Clinical significance of Immunological tests

BCM year III

Patient 1

- F/30, good past health
- Joint pain and swelling for 2 months
- Photosensitivity +
- No skin rash
- Urine x protein –ve
- CBP, RFT, LFT normal
- FH: no lupus

Patient 1 (cont'd)

- ANA 1/360, homogenous
- Anti-dsDNA 30 iu/ml (negative)
- Rheumatoid factor 15 iu/ml (negative)
- Anti-ENA
  - Anti-Sm^-, anti-$nRNP^-$, anti-Ro^+, anti-La^-
- C3 86 mg/dl, C4 18 mg/dl (normal)
- C-reactive protein <0.3 mg/dl (normal)

Anti-nuclear antibodies

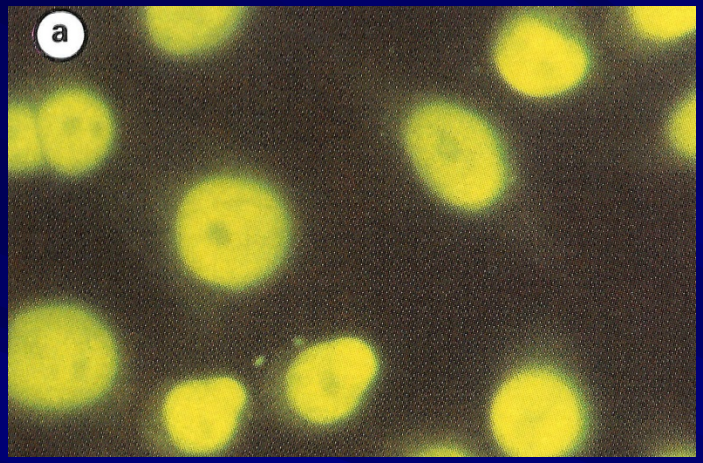
- a group of autoantibodies against nuclear constituents
- Usually tested by indirect immunofluorescence (IIF)
- Clinical use
  - Systemic lupus erythematosus 95%
    sensitive but not specific
  - Rheumatoid arthritis 60%
  - Scleroderma 90%
  - many other diseases
  - normal 3-4%

Pattern (ANA)

- useful guide to Ab specificity and sometimes to clinical disease

- Diffuse
  - Deoxyribonucleoprotein
  - Nonspecific
- Speckled
  - ENA
  - rheumatic diseases

- Rim
  - dsDNA
  - SLE
- Centromere
  - CREST
- Nucleolar
  - Ribonucleoprotein precursors
  - Scleroderma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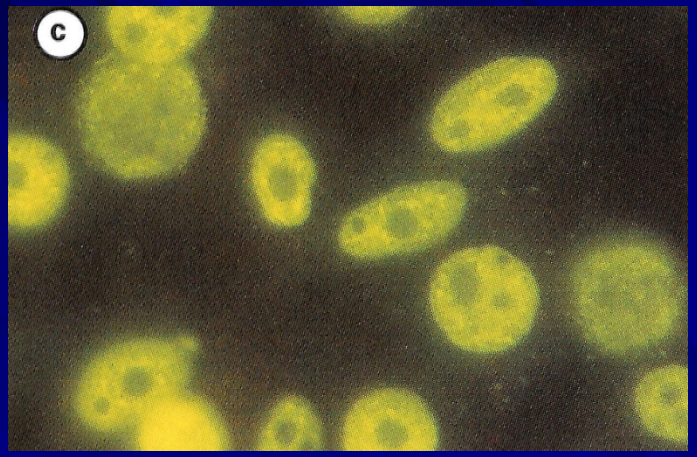
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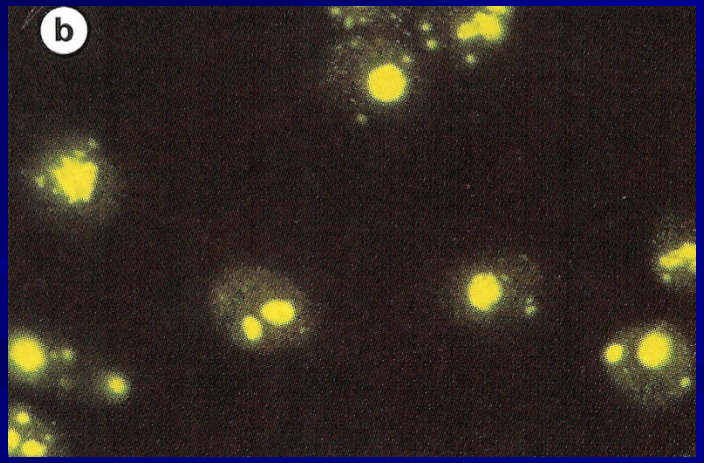
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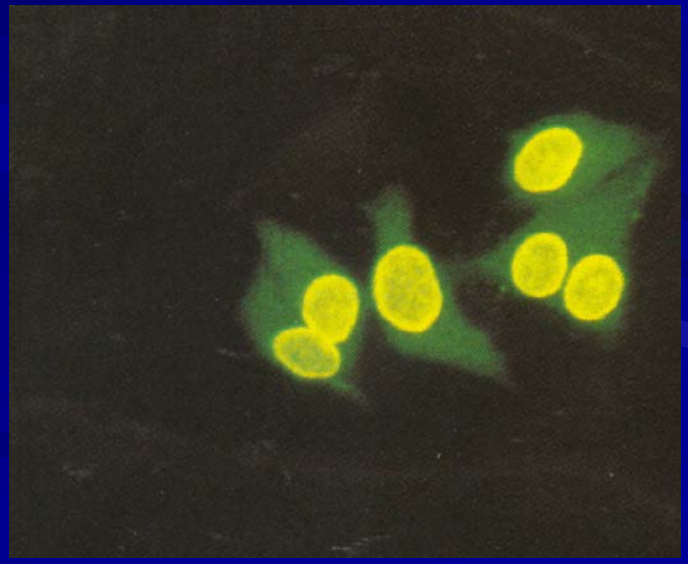


