

Antineutrophil cytoplasmic autoantibodies (ANCA) versus other neutrophil specific autoantibodies.

Main message: ANCA is just one small but important piece in the "diagnostic mosaic" of vasculitis

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ANCA belong to the family of neutrophil-specific antibodies (NSA): what is the difference between ANCA and NSA?

- **Anti-neutrophil cytoplasm antibodies (ANCA) in vasculitis, infections, and drug-induced syndromes:**
Are defined by antigen target in neutrophil granules!
- **Neutrophil-specific antibodies (NSA) in non-vasculitic diseases i.e. rheumatoid arthritis, ulcerative colitis, sclerosing cholangitis, chronic active hepatitis, autoimmune neutropenia, Felty's syndrome, Sweet's syndrome, etc:**
Show no clear neutrophil antigen specificity (negative or weak positive in routine testing for ANCA antigens)!

Distinguishing the term ANCA from NSA is only proposed to avoid unintended/misunderstood clinical use of an "ANCA" result!!!

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Consensus-agreed definition of vasculitis-associated ANCA.

- 1. Presence of NSA as shown by specific neutrophil (monocyte) reactivity using indirect immunofluorescence (IIF) on human leukocyte substrates. +**
- 2. Clear reactivity of the NSA with target(s) in neutrophil granule(s): most often PR3, MPO, BPI, EL.**

Savige J et al. Am J Clin Pathol 111:507-13,1999.

Savige J et al. Am J Clin Pathol 120:312-20,2003.

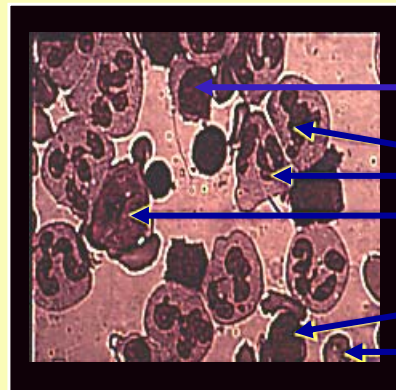
Note! Many publications that used this definition of ANCA have substantiated that patients harbouring such antibodies fulfill criteria for vasculitis/DI-LE!! 2008

Methods to detect IgG class neutrophil-specific antibodies

- **Indirect immunofluorescence (screen)**
- Direct enzyme - immunoassay
- Capture enzyme - immunoassay
- Radio - ligand immunoprecipitation
- Addressable laser bead immuno-assay
- EliA ANCA assay

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Ideally: Use whole buffy coat smear.



