Laboratory Testing used in diagnosis and evaluation of Vasculitis

Eisa Salehi PhD

Assistant Professor

Tehran University of Medical Sciences

Classification of vasculitis

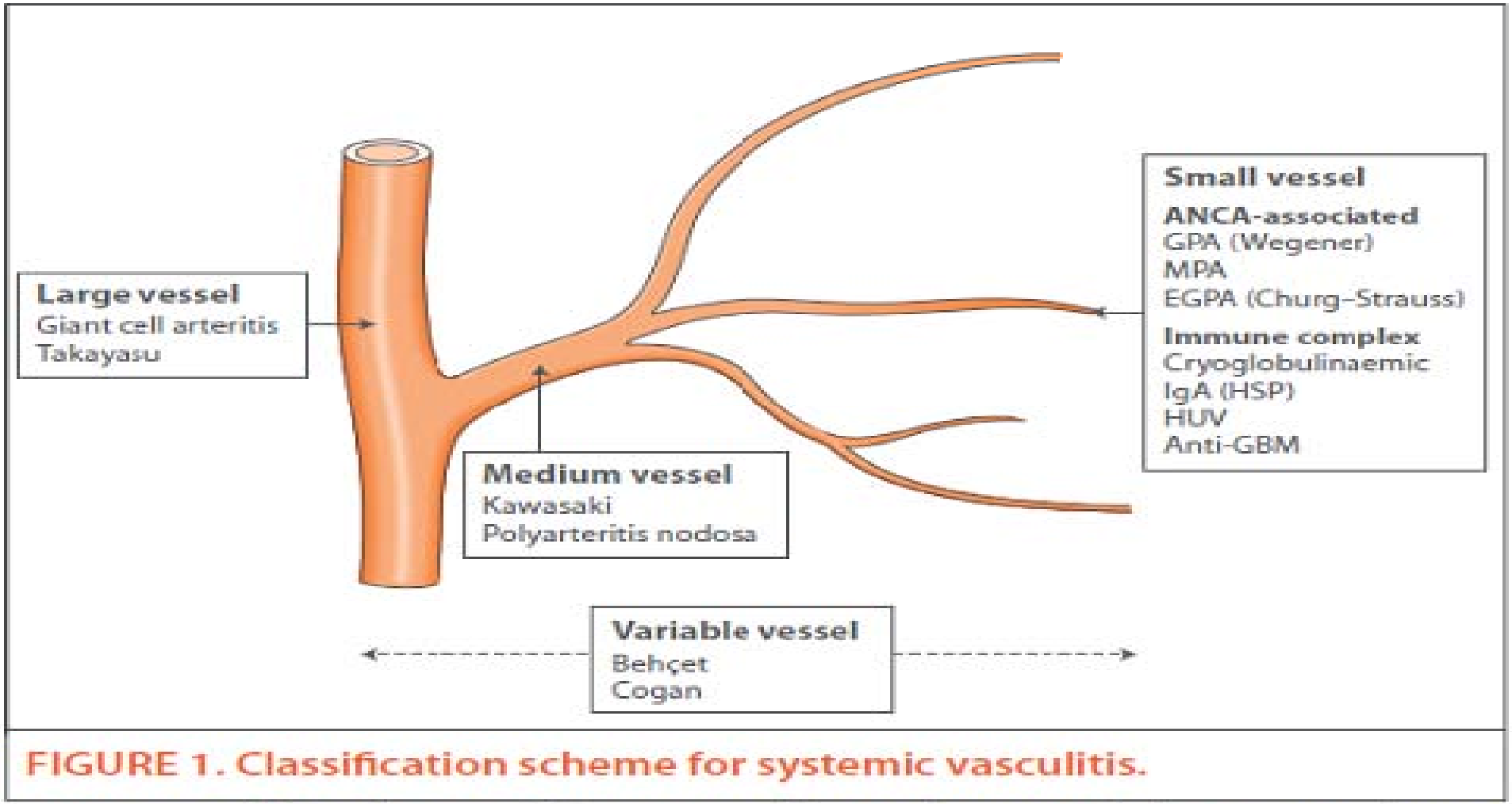


FIGURE 1. Classification scheme for systemic vasculitis.