Titre

- helpful in diagnosis (high titre - more significant)
- no clinical value of monitoring ANA titre

Anti-dsDNA

- specific for SLE but present only in 70% of active disease patients
- level correlate with disease activity in most patients

- A significant number of anti-dsDNA –ve SLE patients have anti-nucleosome antibody; hence a combination of the 2 antibodies raises the sensitivity to 90%

techniques (anti-dsDNA)

- ELISA: generally good, detection of low affinity Ab hence may not be specific
- Farr assay: sensitivity and reproducibility similar to ELISA
  - a radioimmunoassay
  - low affinity Ab dissociated during precipitation step
- IIF using Crithidia luciliae:
  - specific and simple but not sensitive
  - also quantitation not accurate

Clinical significance of antibody level

- calibrated against Wo/80: IU/ml
- level in general reflect disease activity except
- up to 30% of patient has no correlation
- may be a delay of weeks to months between increased titre and increased clinical activity

Anti-ENA (extractable nuclear antigens)

- at least 30 different specificities
- detected by
  - double immunodiffusion, Counter-current immunoelectrophoresis
  - ELISA, Western blotting, immunoprecipitation

Anti-ENA

- Sm
  - Smith
  - highly specific for SLE
  - present in 30%
- nRNP
  - Nuclear ribo-nucleoproteins
  - high titre associated with mixed connective tissue disease (MCTD)
  - lower titre in SLE and other connective tissue diseases

