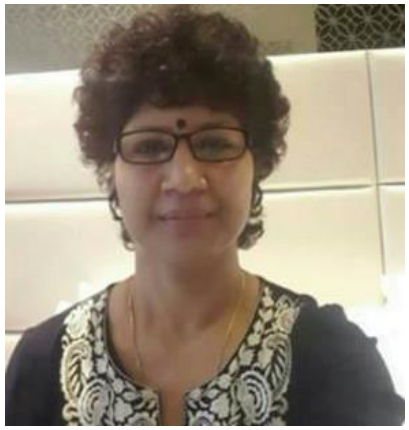


Prof. Uma Kumar
Head, Department of Rheumatology
All India Institute of Medical Sciences
New Delhi
WHO Fellow (IEC)
President-Elect Delhi Rheumatology Association

- Recipient GEM OF INDIA award for academic excellence, ARA-APLAR International Scholarship (Australia), Japan College of Rheumatology International Scholarship (twice), EULAR travel bursary (Spain, Austria), Zydus Oration, Kolkon Oration, R K Jalota Oration,Bhartiya Manav Adhikar Samman 2013 .
- Editorial board Member of IJRD, IJR, IJMR.
- Ex- Associate Editor of IJR.
- Publications : 101
- Current research interest : air pollution & autoimmunity (DST), and role of nanotechnology in Rheumatology (DBT).

Interpretation of Immunological Investigations in Clinical Practice

Case Vignette -1

- A 35-yr-old non-diabetic hypertensive lady
- Polyarthritis - asymmetrical, small joints of left hand with significant EMS – 6 weeks.
- O/E – left 4 PIPs , 2 MCPs & wrist arthritis.
- Investigations – ESR ↑ RF >256 IU

  Anti CCP –Neg X-Ray hands- N

Early RA started on MTX

- **At 3 months – Partial response**

  Transaminitis

  Tingling numbness left hand
- **MTX stopped left wrist injected with steroid**
- **HBV markers positive**
- **NCV- mononeuritis multiplex**
- **Sural nerve biopsy- Medium vessel vasculitis**

“ Polyarteritis Nodosa”

Odd points

1. Asymmetrical arthritis

2. Development of mononeuritis multiplex

3. Hypertension

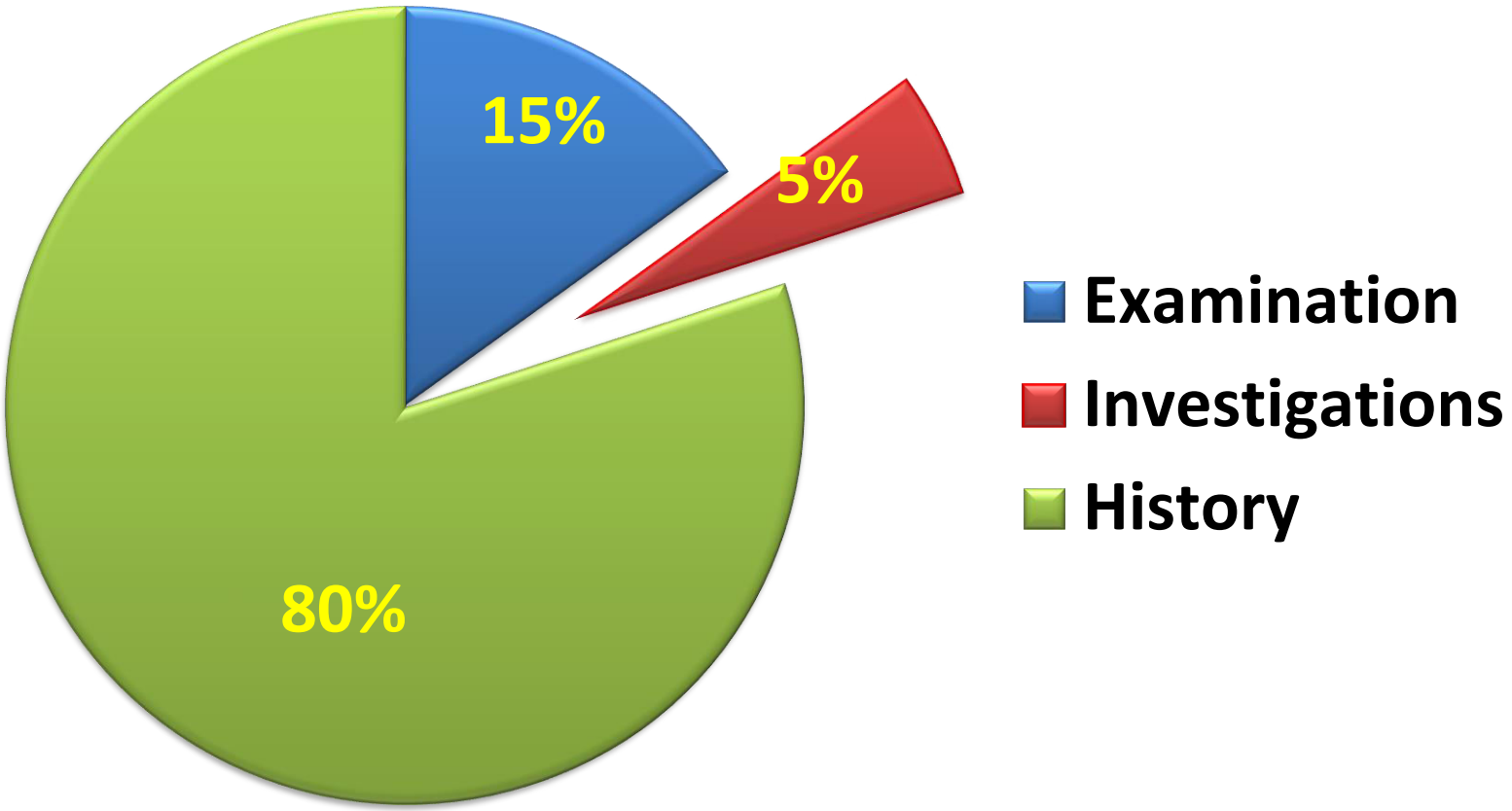
Don’t be in a hurry to give diagnosis.