ANCA antineutrophil cytoplasmic antibody; EGPA eosinophilic granulomatosis with polyangiitis; GBM glomerular basement membrane; GPA granulomatosis with polyangiitis; HSP Henoch-Schönlein purpura; HUV hypocomplementaemic urticarial vasculitis; IgA immunoglobulin A; MPA microscopic polyangiitis

Rationales of laboratory testing in vasculitis:

- Check the general inflammation
- Differential diagnosis
- Check the disease activity
- Check the affected organs for the extent of injury
- Treatment follow up

Lab testing in small vessels vasculitis

- BUN/Cre
- ANCA
- ESR
- CRP
- CBC-D
- Urinalysis, Uprot/cr
- vWAg

ANCA

Anti-Neutrophil Cytoplasmic Antibodies

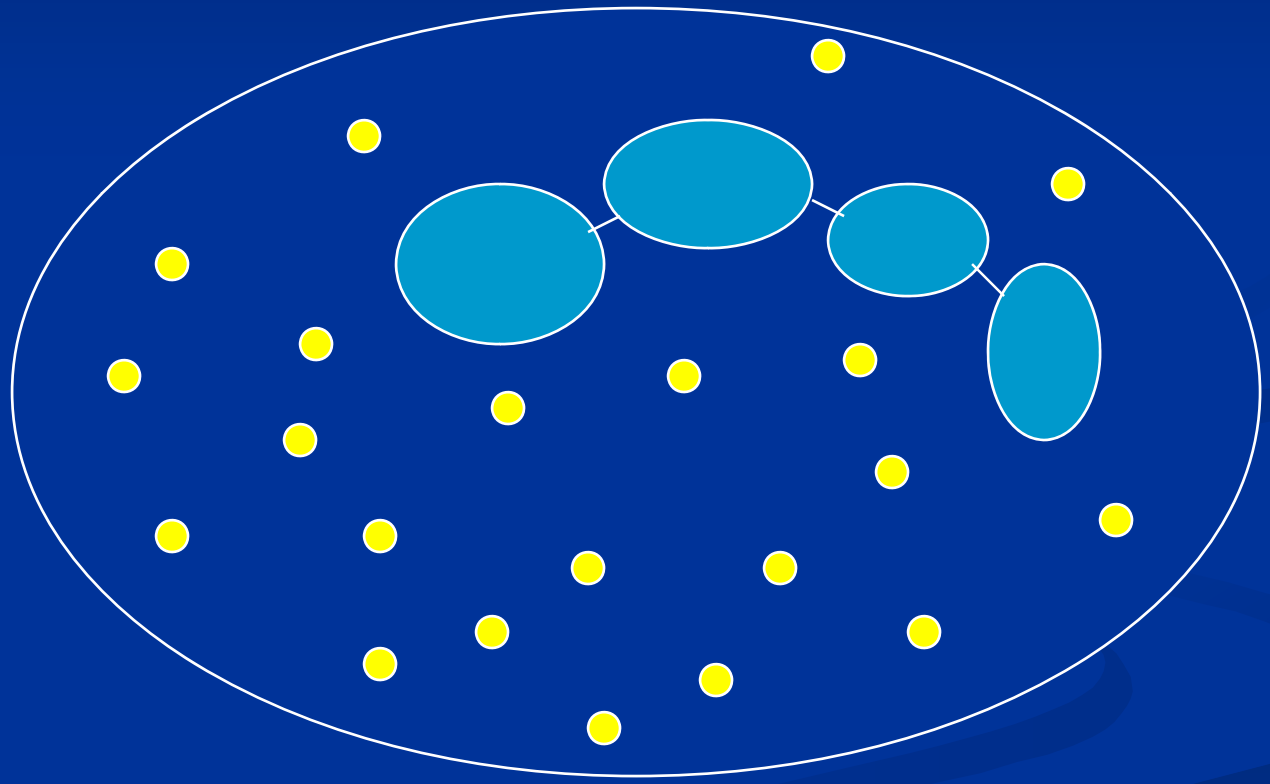
ANCAs were originally described in Davies *et al.* in 1982 in segmental necrotising glomerulonephritis,[14] and by van der Woude *et al.*in 1985 in Wegener's.[15]