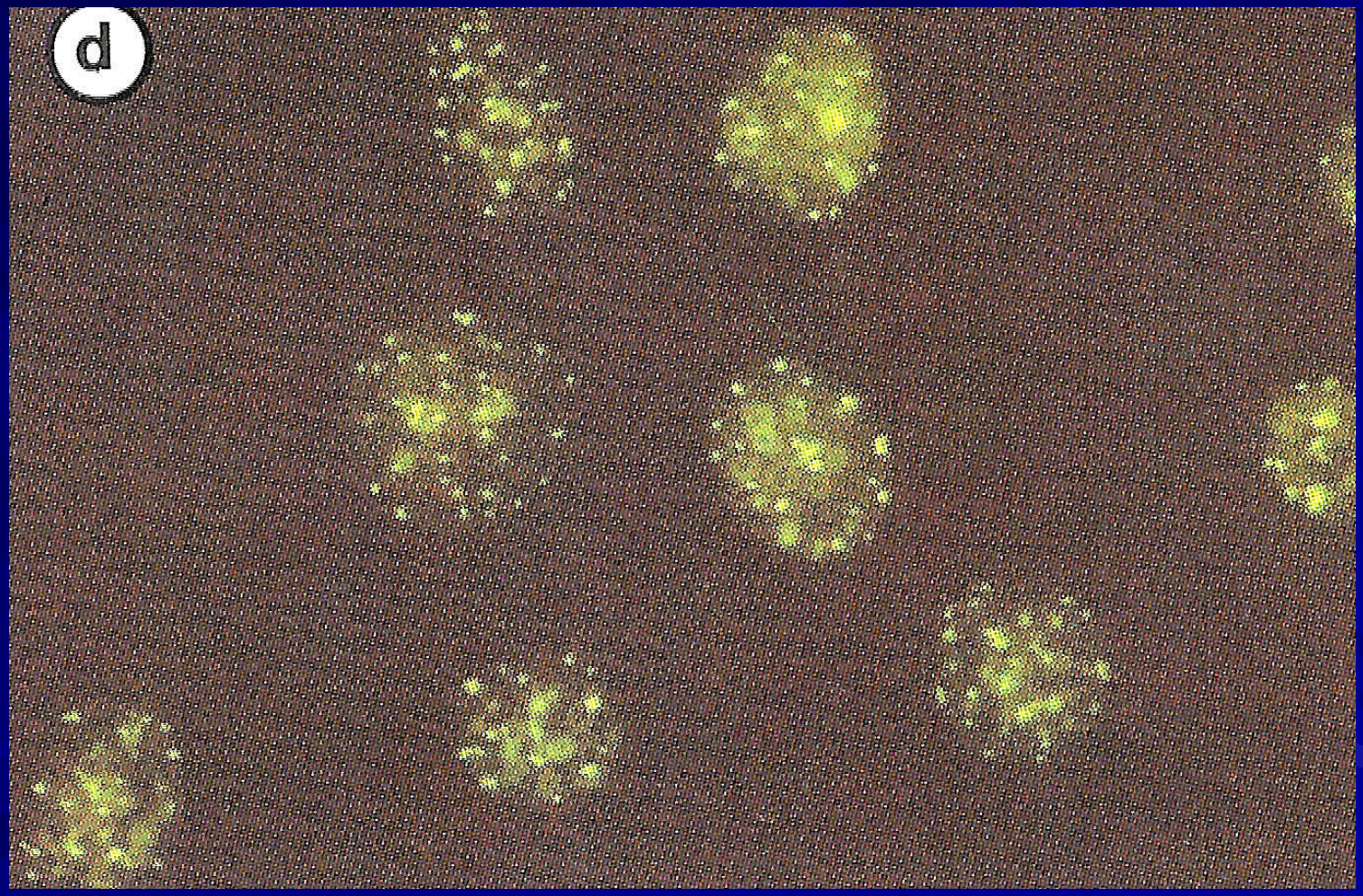
Anti-ENA

- Ro (SS-A)- associated with heterogeneous clinical manifestations:
  - subacute cutaneous LE
  - pregnancy: neonatal lupus
    - photosensitive dermatitis
    - congenital heart block
  - Sjogren's 60%
  - SLE + Sjogren 60%
- La (SS-B)- Ro and La antigens have physical affinity to one another
  - patients frequently have Anti-Ro also
  - Similar disease association
  - Sjogren's syndrome (40%), SLE (15%)

anti-centromere

- kinetochores of centromere

- CREST =
  - Calcinosis
  - Raynaud's phenomenon
  - Esophagus dysmotility
  - Sclerodactyly
  - Telangiectases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C3 and C4 - clinical indications

1. decrease in levels:
- primary deficiency - rare
- secondary (consumptive) - common
  - decrease in C3 only - AP
  - decrease in C3 and C4 - CP
- secondary (decreased production)
  - chronic liver diseases

2. increase in levels:
- in acute phase response - not useful

Causes of decreased C3 and/or C4

- many
- SLE (C3 and C4)
- liver parenchymal diseases (C3 and C4)
- post-infectious glomerulonephritis (C3)
- C1-inhibitor deficiency (C4)

Patient 2

- F/23, good past health
- Neck swelling – found to have a goitre
- Associated with heat intolerance, diarrhoea, weight loss
- Found to have fine hand tremour, exophthalmosis
- FH: no thyroid disease

Patient 2 (cont'd)

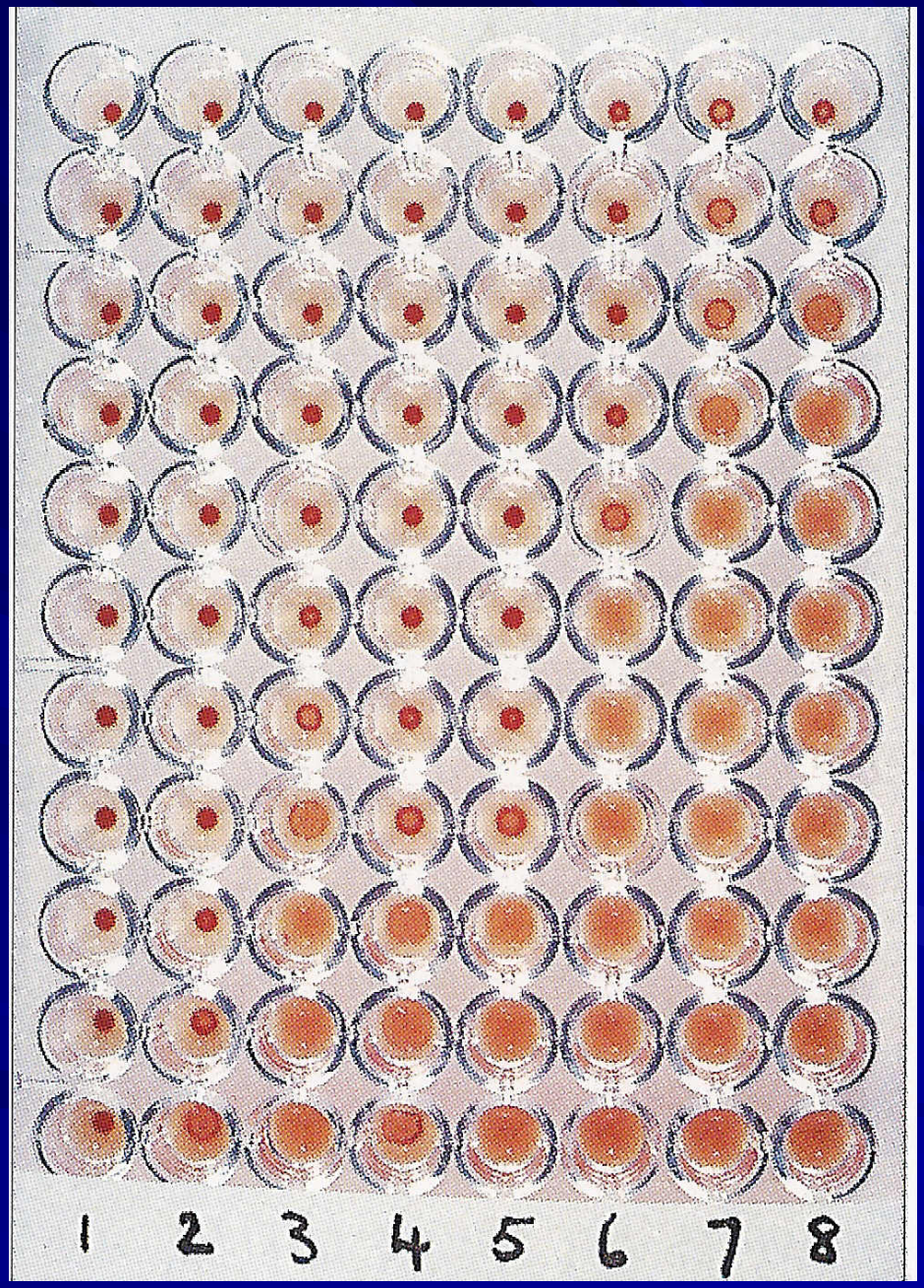
- Thyroid stimulating hormone (TSH) 0.1 mU/l (0.4-4.0)
- Total T3 3.8 nmol/L (1.5-2.6)
- Total T4 220 nmol/L (85-160)
- Anti-thyroid microsomal Ab 1/25600 (<1/100)
- Anti-thyroglobulin Ab 1/12800 (<1/100)
- Anti-TSH receptor Ab 7.2 IU/L (<1.8)

