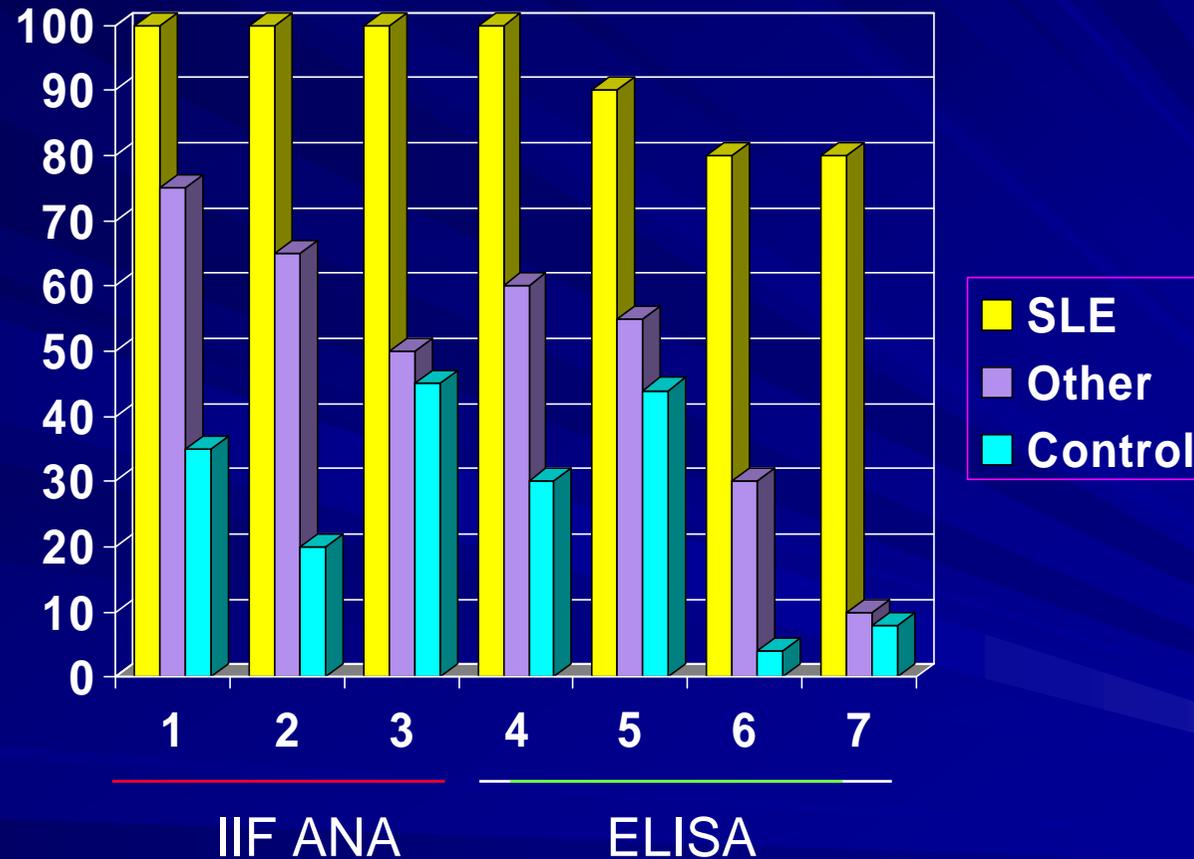
d) nucleolar



ANA patterns

Positive ANA test in SLE, other autoimmune diseases, and healthy controls

% positive

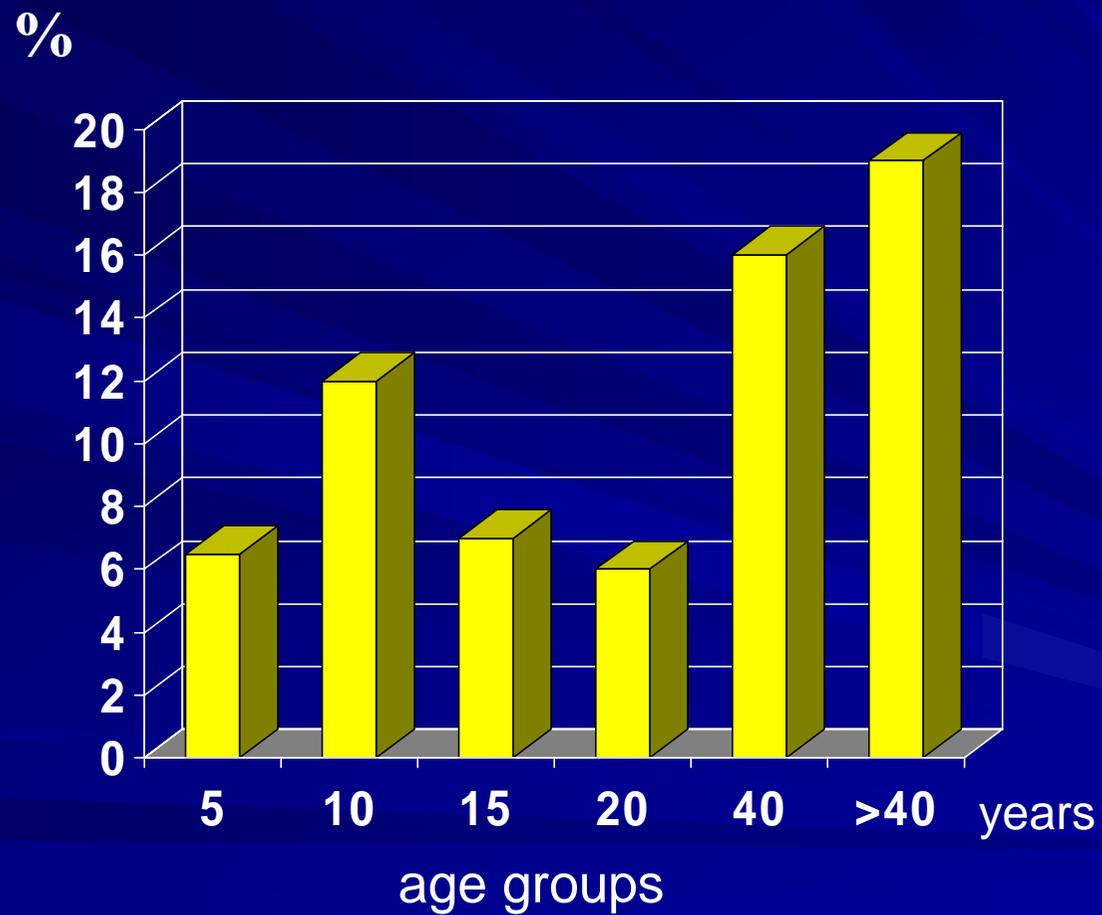


Emlen & Neill, Arthritis Rheum 40:1612, 1997

Diagnostic role: ANA is frequently positive in systemic autoimmune diseases. ANA test should be performed in case of suspicion of systemic autoimmune disease. Diagnostic criterium in SLE, frequently positive in Sjögren's syndrome, mixed connective disease, scleroderma and others. Both specificity and sensitivity are not too high; a positive ANA test may be found in neoplasms, infections and other diseases:

SLE	80-100%
Drug-induced LE	70%
MCTD	100%
Sjögren's syndrome	70%
Felty syndrome	70-100%
Scleroderma	75-85%
Myositis	30%
RA	15-30%
Chronic hepatitis	40-60%
Pulmonary fibrosis	20%
Tuberculosis	50%
Neoplasms	10%
Control	<5%

ANA positivity in healthy children and adults (IIF HEp-2, at 1:32 titre)



Craig et al, J Rheumatol 26:914, 1999

ANA includes many antibodies:

Anti-(double-stranded/native) DNA antibody

Histone antibody

Centromere antibody

ENA (antibodies against extractable nuclear antigens)