Remember autoantibodies are surrogate markers for diagnosing autoimmune diseases rather than being diagnostic markers!

“False positivity of autoimmune markers”

The most important biomarker in rheumatology is clinical evaluation (history and physical examination)

Rheumatology – A strong clinical science



Prior to ordering immunological investigations important to know

- Clear indication for test.
- Anticipated response to outcome.
- Sensitivity/specificity of the test.
- Positive & negative predictive value of test.

"If test will not alter diagnosis,therapy, and prognosis then it may be unnecessary"

Problems of interpreting lab tests in rheumatology

(A) measurement errors

(1) quality of sample provided

(2) quantity of analyte available

(B) poor understanding of the assay resulting in misinterpretation

(1) False positive results

(2) False negative results

Sensitivity

The sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease.

$$\frac{\text{True Positive}}{\text{True Positive + False Negative}}$$

Specificity

The specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease.

$$\frac{\text{True Negative}}{\text{True negative+ False Positive}}$$

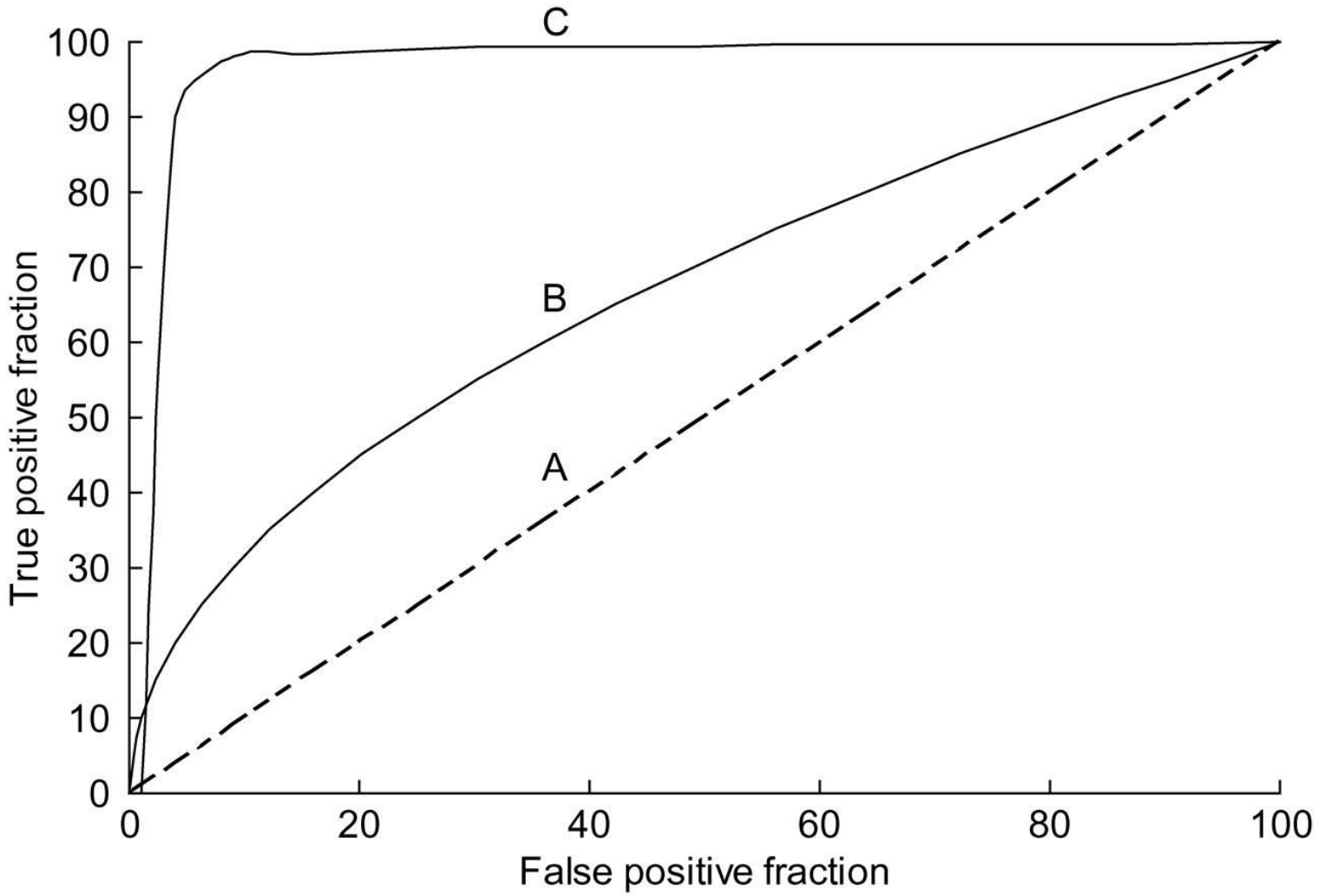
If the test is positive,how likely is it that this patient has the disease ?

$$PPV = \frac{\text{True Positive}}{\text{True Positive + False positive}}$$

If the test is negative ,how likely is it that this patient has no disease ?

$$NPV = \frac{\text{True Negative}}{\text{True Negative + False Negative}}$$

Receiver operator curves: (A) line of zero discrimination (AUC=0.5); (B) typical clinical test (AUC=0.5–1.0); perfect test (AUC=1.0).

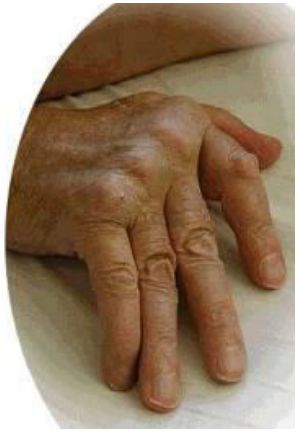


Abdul Ghaaliq Lalkhen, and Anthony McCluskey Crit Care Pain 2008;8:221-223

Common Immunological Investigations in Clinical Practice

- RF
- Anti CCP antibody
- ANA
- ENA (Extractable Nuclear Antibodies)
- ANCA (Anti Neutrophil Cytoplasmic Antibody)
- Scleroderma Antibodies
- HLA – B 27
- ESR and CRP

Rheumatoid factor (RF)



- RF positive in 1% of normal population
- RA occurs in 0.5-1% population
- RF found in 70-90% of RA adults

J Rheumatol 1991;18:989-93.