Lymphocyte

Neutrophil

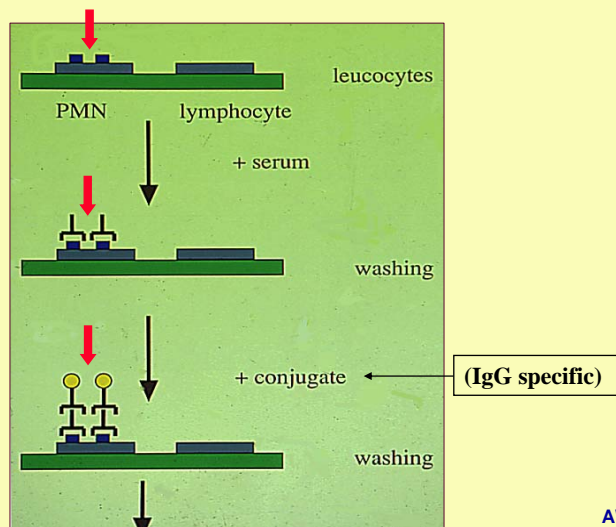
Monocyte

Lymphocyte

Erythrocyte

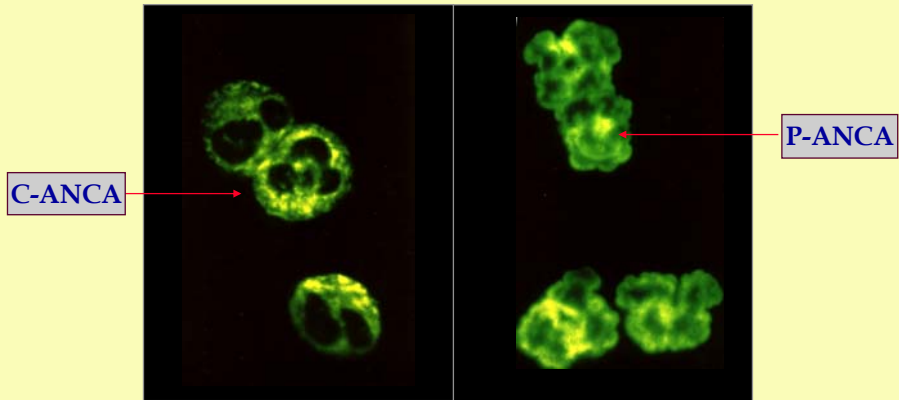
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Indirect immunofluorescence



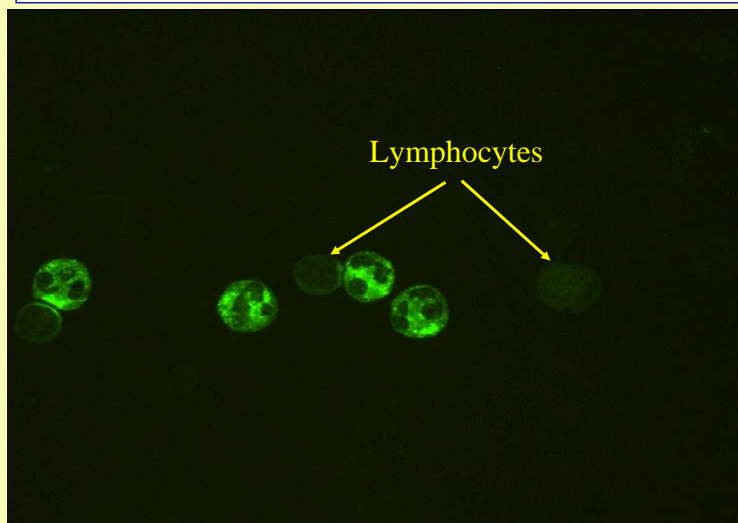
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**ANCA associated with necrotizing
vasculitides (Pr3-ANCA, MPO-ANCA)**



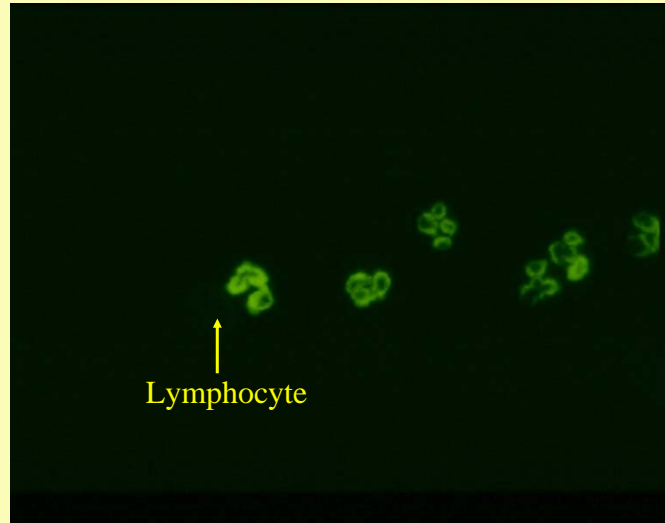
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IgG class C-ANCA



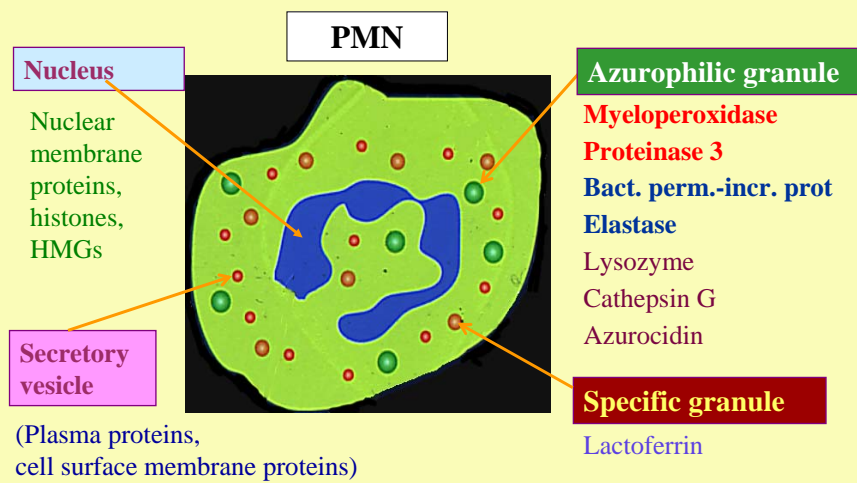
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IgG class P-ANCA