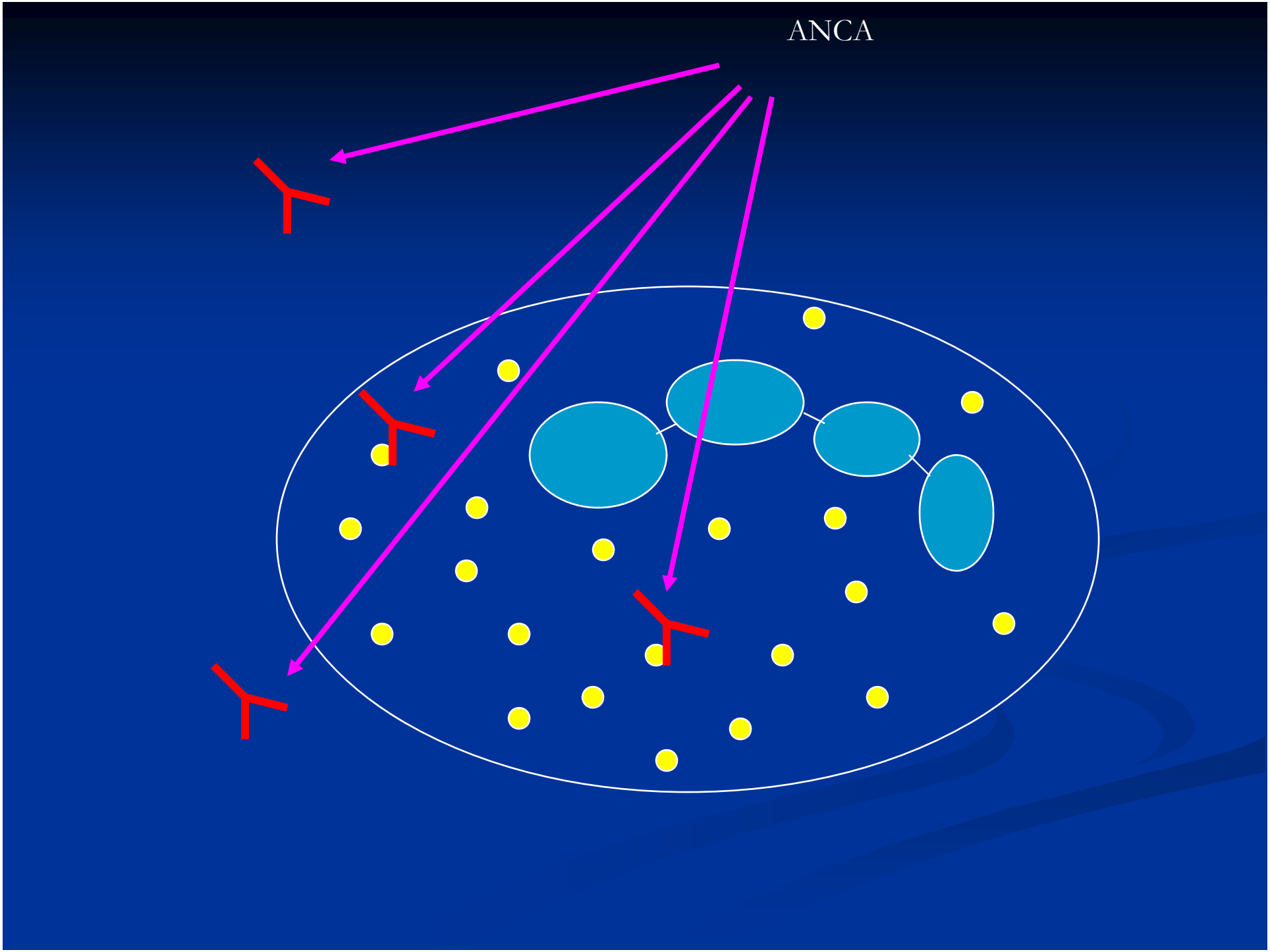
The Second International ANCA Workshop, held in The Netherlands in May 1989, fixed the nomenclature on perinuclear vs. cytoplasmic patterns, and the antigens MPO and PR3 were discovered in 1988 and 1989, respectively.[16]

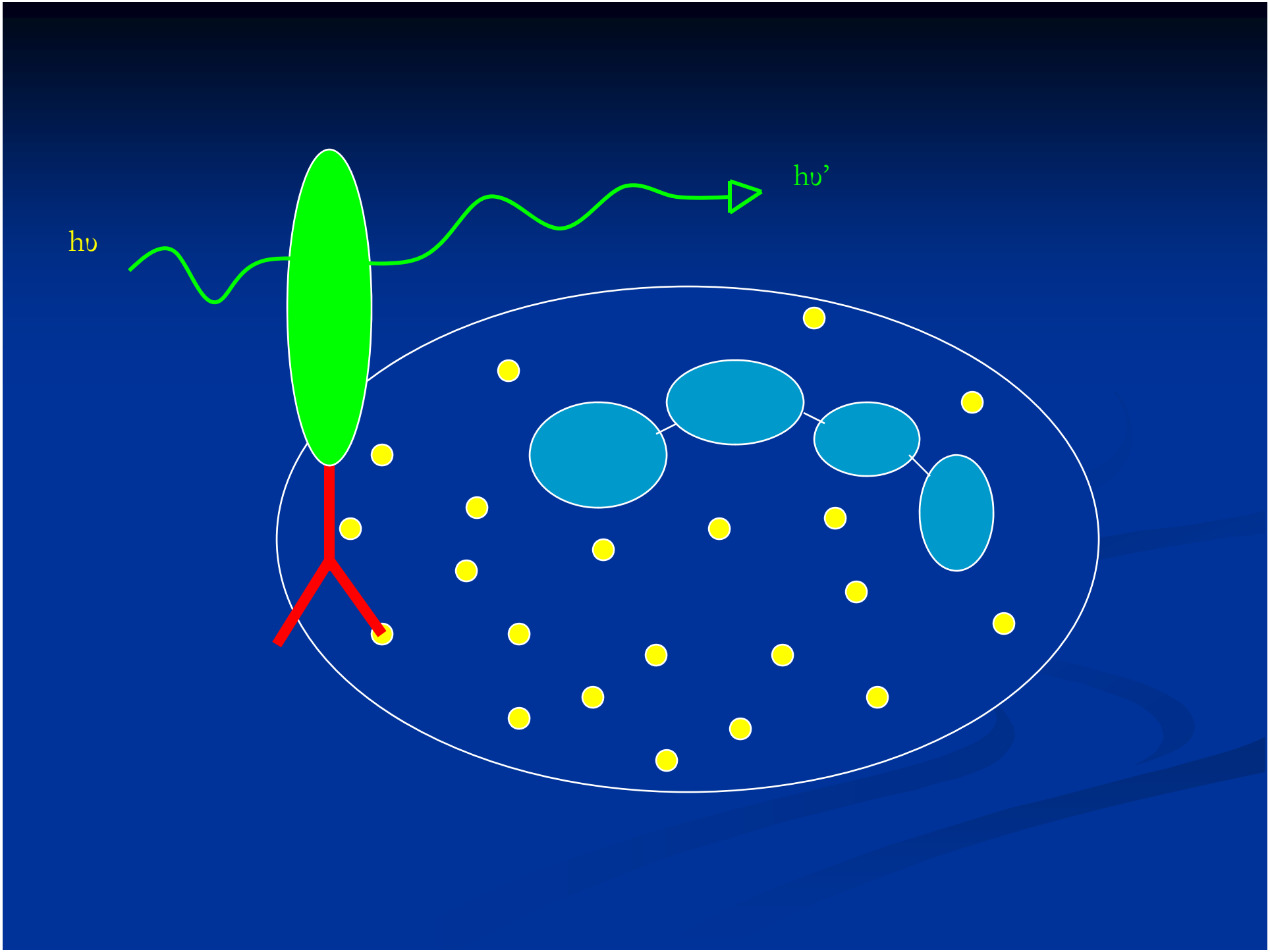
International ANCA Workshops have been carried out every two years.