PCNA antibody

Ku antibody

Chromatin antibodies

and many others, e.g. coilin, DEK, single-stranded-DNA,
IFI 16, Mi-2, nucleosoma, nucleolar antibodies

Type of ANA should be determined if the ANA test is positive

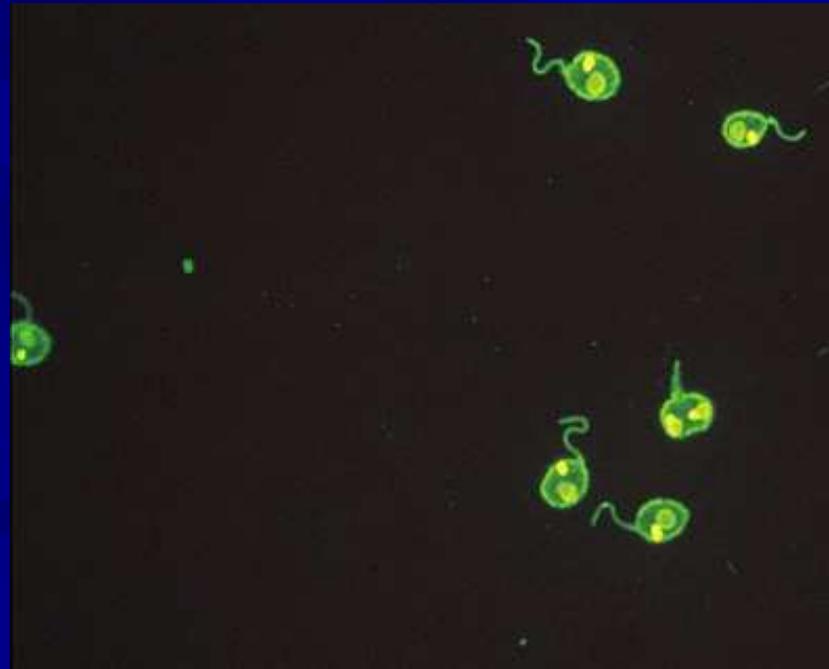
Anti-(native) DNA antibody

Target: double-strand DNA

Determination: ELISA; confirmation by Crithidia test or Western blot (false positive test may be due to presence of high titre single-strand DNA)

Diagnostic role: required for the diagnosis of SLE; also for monitoring disease (lupus nephritis) activity. A negative test excludes active SLE.