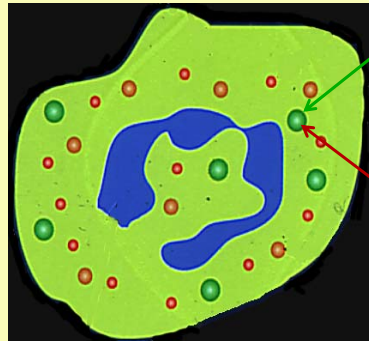
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Neutrophil autoantigens



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ANCA specificities in prim. vasculitis:
 Percentages derived from Western European vasculitis literature



Myeloperoxidase

- Microscopic polyangiitis 60 %
- Renal-limited RPGN 60 %
- Churg-Strauss syndr. 30 %
- Wegener's granulomatosis 15 %

Proteinase 3

- Microscopic polyangiitis 30 %
- Renal-limited RPGN 30 %
- Churg-Strauss syndr. 30 %
- Wegener's Granulomatosis 75 %

NB! These percentages may vary a lot in different populations!

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Ig classes of ANCA

- **IgG** **1,2,4 remission**
- **IgG** **1,2,3 active disease**
- **IgM + G** **acute reno-pulmonary vasculitis**

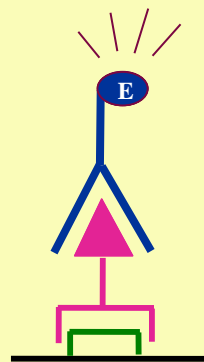
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Methods to detect IgG ANCA

- Indirect immunofluorescence (screen)
- **Direct enzyme - immunoassay**
- Capture enzyme - immunoassay
- Radio - ligand immunoprecipitation
- Addressable laser bead immuno-assay
- ELiA ANCA assay