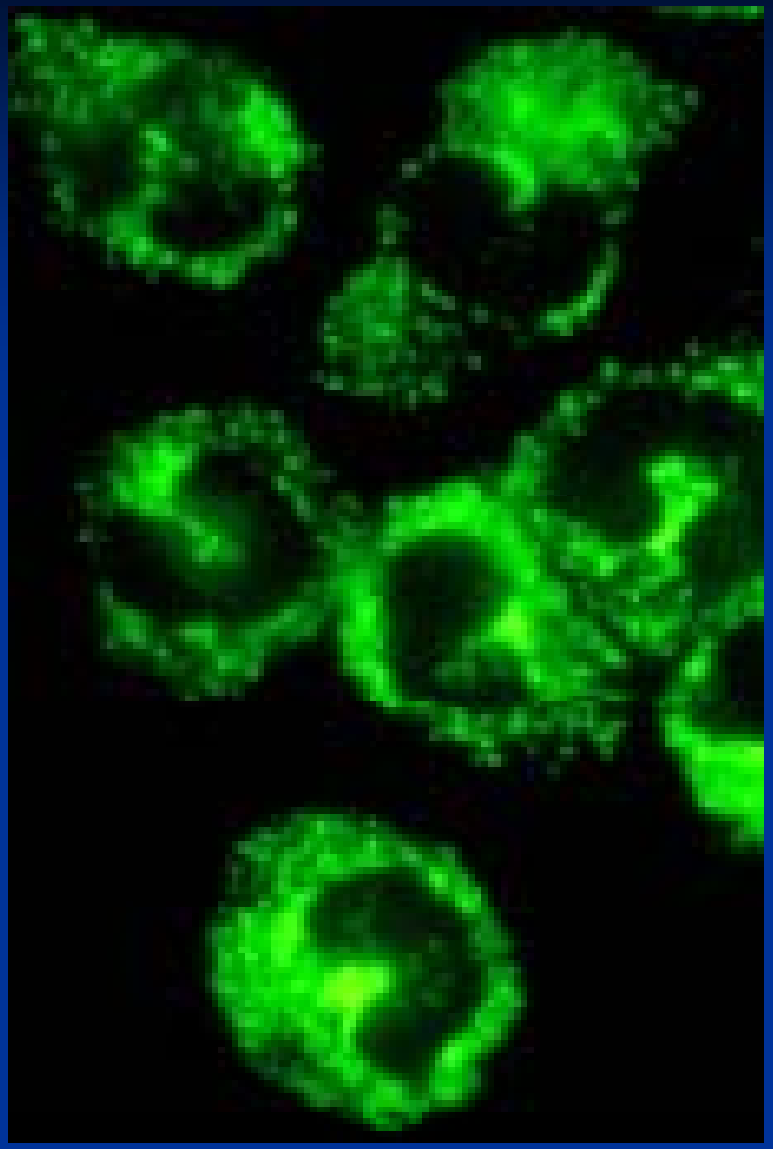
Pathogenetic role of ANCA

- **Clinical evidence**
  Correlation between ANCA values and activity of vasculitis, as well as relapses
  Drug-induced ANCA vasculitis
- ***In vitro* studies**
  Activation of neutrophils by binding of ANCAs → release of destructive enzymes and toxic reactive oxygen radicals, as well as neutrophil extracellular traps;
  factors released by activated neutrophils activate the alternative complement pathway;
  ANCAs bind also to ANCA antigens adsorbed to anionic endothelium and GBM, enhancing complement dependent cytotoxicity;
  ANCAs disregulate neutrophil apoptosis → necrosis;
  specific T lymphocytes for PR3
- **Several animal models of ANCA disease**
  MPO-ANCA, PR3-ANCA, anti-LAMP-2 antibodies