Autoimmune thyroiditis

- anti-thyroglobulin Ab
- anti-thyroid microsomal Ab

- Methodology:
  - particle agglutination
  - ELISA

- Clinical use: presence of these Ab is associated with thyroid autoimmunity; not specific and can be found in thyroid malignancy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prevalence depends much on type of assays

	anti-Tm	anti-Tg
Grave's	60%	20%
Hashimoto's	90%	45%
primary myxoedema	80%	55%
normal males	3%	1%
normal females	10%	4%

Patients 3 & 4

- F/46
- Progressive jaundice & pruritus
- Dragging feeling in RUQ of abdomen
- LFT
  - albumin 42 g/l (N)
  - Globuin 75 g/l (inc.)

- F/53
- Progressive jaundice & pruritus
- Loss of apeptite and weight
- LFT
  - albumin 41 g/l (N)
  - Globuin 93 g/l (inc.)

Patients 3 & 4 (cont'd)

- Bilirubin 38 μmol/ (inc)
- ALT 166 iu/l (5-30)
- AST 121 iu/l (5-45)
- ALP 1050 iu/l (20-85)

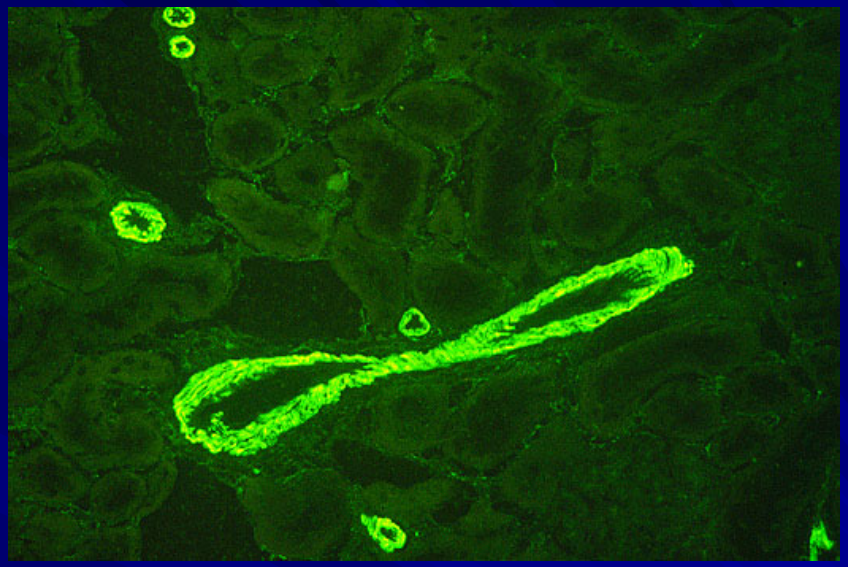
- IgM 400 mg/dl (inc)
- IgA, IgG normal
- ANA -ve
- Anti-mitochondria Ab +ve