RF in rheumatological and non-rheumatological conditions

Rheumatic conditions (prevalence)	Nonrheumatic conditions
Rheumatoid arthritis (50 to 90%)	Aging
Systemic lupus erythematosus (15 to 35%)	Infection: bacterial endocarditis, liver disease, tuberculosis, syphilis, viral infections , parasitic diseases
Sjögren's syndrome (75 to 95%)	Pulmonary disease: sarcoidosis, IPF, silicosis, asbestosis
Systemic sclerosis (20 to 30%)	Miscellaneous diseases : PBC, malignancy (especially leukemia and colon cancer)
Cryoglobulinemia (40 to 100%)	
Mixed connective tissue disease (50 to 60%)	

The rheumatoid factor: an analysis of clinical utility. *Am J Med 1991;91:528-34.*

RF

- Absence of RF in presence of progressive clinical & radiological disease is seen in 10-30% patients (seronegative).

- Patients who become seropositive often do not have RF at presentation (33% in first 3 months & 60% in first 6 months)

- Not useful for measuring disease activity.

Case Vignette 2

- 30 –yr-old obese lady
- Significant polyarthralgia, EMS 10min – one year
- Tenderness few PIPs
- ESR 32 in first hr
- RFT - WNL
- LFT - SAP 346 IU/L
  rest normal
- RF – 64 IU/ml
- ANA – Negative
- ACPA – Negative
- TSH - WNL

Very Early RA

S. Calcium 7.4 mg/dl
S.Phosphate – 4.5mg/dl
S. Vit D - Reduced

Osteomalacia

Antinuclear antibody (ANA)

"Can be viewed as ESR of autoimmunity"

- It is nonspecific but valuable because of its sensitivity.

- Reporting – Titre & Pattern.

- ANA titre may provide more specific information than mere positivity.

ANA

SLE
MCTD
Drug induced lupus
Scleroderma
AIH
Sjogren's syndrome
Myositis, RA
Malignancy
HIV
Bacterial endicarditis, DM
DLE
Drugs,ILD,silicone implants
Normal women, elderly

ANA

Titer	Population
1:40	32%
1:80	13%
1:160	5%

Arthritis Rheum 1997;40:1601-11

Population	% ANA Positivity
SLE adults	98%
Other CTD	40-70%
Autoimmune thyroiditis	20%
Healthy	5%

- ✓Only 0.1% population have SLE
- ✓SLE can be ruled out if ANA is negative
- ✓ANA valueless in monitoring disease activity
  -should not be repeated

Patterns

"pattern reflects the intracellular target of ANA"

- It may help in deciding most appropriate antibody by second line tests (Immunonlot)

ANA pattern	Antibody to	Disease
Homogeneous	DNA, Histone	SLE, DLE
Speckled	RNP, Sm SSA/Ro,SSB/La	SLE SLE, SS
Diffuse grainy	Scl-70 (topoisomerase)	dcSSc
Centromeric	Centromere	lcSSc
Nucleolar	PM/Scl, RNA-pol1,U1RNP,others	SSc, SLE, SS
Speckeled cytoplasmic	Jo-1, SRP, mitochondria	PM/DM PBC
Diffuse cytoplasmic	Ribosome	SLE

Clin Exp Rheumatol 2004;22:349-355

Extractable Nuclear Antibodies

- Directed against small nuclear ribonucleoproteins involved in RNA processing

- Reported as positive/negative

- Useful in diagnosis but not for monitoring disease activity

-Anti Sm

-Anti U1 RNP

- Anti Ro (SS-A)
- Anti La (SS-B)
- Anti Scl 70 any many others

ENA

- Should be ordered only if there is a suspected or known CTD & ANA test positive in significant titer.
- Negative ENA is not very helpful as it has low sensitivity

1. **Can IIF-ANA be positive without anti-ENA positivity ?**

- Yes e.g. IIF-ANA can be positive in RA without corresponding anti-ENA positivity

2. **Can anti-ENA be positive without IIF-ANA being positive?**

- Yes

- Given the relative scarcity of the SSA/Ro and Jo-1 antigens in the cell substrate ANA may be negative even when antibodies are present in the serum.

- Therefore, in presence of clinical findings highly suggestive of Sjogren's syndrome or congenital heart block in neoborn or polymyositis anti- ENA is recommended.

Should ANA or ENA be repeated?