ANCA

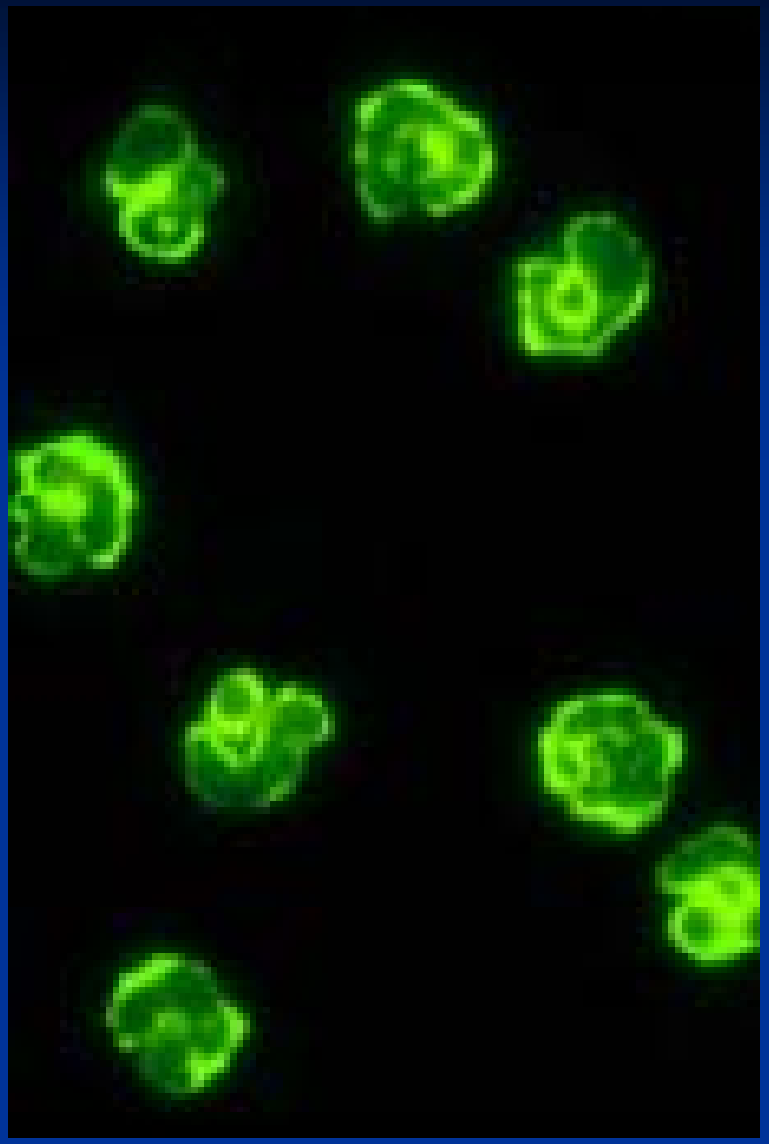

hυ
hυ’



c-ANCA:

Cytoplasmatisch

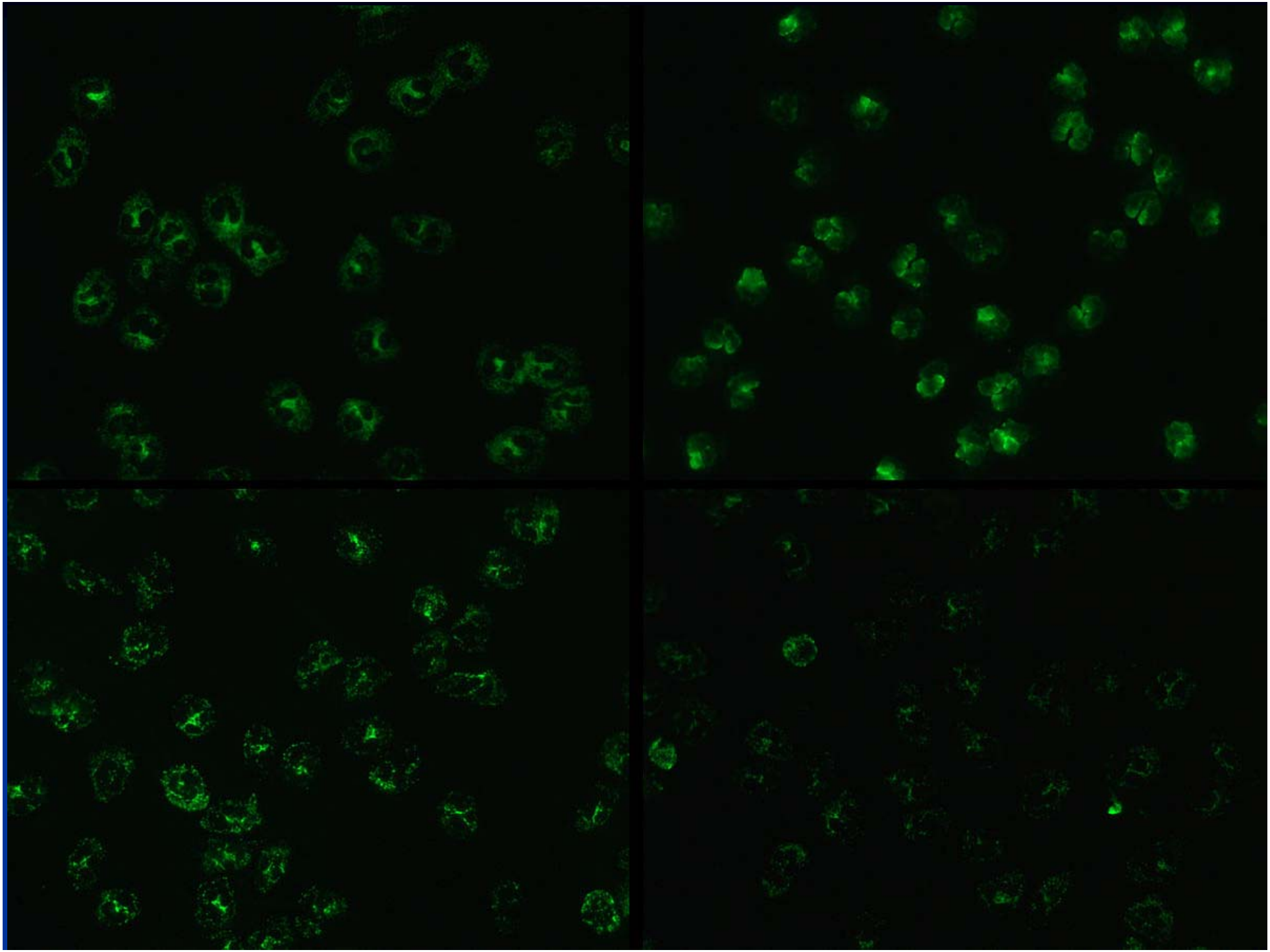
Diffuus, granulair,
cytoplasmatisch patroon.



p-ANCA:

Perinucleair

Perinucleaire tot nucleaire aankleuring.



ANCA can be divided into

- c-ANCA----------PR3
- c-ANCA (atypical)---------- PR3 + BPI
- Perinuclear ANCA (p-ANCA)
  - classical p-ANCA----------MPO
  - p-ANCA without nuclear extension ---- BPI,cathepsin G, elastase, lactoferrin and lysozyme
  - granulocyte specific-antinuclear antibody (GS-ANA).
- atypical ANCA (a-ANCA or x-ANCA).

Other less common antigens include:

- HMG1 (p-ANCA pattern),
- HMG2 (p-ANCA pattern),
- alpha enolase (p and c-ANCA pattern),
- catalase (p and c-ANCA pattern),
- beta glucuronidase (p-ANCA pattern),
- azurocidin (p and c-ANCA pattern),
- actin (p and a-ANCA)
- h-lamp-2 (c-ANCA)