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Direct enzyme-immunoassay



Substrate

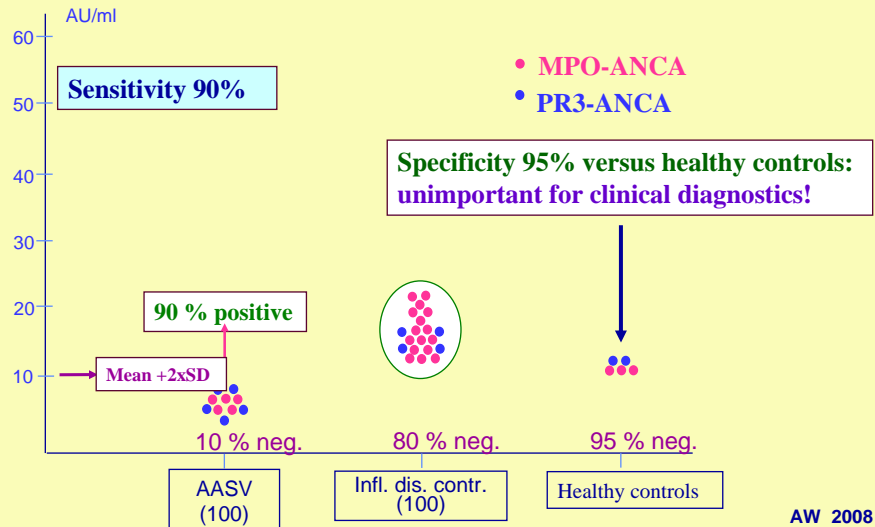
Enzyme-
conjugate

ANCA

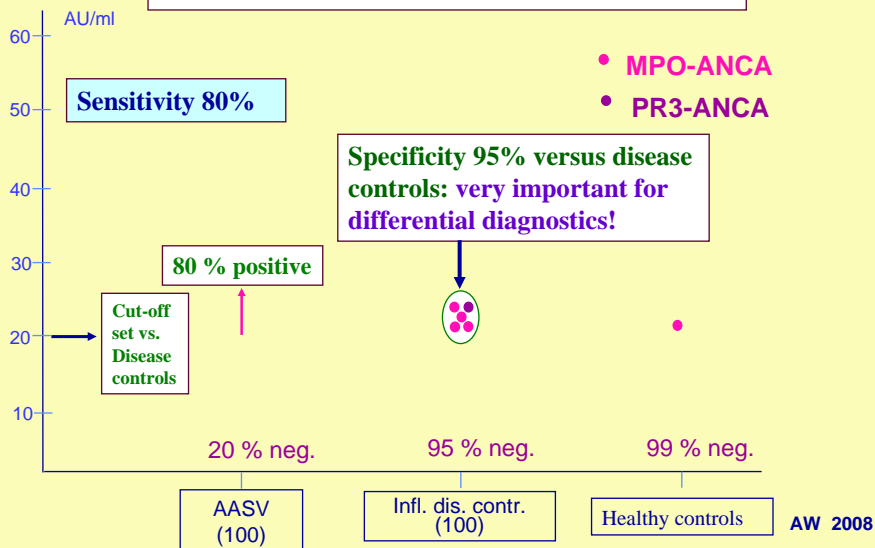
PR3 or MPO,
EL or BPI

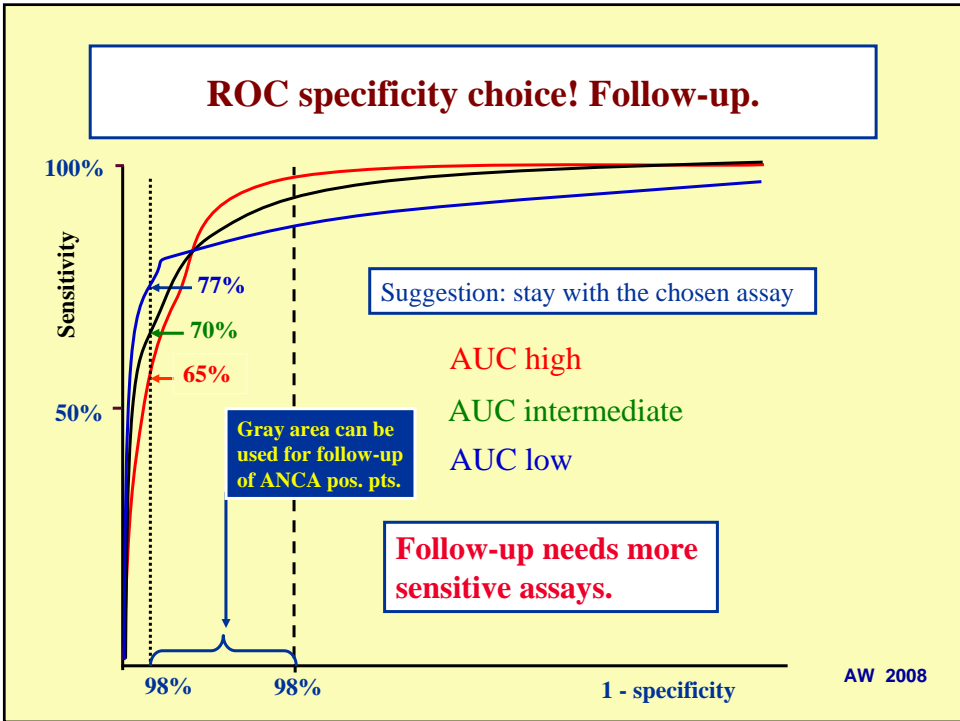
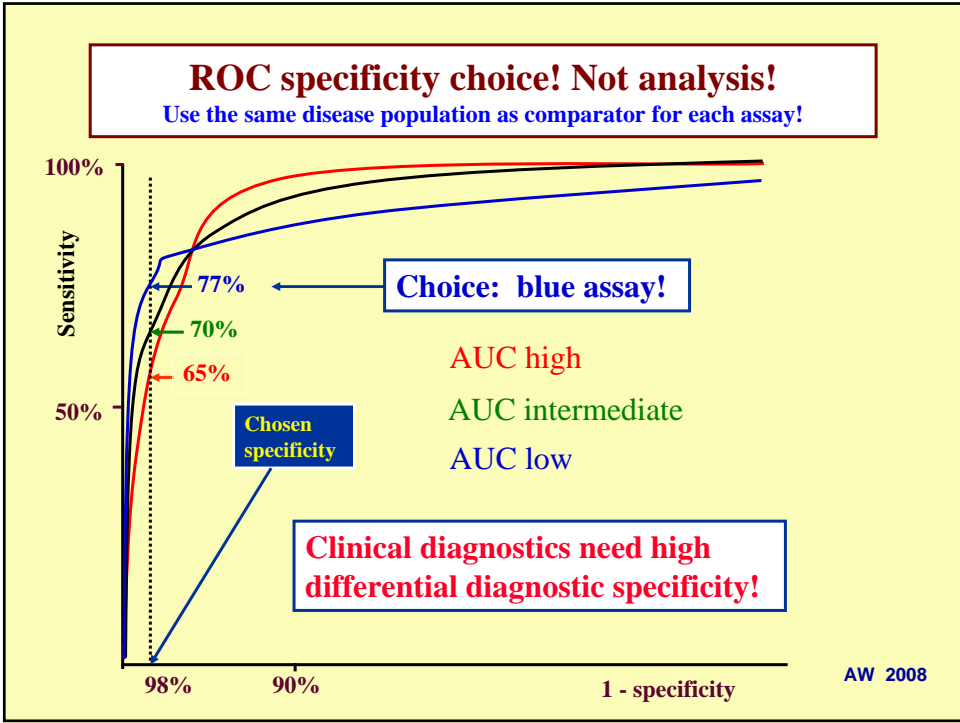
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High sensitivity cut-off setting for e.g. ANCA screening