- Repeat ANA or ENA is NOT indicated unless there is any change in clinical condition of the patient raising suspicion of another disease or appearance of new rheumatic disease.

Anti Ds DNA

- Specificity 95% but sensitivity 60%for SLE

Rheum Dis Clin North Am 1994;20:1-28

- Should never be performed in patients with routine screening process for patients with aches and pains.
- Testing is not recommended in patients with a negative ANA test.

Antibody avidity and affinity

- A 65-year-old man with arthralgia and hair loss for 6 months was found to have high titer anti-dsDNA & ANA 1:80 using rat liver sections as substrate.
- Likely diagnosis on the basis of clinical examination and radiological investigation was osteoarthritis.
- On repeating the test ANA was negative on HEp2 substrate. ds-DNA antibodies were positive in a similar but different ELISA assay

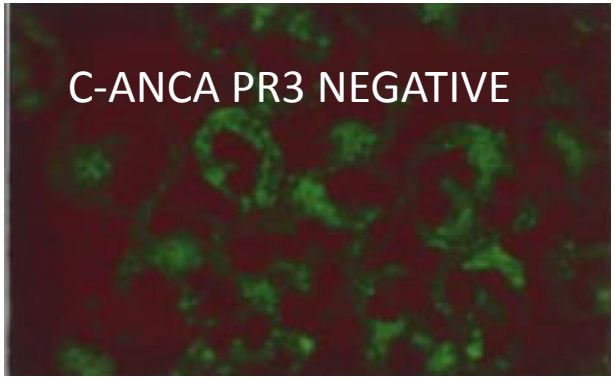
But IIF testing using Crithidia luciliae as substrate was negative

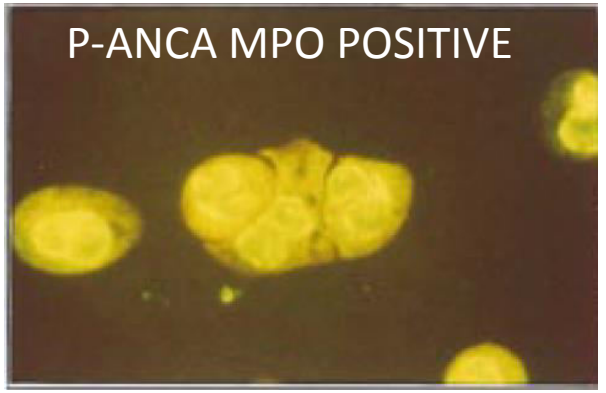
Use of advanced ELISA system improves sensitivity by detection of low affinity antibodies but reduced specificity and PPV of test

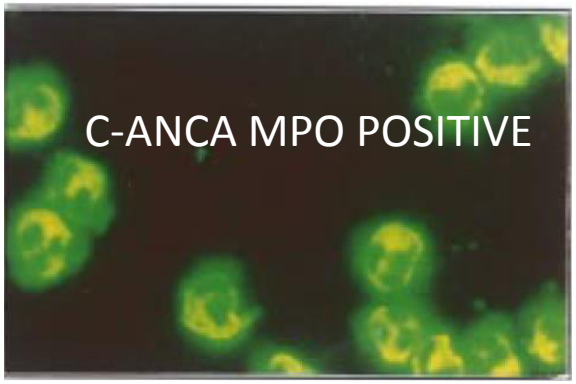
J Clin Pathol 2000;53:424–32 , J Clin Pathol 2001;54:187–90