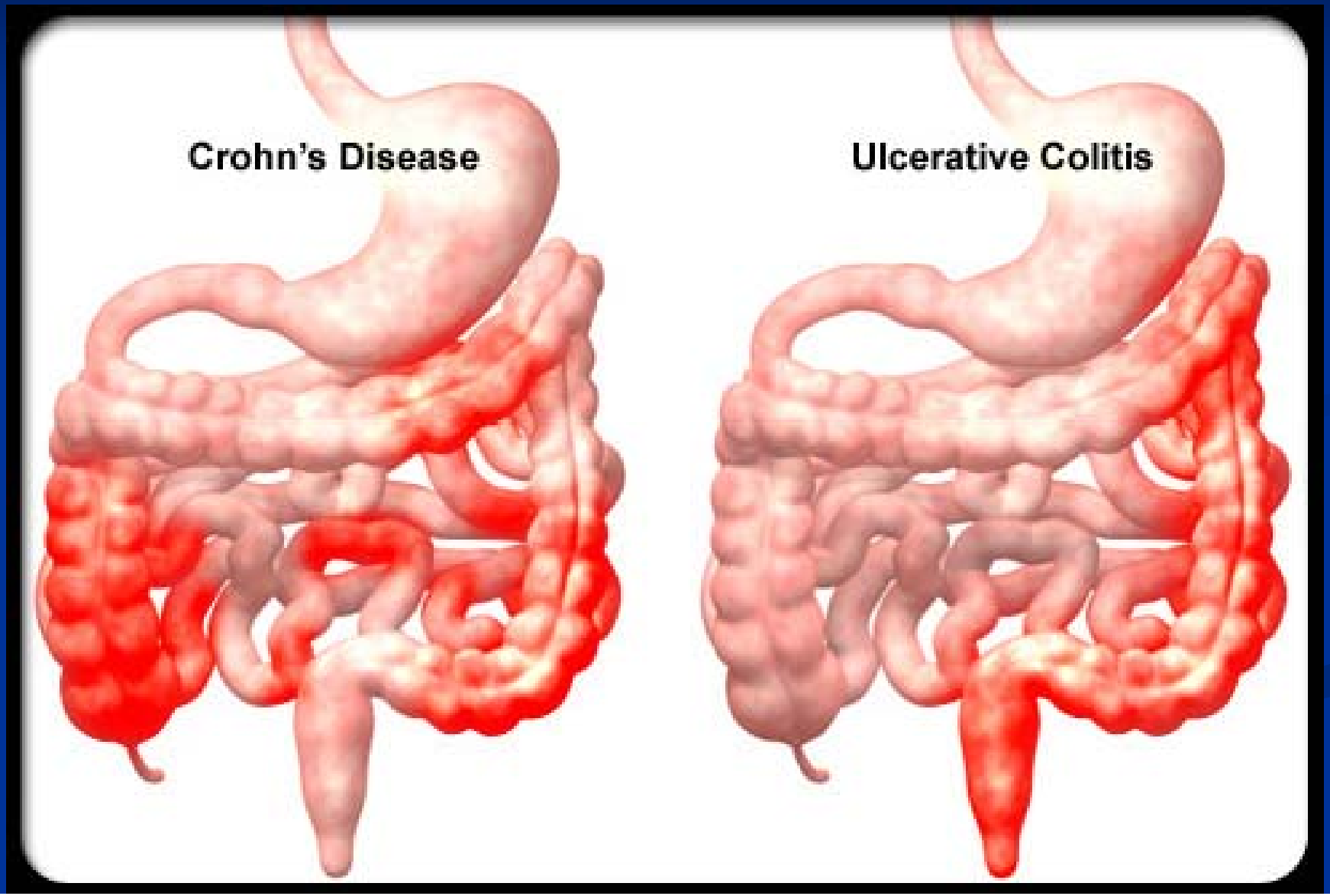
- 85 to 90% of WG patients are positive for ANCA, among them 80% are cANCA positive and 20% are pANCA positive
- In MPA 80% among them 80% are pANCA positive and 20% are cANCA positive
- CSS 40% positive mostly anti MPO

Sensitivity and specificity of ANCA (IIF + ELISA for PR3, MPO) from different studies in the literature

Disease	Sensitivity of ANCA
Limited Wegener's granulomatosis	50-66 %
Generalized Wegener's granulomatosis	80-98 %
Microscopic polyangiitis	82-90 %
Pauci-immune necrotizing extracap GN	90-95 %
Churg-Strauss syndrome	60-70 %

Control group	Specificity of ANCA
Patients with various other diseases	76-91 %
Healthy subjects	94-99 %