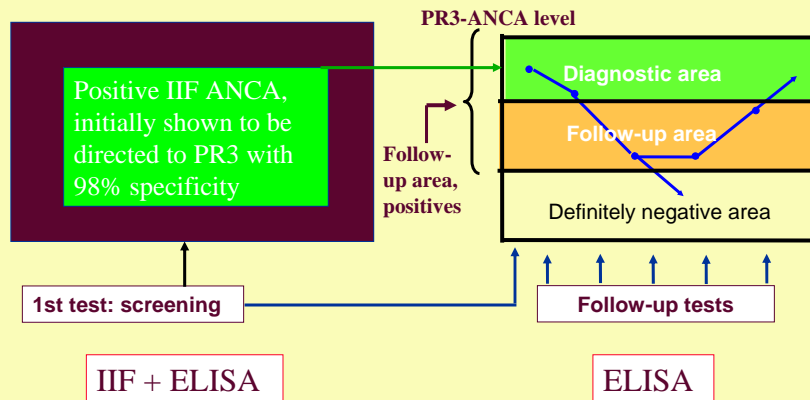


Vasculitis-specific cut-off setting for ANCA ELISAs





Testing for and follow-up of positive PR3-ANCA



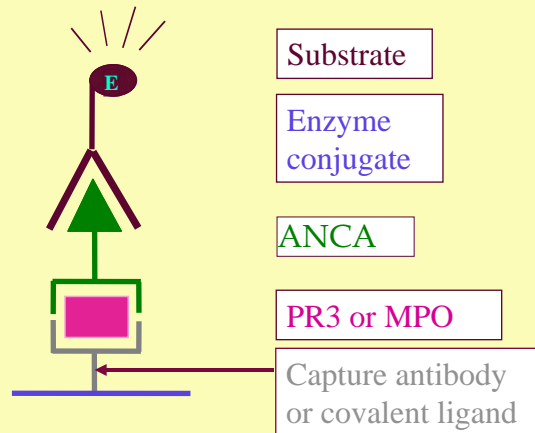
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Methods to detect IgG ANCA

- Indirect immunofluorescence (screen)
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- **Capture enzyme - immunoassay**
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- EliA ANCA assay