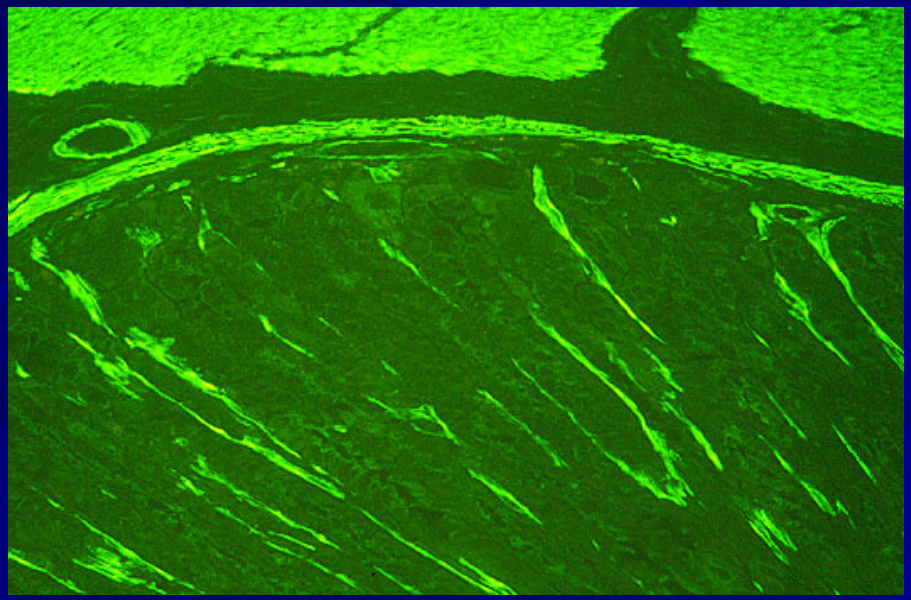
- Bilirubin 39 μmol/ (inc)
- ALT 152 iu/l (5-30)
- AST 164 iu/l (5-45)
- ALP 83 iu/l (N)

- IgG 4400 mg/dl (inc)
- IgA, IgM normal
- ANA 1/1280
- Anti-smooth muscle Ab +ve

Anti-Smooth Muscle Antibodies

- found in
  - chronic active hepatitis (CAH) 70%
  - primary biliary cirrhosis (PBC) 20%
  - cryptogenic cirrhosis 30%

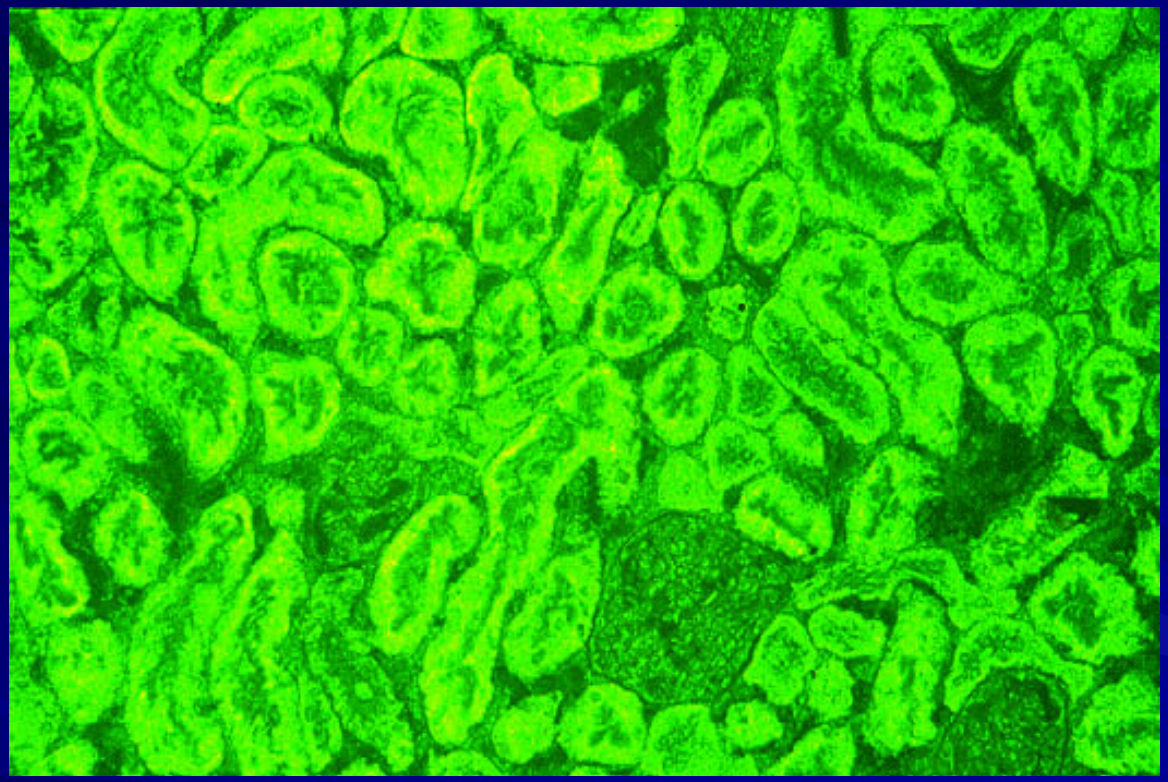




Anti-Mitochondrial Antibodies

- found in
  - Primary biliary cirrhosis 90%
  - chronic active hepatitis 25%
  - cryptogenic cirrhosis 25%