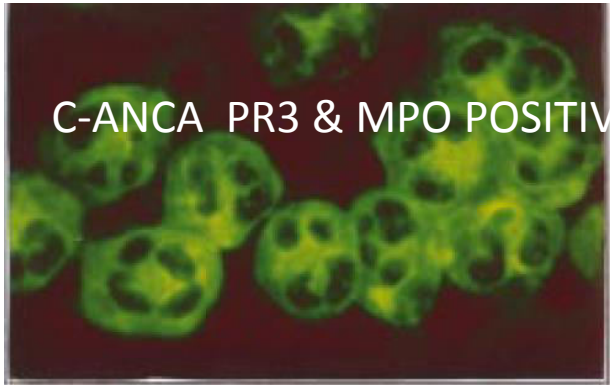
ANCA

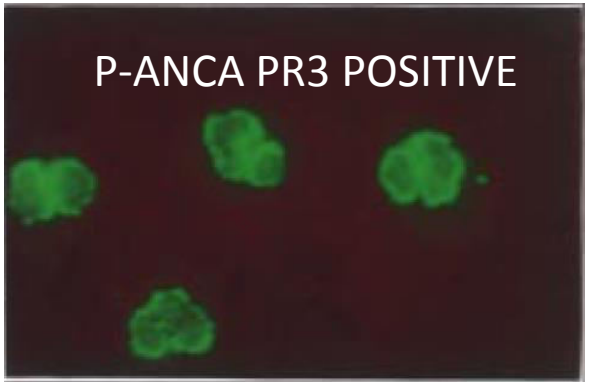
"autoantibodies against antigens in cytoplasmic granules of neutrophils and monocytes"