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Capture enzyme-immunoassay



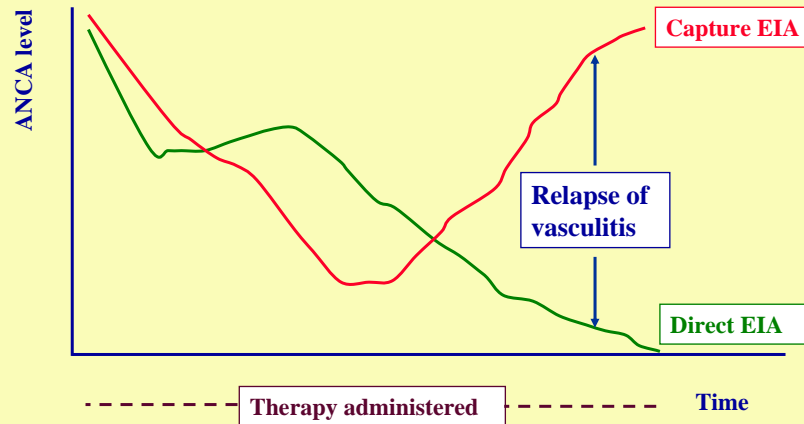
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Advantages of capture EIA

- **Reaction with conformational epitopes**
- **Especially reactive in phases of active disease**
- **More sensitive than direct EIA**
- **Parallels IIF-ANCA better**
- **Large critical control studies are needed (study of differential diagnostic disease controls that mimic vasculitis)**

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Fluctuations of PR3-ANCA levels in Wegener's granulomatosis: potential differences between direct and capture Elisa results



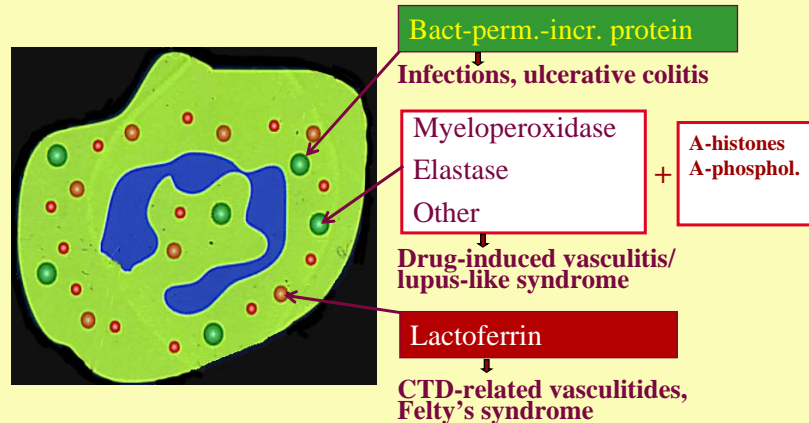
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Methods to detect IgG ANCA

- Indirect immunofluorescence
- Direct enzyme – immuno assay
- Capture enzyme - immuno assay
- Radio - ligand immunoprecipitation
- Adressable laser bead immuno-assay
- **EliA ANCA assay**

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ANCA antigens in other vasculitides



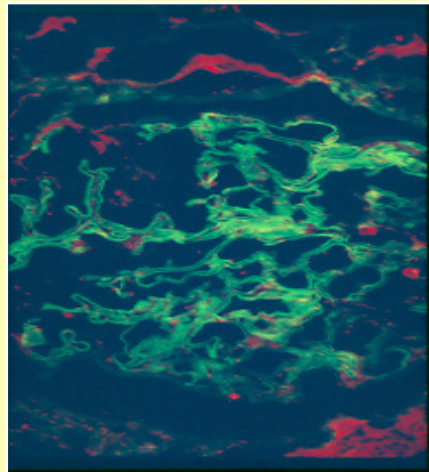
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Characteristics of some rare ANCA

- Note that ANCA directed to **BPI** are characteristic of an **infectious condition** (cystic fibrosis, chronic bronchitis, heart valve infection, ulcerative colitis) etc.
- ANCA directed to **more than one classical granule component** (PR3, MPO, EL, Azurocidin etc.) are found in **drug-induced syndromes** (vasculitis or lupus-like syndrome)
- **EL-ANCA** are characteristically found in patients with **cocaine-induced nasal destruction** but also in some drug-induced syndromes

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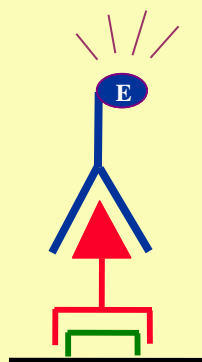
Remember to test for anti-glomerular basement membrane antibodies!