  - very rare in extrahepatic biliary obstruction and normal subjects



Patient 5

- F/36
- 3 month history of joint pain and swelling
- Morning stiffness +
- No skin rash, alopecia, photosensitivity

Patient 5 (cont'd)

- ANA 1/120, homogenous
- Anti-dsDNA 15 iu/ml (negative)
- Rheumatoid factor 115 iu/ml (<15)
- Anti-cyclic citrullinated peptide (CCP) 50 units/L (<20)
- Anti-ENA -ve
- C3 136 mg/dl (75-150), C4 18 mg/dl (9-35)
- C-reactive protein 1.8 mg/dl (<0.3)

RHEUMATOID FACTOR

- IgM against IgG Fc
- Methods
  - nephelometry
  - Agglutination method
  - ELISA

Diagnostic significance

- RA - not specific nor sensitive
  - present in 70% of patients
  - other AI diseases: SLE, scleroderma, CAH
  - chronic infections: TB, osteomyelitis
  - normal: increase incidence with age, 5% of general population
- high titre associated with active disease and extra-articular manifestations (Sjogren's syndrome, pulmonary disease, Felty's syndrome)
- very high titre in rheumatoid vasculitis

- Anti-CCP (cyclic citrullinated peptide)
- Highly specific but not sensitive (50-70%)

Patient 6

- M/54
- Non-smoker, good past health
- Sudden onset of shortness of breath
- Fever 38.4^{o}C
- No haemoptysis, chest pain

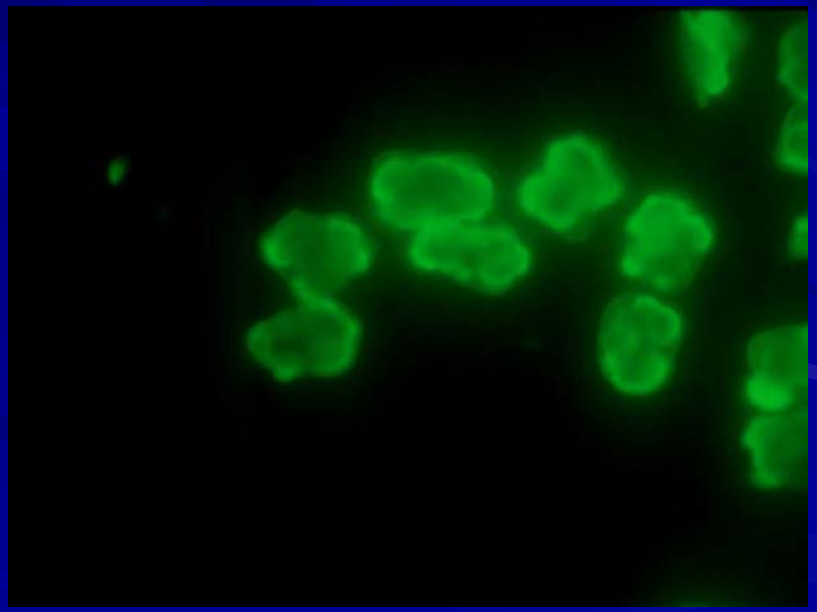
Patient 6 (cont'd)

- ESR 110 mm/hr
- C-reactive protein 10 mg/dl (<0.8)
- ANCA – cANCA positive
- PR3-ANCA positive
- Anti-glomerular basement membrance Ab negative

Anti-neutrophil cytoplasmic Ab (ANCA)

- method: IIF
- substrate: human leucocytes, ethanol-fixed
- conjugate: anti-human IgG
- 2 staining patterns: cytoplasmic, perinuclear
- perinuclear pattern is an artefact of ethanol fixation
- cANCA: proteinase III
- pANCA: myeloperoxidase, others

ELISA available using purified antigens



clinical uses of ANCA

- diagnosis of small vessel vasculitis
- cANCA - Wegener's granulomatosus
- pANCA - microscopic polyarteritis, Churg-Strauss syndrome, pauci-immune glomerulonephritis
- IIF titre of some value in monitoring disease activity, prefer to use ELISA results

Patient 7

- F/32
- Recurrent spontaneous abortions x4
- Livedo reticularis +
- No history of stroke, deep venous thrombosis, transient ischaemic attack
- No joint pain, skin rash, alopecia, photosensitivity, oral ulcers

Patient 7 (cont'd)

- CBP: platelet 100 x 10^6/l
- Anti-cardiolipin Ab
  - IgG 56 GPL/ml (<10)
  - IgM 10 MPL/ml (<10)
- Lupus anti-coagulant positive
- Anti-β2-glycoprotein I Ab 40 units/L (<20)
- VDRL positive