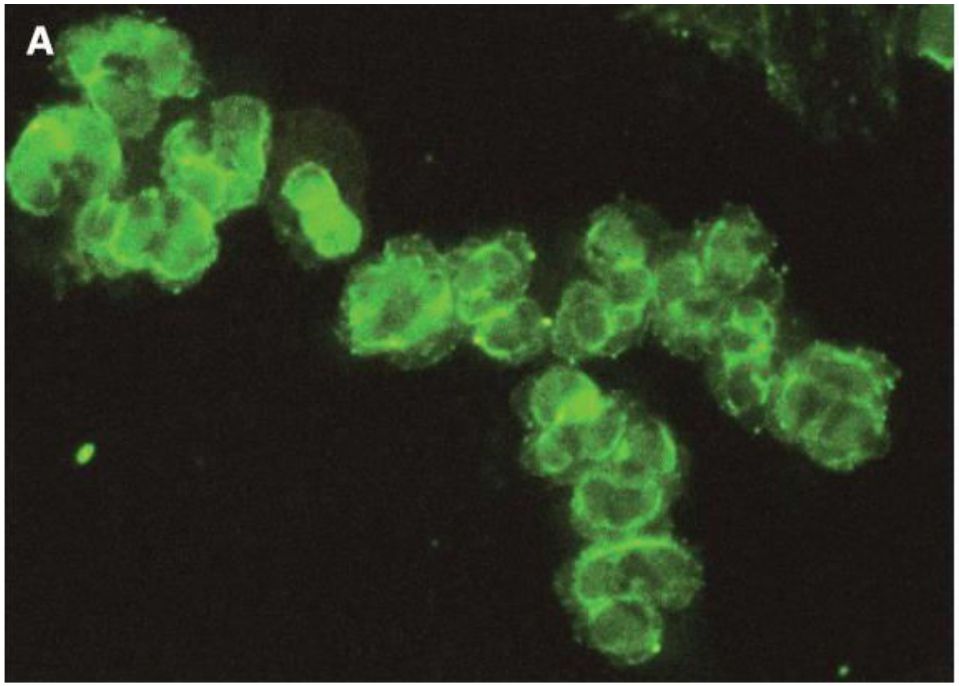






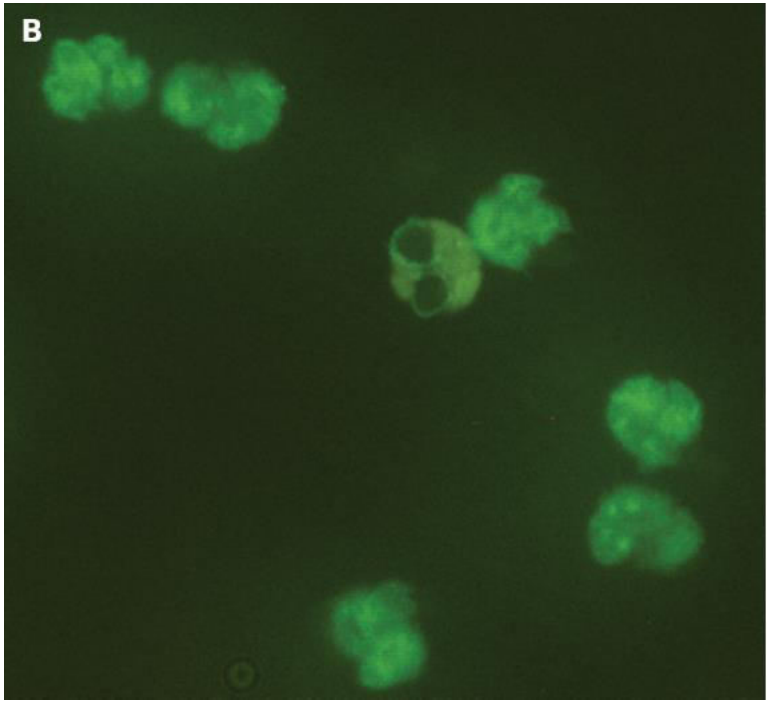
Misinterpretation errors

Interference in ANCA assays by ANA



Perinuclear ANA in SLE was completely abrogated on formalin fixed neutrophils

Mimic ANCA



High titre ANA obscuring underlying MPO-ANCA in RPGN (central eosinophil shows weak peripheral stain only in nucleus).

Mask ANCA

Consensus

- Any serum giving an immunofluorescence pattern seen on ethanol fixed neutrophils demonstrating ANCA, any other cytoplasmic stain or homogenous or perinuclear ANA should be investigated for antibodies to PR3 and MPO

International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). Am J Kidney Dis 1999;111:507–13

AASV & ANCA

	Sensitivity	Specificity	PPV	Likelihood Ratio
IIF	67%	93%	45%	9.4%
IIF/ELISA	52%	99%	88%	88.1%

Kidney Int 1998;53:743-53

	WG	MPA	iRPGN
Sensitivity	73%	67%	82%
Specificity		98%-100%	

Arthritis Care Res 2000;13:424-34

- ANCA should not be used as screening tool or diagnostic test in patients with suspected vasculitis.