Crithidia luciliae
test



Histone antibody

Target: histones (H1, H2, H3, H4) in nucleosomes

Determination: ELISA

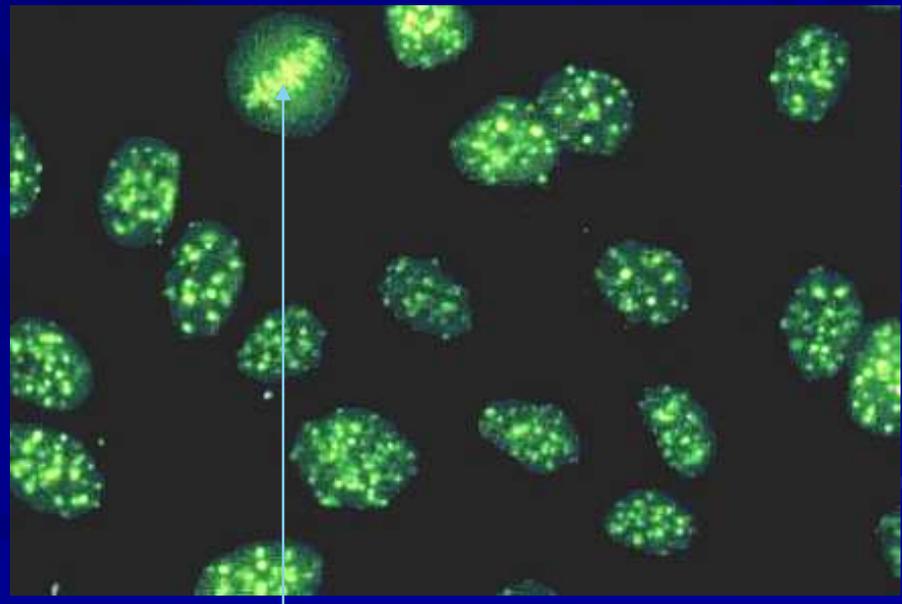
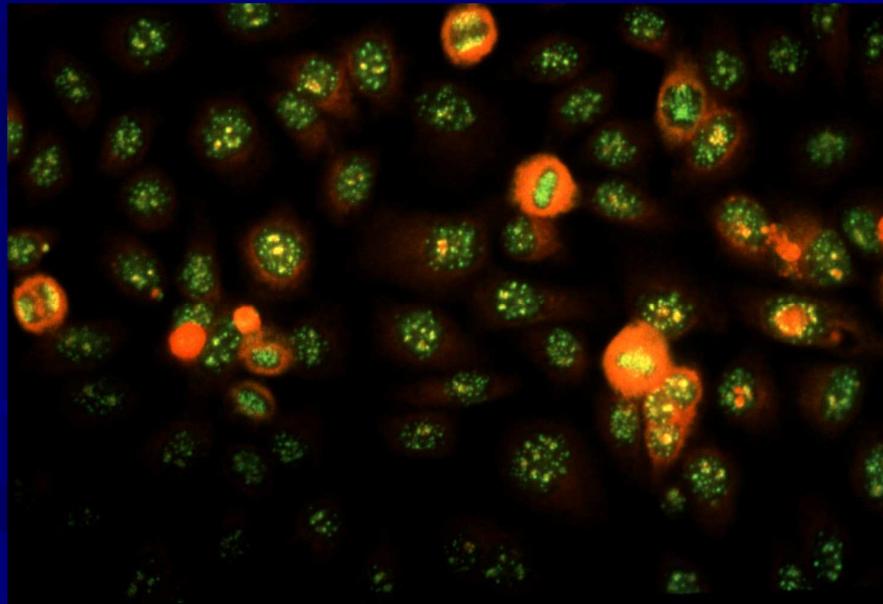
Diagnostic role: histone antibodies may be present in many systemic autoimmune diseases, e.g. in SLE, drug-induced lupus, RA, juvenile idiopathic arthritis (JIA), systemic scleroderma, etc. Diagnostic significance: diagnosis of drug-induced lupus.

Centromere antibody:

Target: centromere (80 kD) protein

Determination: indirect immunofluorescence, ELISA

Diagnostic role: diagnosis and differential-diagnosis of scleroderma (positive in CREST syndrome, a limited form of scleroderma, not involving kidneys)



Special chromatin pattern

Antibodies to Extractable Nuclear Antigens (ENA)

A group of nuclear and non-nuclear antigens traditionally named as ENA:

- SS-A (Ro) and SS-B (La)
- Scl-70
- Jo-1
- RNP (U1-RNP)
- Sm
- Others: PCNA, Ku, MI-2, PMScI, PM-1.

SS-A (Ro) & SS-B (La) antibodies

Target: non-histone nuclear proteins

Determination: ELISA

Diagnostic role: diagnostic criterium for primary or secondary Sjögren's syndrome (SS), frequently positive in SLE, subacute cutaneous lupus (SCLE) and neonatal lupus. SS-A and B antibodies usually occur together.

Scl-70 antibody

Target: DNA topoisomerase I

Determination: ELISA

Diagnostic role: positive in 50-70% of systemic forms of scleroderma (PSS); indicate a poor prognosis. Some patients with 'primary' Raynaud's syndrome are positive; they have a high PSS risk

Jo-1 antibody

Target: aminoacyl tRNA synthetase

Determination: ELISA

Diagnostic role: positive in certain forms of myositis (polymyositis and/or dermatomyositis); such cases are also called anti-synthetase syndrome. Can be used for diagnosis/classification of myositis, and fibrosing alveolitis.