Tests for Anti-phospholipid antibodies

- basically VDRL, lupus anticoagulant (LA), solid-phase assays
- since each test identifies a slightly different subgroup, best to do all 3 tests

- VDRL: least sensitive
- when +ve, only at low titre
- LA: most specific; but only a functional assay
  - specificity of Ab involved not directly tested
  - also a variety of methods available
- Anti-cardiolipin (ACL) antibody Test - by ELISA
- Anti-β2-glycoprotein I antibody test – by ELISA, also specific

Patient 8

- M/76
- Chronic smoker
- Sudden onset of low back pain
- No history of infections apart from a few episodes of flu in past 2 years
- LFT: elevated globulin 64 g/l
- CBP: high ESR 120 mm/hr., mild anaemia 9.8 g/l

Patient 8 (cont'd)

- IgG 6180 mg/dl (819-1725)
- IgA 20 mg/dl (70-386)
- IgM 15 mg/dl (55-307)
- Serum protein electrophoresis: M band positive
- Immunofixation: IgG/kappa band

Definition of paraprotein

- abnormal discrete band(s) seen on electrophoresis of serum or urine
- comprise intact or fragmented homogenous Ig derived from a single clone of B cells
- progressive (malignant) or self-limiting (benign)

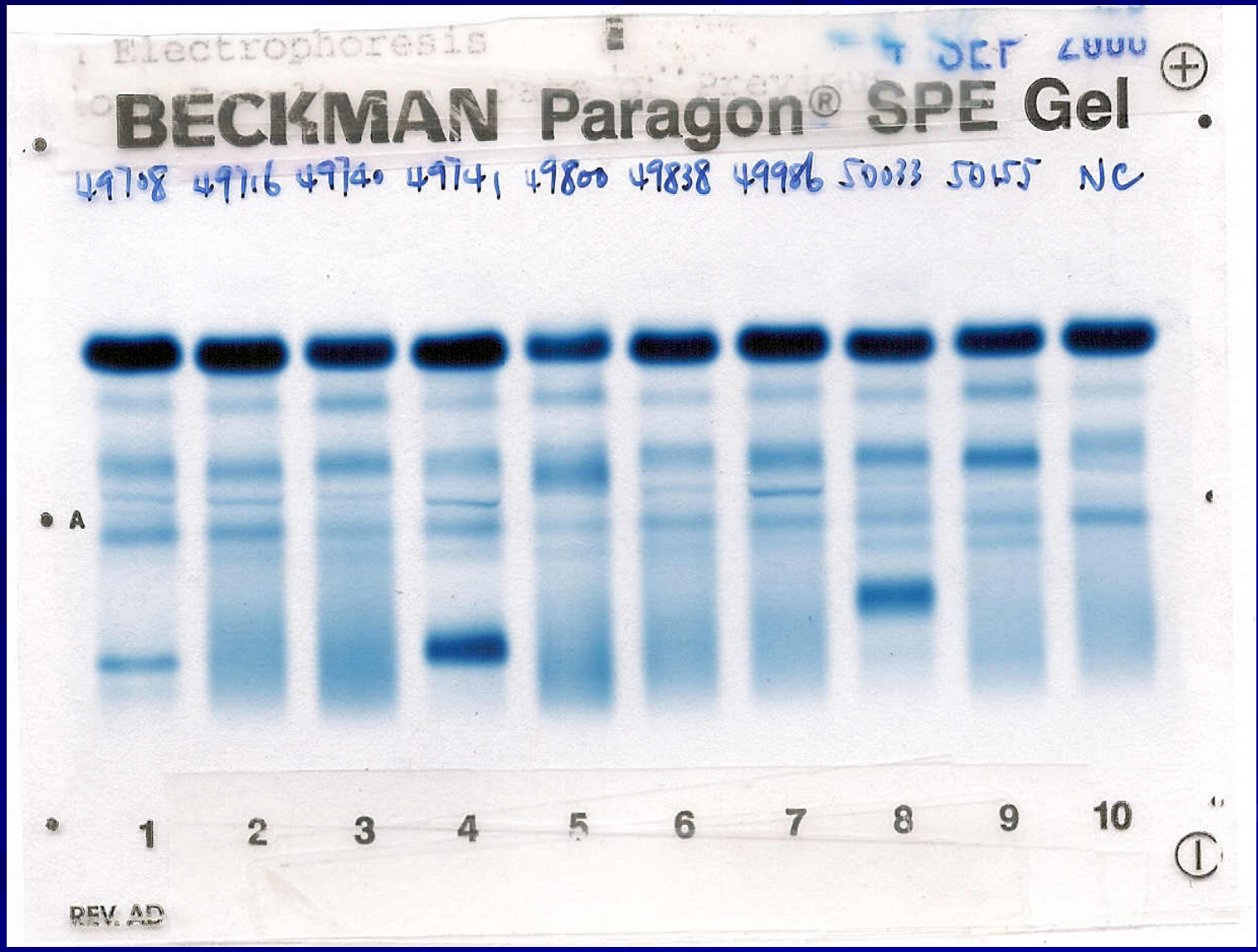
Diseases associated with paraproteins

1. malignant
- multiple myeloma
- B cell lymphoma including Waldenstrom's macroglobulinaemia
- heavy-chain disease
- amyloidosis