Especially in cases with renal and pulmonary manifestations

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Direct enzyme-immunoassay (anti-GBM)



Substrate

Enzyme-conjugate

Anti-GBM

Alpha-3 domain of type IV collagen

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Chapel Hill Nomenclature

Large vessel vasculitis

- Giant cell (temporal) arteritis
- Takayasu arteritis

Medium vessel vasculitis

- Polyarteritis nodosa
- Kawasaki disease

Small vessel vasculitis

- **Wegener's granulomatosis ***
- **Churg-Strauss syndrome ***
- **Microscopic polyangiitis ***
- Henoch-Schönlein purpura
- Cryoglobulinaemic vasculitis
- **Anti-GBM disease ****
- Cutaneous leucocytoclastic vasculitis

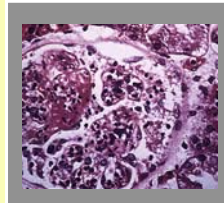
* Common presence of ANCA

**Presence of a-GBM

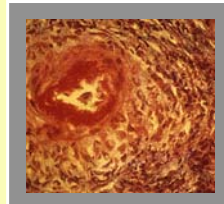
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ANCA-associated vasculitides

Necrotizing capillaritis



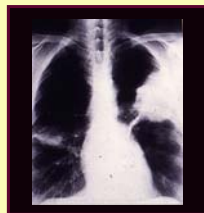
Necrotizing venulitis/arteriolitis



Histopathology common to all necrotizing vasculitides!

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Wegener's granulomatosis

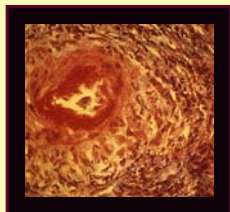
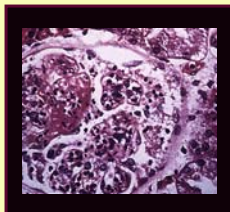


DEFINITION: Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels (e.g., capillaries, venules, arterioles, and arteries).

Necrotizing glomerulonephritis is common.

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Microscopic polyangiitis



DEFINITION: Necrotizing vasculitis, with few or no immune deposits, affecting small vessels (i.e., capillaries, venules, or arterioles). No granulomas. *Necrotizing arteritis involving small and medium-sized arteries may be present. Necrotizing glomerulo-nephritis is very common. Pulmonary capillaritis often occurs.*

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Future of ANCA in diagnostics?

New diagnostic criteria are now being discussed in a collaborative effort between the European League against Rheumatism and the American College of Rheumatology:

-Presence of ANCA determined by standardized and clinically validated technology is likely to become an important criterion for primary small vessel vasculitis.

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New reference reagents for PR3-ANCA and MPO-ANCA

-New reference reagents are now available from Centers of Disease Control and Prevention in Atlanta, Georgia, USA.

-They have been elaborated by the IUIS/WHO/AF/CDC Committee for the Standardization of Autoantibodies in Rheumatic and Related Diseases.

-The reagents can be ordered from the address -

Biological Reference Reagents, NCID/SRP/BRR, Mailstop C-21, Centers for Disease Control and Prevention (CDC), 1600 Clifton Rd. N.E. Atlanta, GA 30333, USA, Phone 404-639-3354, E-mail: Subject -ANA rvogt@cdc.gov

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Conclusions:

- Do not report presence of ANCA in case you have only detected NSA!
- Use whole buffy coat cell mixture as cell substrate to confirm neutrophil specificity.
- Both IIF and ELISA results must be positive to call them ANCA, and strive to report them together.
- IIF is a useful method to screen for NSA, but ANCA antigen specific assays are necessary to reveal their nature.

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Conclusions:

- Presence of ANCA indicates ongoing necrotizing inflammation in small to medium size vessels.
- If ANCA are found in a patient suspect of suffering from polyarteritis nodosa (PAN) the patient has small vessel involvement and thus should be diagnosed as microscopic polyangiitis (MPA) or Wegener's granulomatosis (WG).
- Treatment of the two conditions and the prognosis is very different in most cases.
- True ANCA are credible reporters of vasculitic disease when directed to PR3, MPO

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