(U1)-RNP antibody

Target: various nucleoproteins

Determination: ELISA

Diagnostic role: in MCTD diagnostic criterium, frequently positive in SLE, less frequently in PSS.

Sm antibody

Target: 29 kD nucleoprotein

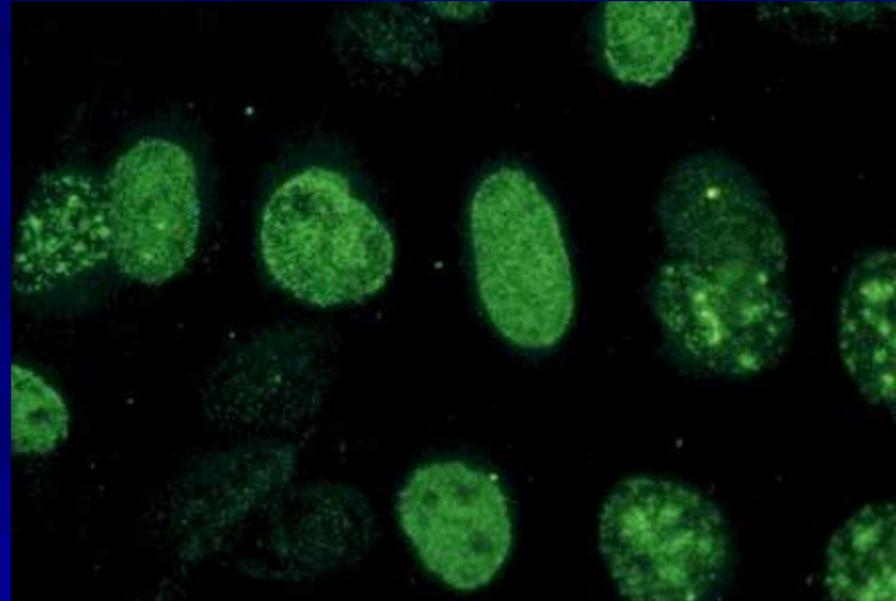
Determinaton: ELISA

Diagnostic role: in SLE diagnostic and prognostic marker (central nervous system (CNS), kidney, skin involvement).

PCNA („proliferating cell nuclear antigen”) antibody:

specific for SLE; PCNA+ patients display renal and CNS involvement. Diagnostic significance: SLE (in particular if anti-DNA and Sm negative).

Typical nuclear staining pattern in PCNA



Ku antibody: in polymyositis/scleroderma overlap syndrome.

Ribosomal protein antibodies: in systemic autoimmune diseases, in particular in SLE.

Rheumatoid factor (RF)

Target: IgG molecule

Determination: turbidimetry (IgM)

Diagnostic role: classification criterium in RA, frequently positive in Sjögren's syndrome and in cryoglobulinaemia. Both specificity and sensitivity are low (frequently negative in RA, and positive in many other autoimmune diseases including infections (e.g. poststreptococcal glomerulonephritis):

RA	50-90
SLE	15-35
Sjögren's syndrome	75-95
scleroderma	20-30
Cryoglobulinaemia	40-100
MCTD	50-60
Viral infections	15-65
Endocarditis	25-50
Liver cirrhosis	15-40
Sarcoidosis	3-33
Pulmonary fibrosis	10-50
Silicosis	30-50
Neoplasms	5-25
Control	<5

Antibody to C1q

Target: C1q complement component

Determination: ELISA

Diagnostic role: positive in hypocomplementaemic urticaria vasculitis (HUV). Positive in 17-58% of SLE cases (mostly in lupus nephritis). Positive in 88% of membranoproliferative glomerulonephritis (MPGN), and also in poststreptococcal glomerulonephritis

C3 Nephritic Factor

Target: activated factor B, a constituent of the C3 convertase, C3b,Bb.