2. benign (usually transient)
- infectious processes
- other conditions associated with hyper-gammaglobulinaemia

Diagnosis of multiple myeloma

1. serum and early-morning urine for presence of paraprotein
   - narrow band seen on electrophoresis
   - shown to be monoclonal by immunochemical methods (eg. immunofixation)
   - presence of free light chain suggest a malignant process
   - suppression of non-paraprotein Ig also suggest a malignant process
2. bone marrow biopsy - increased number of plasma cells with abnormal morphology
3. skeletal X-ray - lytic lesions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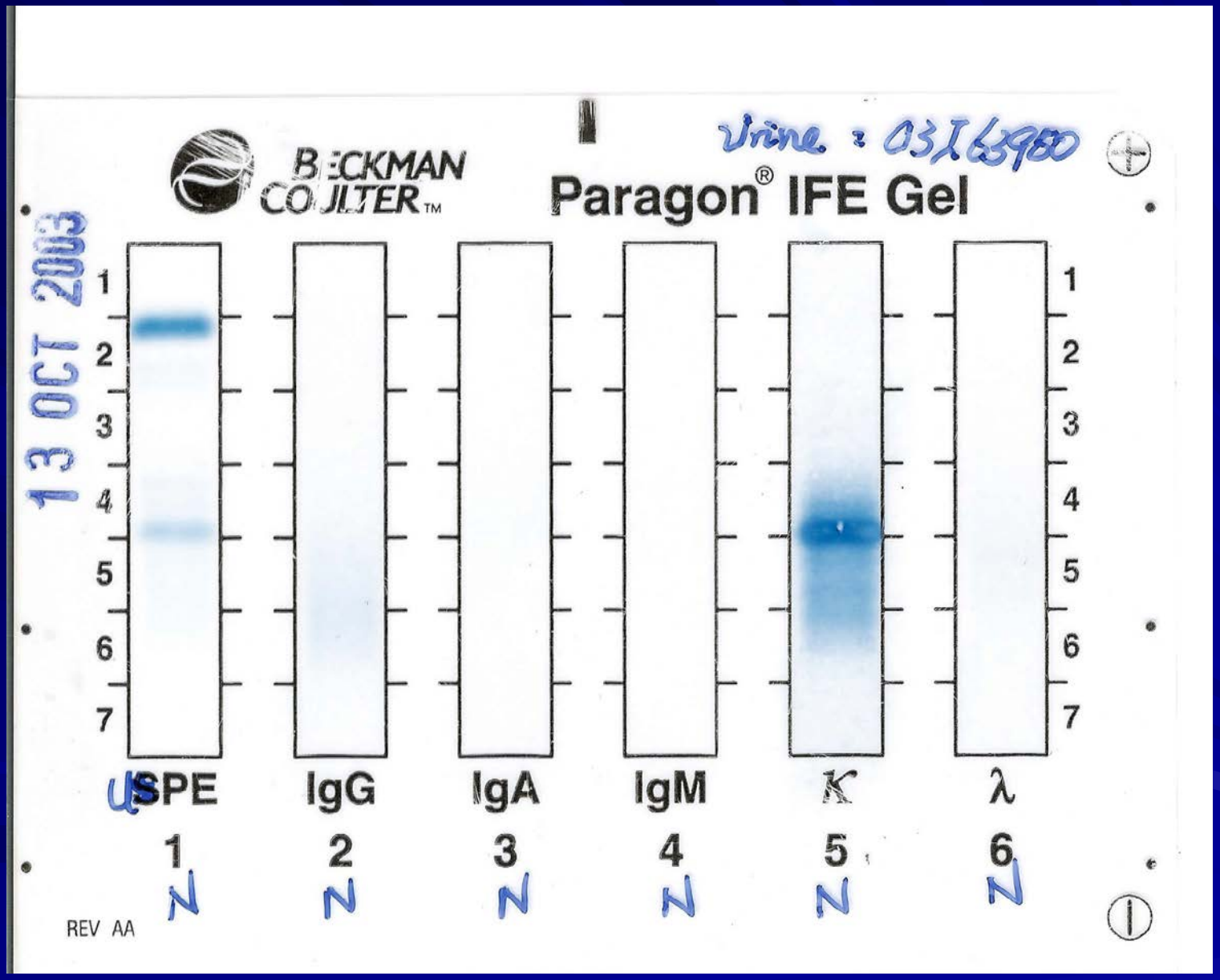
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IgG, IgA, IgM

Clinical indications

1. antibody deficiency , eg.
- Primary: CVID, XLA, selective IgA deficiency
- Secondary: myeloma
- monitor Ig replacement therapy

2. ? quantitation of paraprotein
- cheaper and easier than densitometry
- work in majority of cases
- Can give gross errors, hence not recommended

3. conditions where raised Ig is part of the spectrum of clinical features, eg. chronic liver diseases, AIDS

The End