- Guidelines have been proposed to help increase its diagnostic value.

Q J Med 2001;94:615-621

Guidelines for ANCA testing

COPD
Pulmonary nodule
Subglottic stenosis of the trachea
Retro-orbital mass
Pulmonary-renal syndrome
RPGN
Vasculitis of skin with systemic symptoms
Mononeuritis multiplex
Any other condition that resembles systemic vasculitis

Case Vignette 3

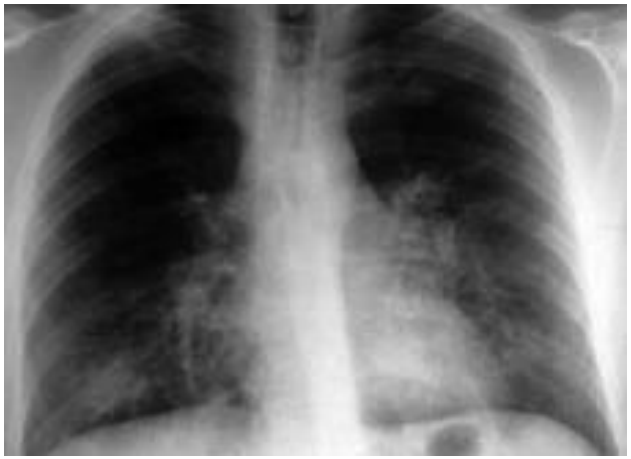
A 45-yr-old male

- Fever, polyarthralgia, fatigability, palatal ulcer, dry cough, nasal crusting - 6 months
- O/E - Anaemia, Multiple skin ulcers
  Basal inspiratory crackles in chest
- Invest – Mild pancytopenia, ESR 92 mm in 1 Hr.
- ANA – Negative, Anti MPO positive C-ANCA pattern

ANCA associated vasculitis
Received steroids, Referred

Skin biopsy-no e/o vasculitis
On evaluation patient did give history of high risk behaviour

HIV positive



ASO (Anti-streptolysin O antibody)

- Usually no relevance in individual more than 15 years
- Also never ask for this test if persistent arthritis for than 6 weeks