Determination: haemolytic assay

Diagnostic role: present in membranoproliferative glomerulonephritis Type II (dense deposit disease), but may also be present in Type I and III; the diagnostic significance of this test is uncertain (performed only rarely)

Antiphospholipid antibodies:

Target: various phospholipids and phospholipid-binding glycoproteins (beta2-glycoprotein I).

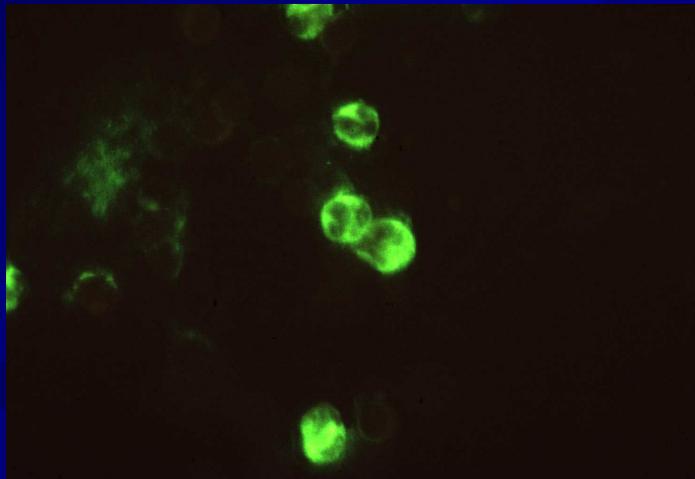
Determination: Antibodies against cardiolipin, phosphatidyl serine, β 2-glycoprotein I and prothrombin are measured by ELISA, while lupus anticoagulant (LA) is measured in a functional (clotting) assay.

Diagnostic significance: Diagnostic criterium (anti-cardiolipin, and anti- β 2GPI antibody and/or lupus anticoagulant) in primary and secondary antiphospholipid syndrome. May be positive after infections (this is why a positive test should be repeated after 12 weeks)

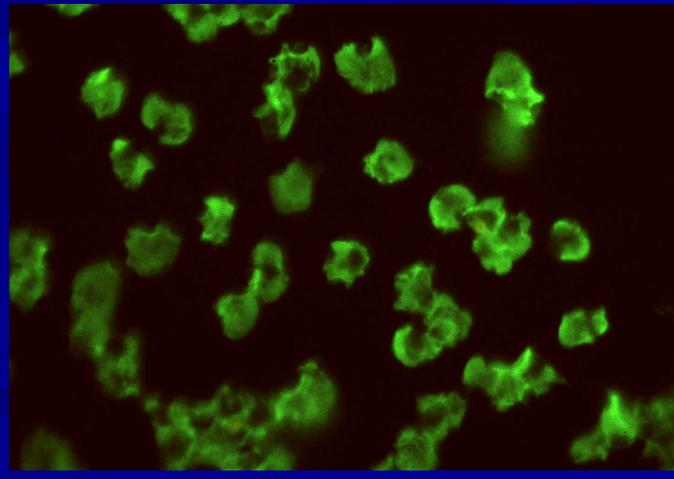
Anti-neutrophil cytoplasmic antibodies (ANCA)

Target: various cytoplasmic antigens of neutrophil granulocytes: proteinase 3 (PR3), myeloperoxidase (MPO), elastase, lactoferrin, bactericidal permeability increasing protein (BPI). There are two staining patterns: cytoplasmic (c) and perinuclear (p). In most cases c indicates PR3, and p indicates MPO.

Determination: screening: indirect immunofluorescence; in case of positivity, a further ELISA confirmatory test is required



c-ANCA



p-ANCA

Indication of ANCA screening: ANCA positive vasculitis group (Wegener's granulomatosis, microscopic polyangiitis), differential-diagnosis of nephritis; inflammatory bowel diseases (IBD), primary sclerosing cholangitis, haemorrhagic alveolitis.

Proteinase 3 (PR3) antibody: highly specific for Wegener's granulomatosis (>95%), even for atypic forms (such as atypic neuritis and necrotising nephritis).

Myeloperoxidase (MPO) antibody: Positive in microscopic polyangiitis (60-80%), in some focal necrotising glomerulonephritis, rapid progression glomerulonephritis (RPGN), rarely in Wegener's granulomatosis, Churg-Strauss syndrome; Goodpasture syndrome, systemic autoimmune diseases.

The role of ANCA tests in monitoring is debated.

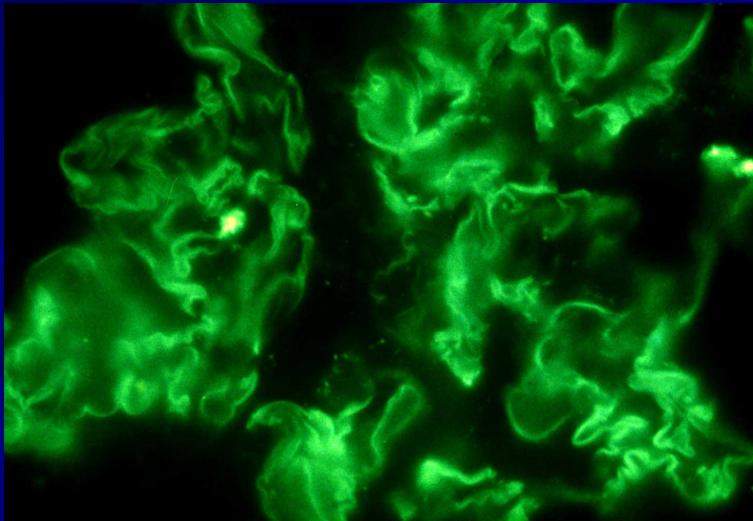
GBM antibody

Anti-GBM disease can present as a disease limited to RPGN or as the classic pulmonary-renal syndrome, known as Goodpasture's syndrome.

Target: glomerular basement membrane (type IV collagen $\alpha 3$ chain)

Determination: ELISA (formerly: IIF in monkey/human kidney)

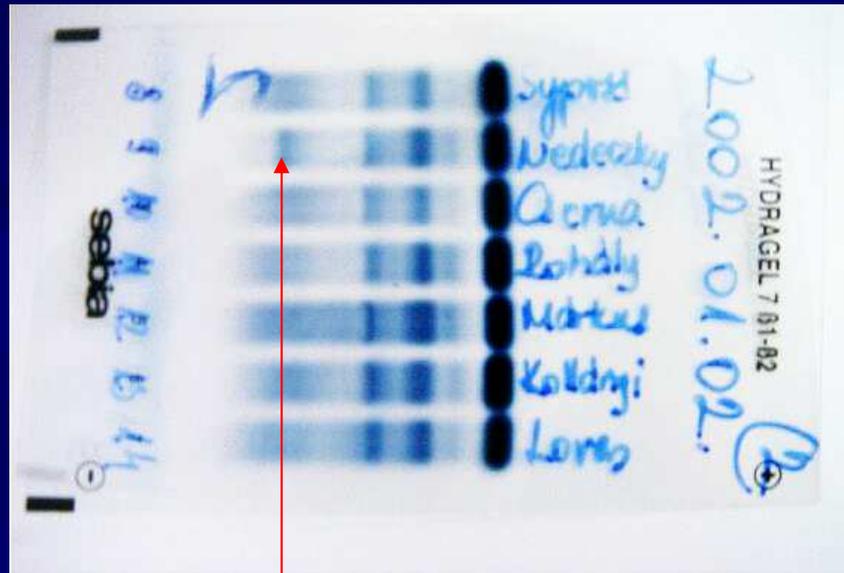
Diagnostic role: confirmation of diagnosis obtained by renal biopsy



GBM antibody as seen by indirect immunofluorescence

Serum/plasma protein assays

a) Determination of M component (in case of amyloidosis):
protein electrophoresis, and immunofixation



agarose gel electrophoresis

M component



immunofixation
(IgG lambda)

b) Serum immunoglobulin concentrations: determined by turbidimetry; diagnostic role in case of M component. Elevated IgA1 levels and IgA-containing immune complexes are of doubtful diagnostic value in IgA nephropathy

c) Cryoglobulin assay: required in primary (essential) mixed cryoglobulinaemia. Cryoprecipitate should be analysed, it may be monoclonal or mixed.