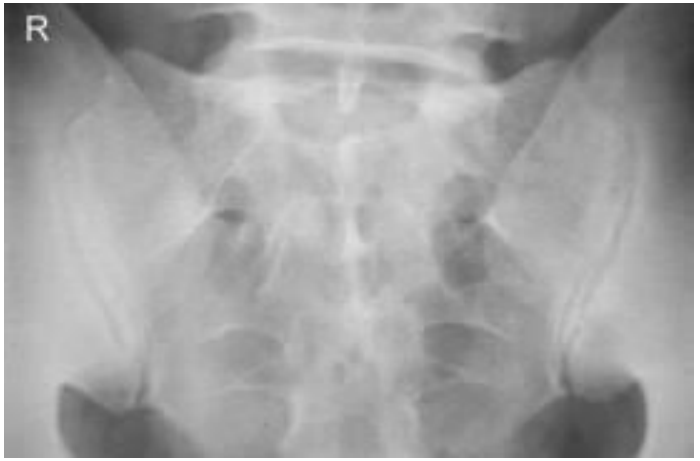
Serum complement

Three measures are available in routine clinical practice

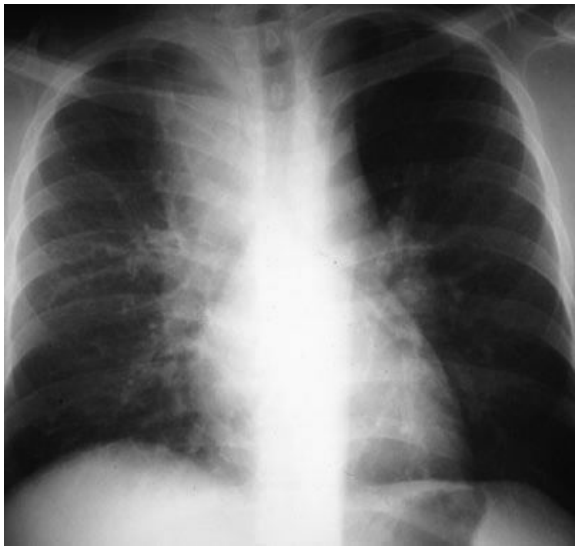
1. Total complement activity (CH50)
2. C3
3. C4

- Storage of sample for more than 6 hrs at room temperature after drawing and coagulation results in complement activation in vitro leading to falsely low results for CH50.
- C3 & C4 does not require careful handling.

Case Vignette



- 35 -yr-old male
- Low back pain ?? typical inflammatory (relief with NSAID, more in morning but persisting) -3 months
- Rt. gluteal pain, weight loss
- left ankle arthritis T.B.
- HLA- B27 positive (PCR).
- Diagnosed SpA started on SSZ and NSAID in Nov 2011



AIIMS Feb 2012 – evaluated had T.B hilar lymphadenopathy & collection in gluteal muscles, mantoux positive.

HLA - B27

- It is present in 5-8% (6%) of general population.
- Found in 95% white AS patients and 50% black AS patients.
- Since there are only 10-20% chances of ever developing the disease in patients first degree relative, asymptomatic family member should not be tested.
- Routine ordering of this test in patients with non-inflammatory back pain will result in false positivity leading to erroneous diagnosis.

- 'Arthritis Panels' OR 'Arthritis Profile' OR Rheumatology Screening Panels

Intellectually inappropriate
&
Higher false positivity

"must be condemned"

ESR/CRP

- Valid but not diagnostic of inflammation
- Can rise with infection, malignancy, anaemia lipid lowering drugs, obesity, stress, elderly
- Does not parallel inflammation
- CRP is slightly more reliable (does not very with age and gender)
- Clinical picture should determine the therapeutic decision

Serum Uric Acid

- Not much helpful in the diagnosis of gout because of high prevalence of asymptomatic hyperuricemia
- 10% of patients with acute gout have normal uric acid levels
- with the use of non-enzymatic method alphamethyldopa, caffeine, theophylline, hyperglycemia, aspirin metabolite can cause pseudohyperuricemia

Take Home Message

1. "No screening tests exists for arthritis".
2. Search for autoantibodies should be made only when there is strong suspicion of autoimmune disease.
3. Immunological investigations should be interpreted cautiously within the context of the patient's clinical condition.

Take Home Message

4.Begin with sensitive tests and if positive use more specific tests to help confirm diagnosis.

5. Most auto-antibody tests need not be serially repeated exceptions ANCA & anti-ds DNA.

6. The practice of ordering a rheumatologic panel or an arthritis panel must be discouraged.

Thank you