This test may be positive in many autoimmune diseases (due to immune complexes in plasma).

To rule out secondary cryoglobulinaemia, HCV antibody assay is mandatory.

A prerequisite of reliable result: to draw blood at 37°C and to store at this temperature until clot formation (for cca 2 hours).

Assay of complement components (C3, C4):

Determination: haemolytic assay, immunodiffusion and turbidimetry

Diagnostic role: For screening of complement deficiencies; monitoring of immune complex diseases (e.g. SLE, acute glomerulonephritis, and certain types of chronic glomerulonephritis)

– in active stage their level decreases, while in inactive stage they tend to normalize.

Complement deteriorates at room temperature in serum or fluid; samples should be brought to the laboratory as soon as possible. Separate serum from clot and freeze at -70°C until test is performed. Both blood and fluid must be processed and frozen within 2 hours after specimen collection. Failure to process the specimen in this manner may lead to falsely decreased functional activity levels.

Low C3 levels:

- Severe recurrent bacterial infections due to C3 homozygous deficiency
- Absence of C3b inactivator factor
- Acute poststreptococcal glomerulonephritis
- Immune complex diseases
- Active SLE
- Membranoproliferative glomerulonephritis (MPGN)
- Some other forms of nephritis
- End-stage liver disease

Low C4 levels:

- SLE (exacerbation)
- Glomerulonephritis (early stage)
- Immune complex diseases
- Cryoglobulinemia
- Inborn C4 deficiency
- Hereditary angioneurotic edema (HANE)

Inflammatory and/or graft rejection markers

The standard care in graft rejection: repeated creatinine measurements and biopsy

- a) C-reactive protein (CRP)
- b) Serum /urine neopterin
- c) Cytokines and their receptors, e.g. soluble IL-2 receptor (sIL-2R)
- d) CD markers (e.g. CD40L, CD69) on blood T lymphocytes
- e) CD markers on urinary cells; cytokines excreted in urine
- f) Many others including combination of tests (mRNA expression analysis, proteomics, etc)

A comprehensive review on this topic has been written by W. Gwinner (World J Urol 2007; 25:445-55)

Proposed tests in order to exclude or verify some systemic autoimmune diseases

SLE: ANA*, anti-DNA*, ENA (=anti-Sm, Scl-70, RNP, SS-A/B), anti-CL/LA, C3, C4

RA: RF, CRP, CCP, ANA, (C3, C4)

Juvenile idiopathic arthritis (JIA): RF, ANA, CRP

Sjögren's syndrome: SS-A*, SS-B*, ANA*, RF

Scleroderma: Scl-70, anti-centromere, ANA, RNP

MCTD: ANA*, ENA (RNP*, Scl-70)

Myositis: ANA (Hep-2), ENA (Jo-1, Scl-70, RNP)

Vasculitis: ANCA, CRP, ANA, RF, cryoglobulin (C3, C4)

* a negative test makes diagnosis unlikely

Conclusions

- A positive serology test may indicate the presence of a specified (autoimmune) disease but is never sufficient to make a diagnosis. Diagnosis is based on both laboratory (histology) and clinical data
- A positive test usually necessitates a confirmatory test, and if positive again, a follow-up test is required
- Positive test may be present in 'healthy' individuals, however, the persistence of an autoantibody indicates a certain risk to develop autoimmune disease
- The laboratory should interpret positive tests (which method, what is the specificity and sensitivity of the test, etc). In case of uncertainty, you should ask laboratory personnel for comment
- It is easier to make a comment if you submit the most relevant clinical and laboratory data to the laboratory (including positive test(s) found earlier; not only purported diagnosis)