Inflammatory bowel disease

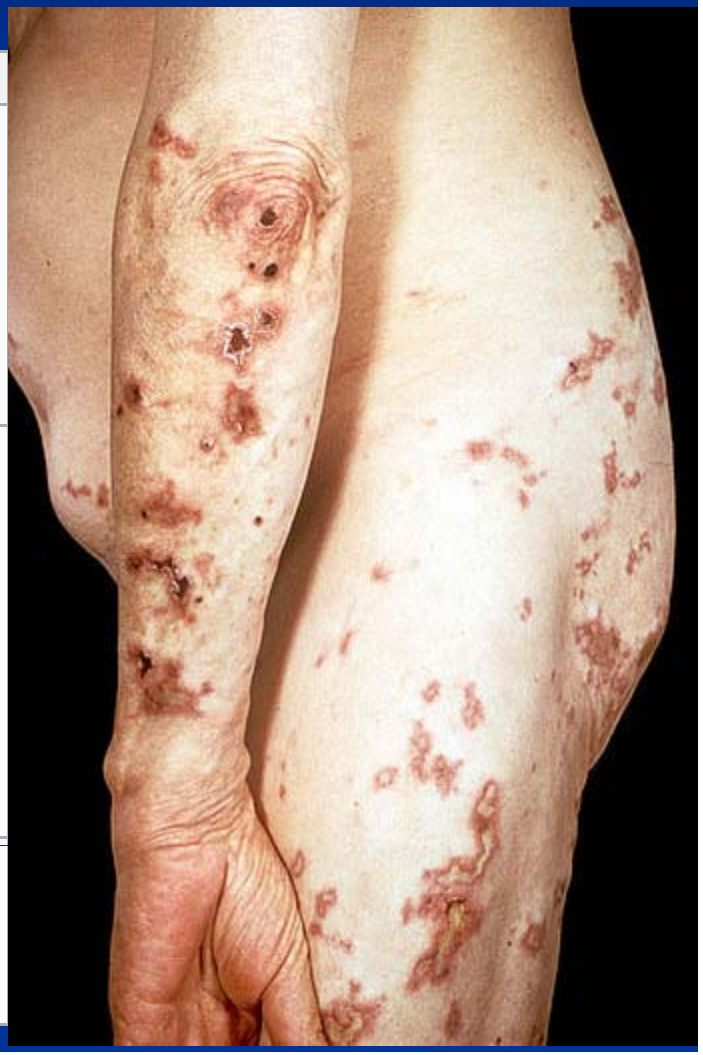


Henoch-Schonlein Purpura Laboratory Findings

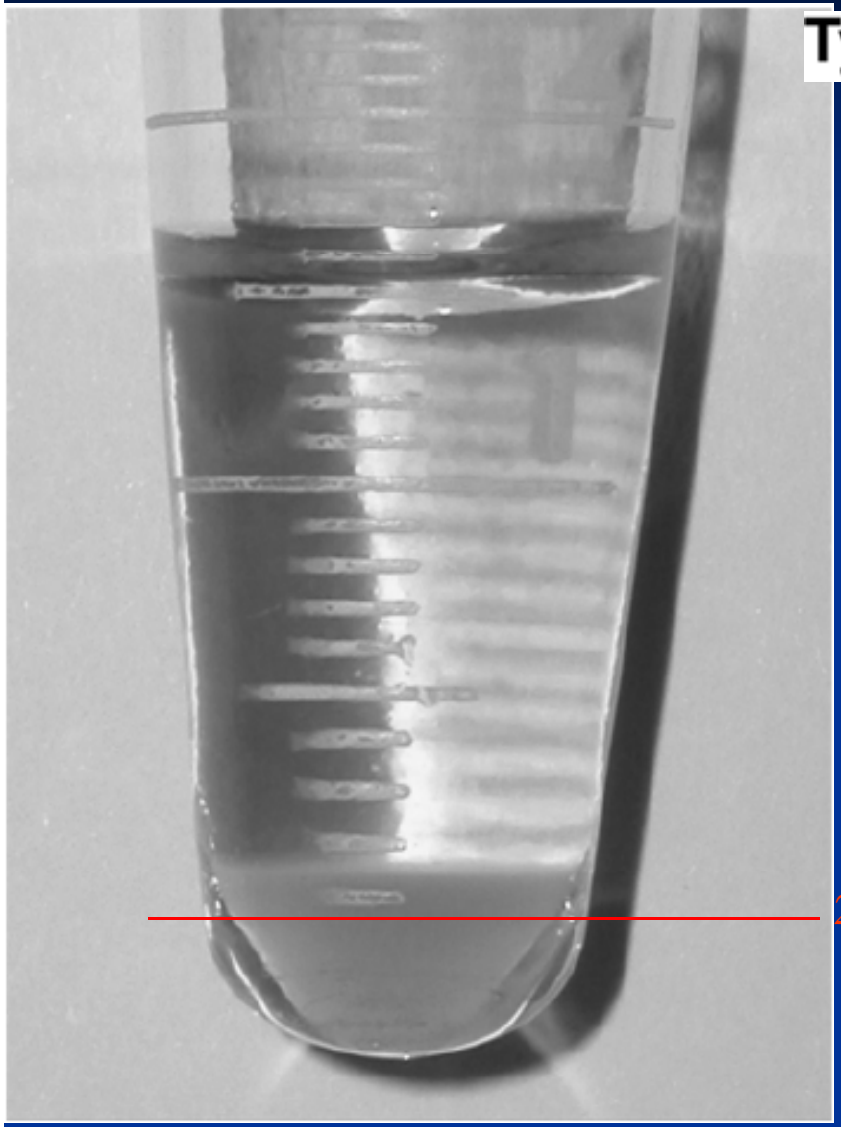
- There is NO definitive diagnostic test.
- **IgA levels** may be elevated in 50-70% of patients.
- Platelet counts and coag studies should be normal.
- Inflammatory markers may be elevated.
- Urinalysis
  - Red cells, white cells, casts, proteinuria
  - May not be present until later in the course
  - Remember to continue UA screenings after the acute phase.
- Negative RF and ANA.

Cryoglobulinemia

Type	Composition	Percent
Type I	isolatedmonoclonalimmunoglobulins	10–15% of the total cases
Type II	immunocomplexesformed bymonoclonal IgM	50–60% of reported cases
Type III	immunocomplexesformed bypolyclonal IgM	25–30% of the reported cases

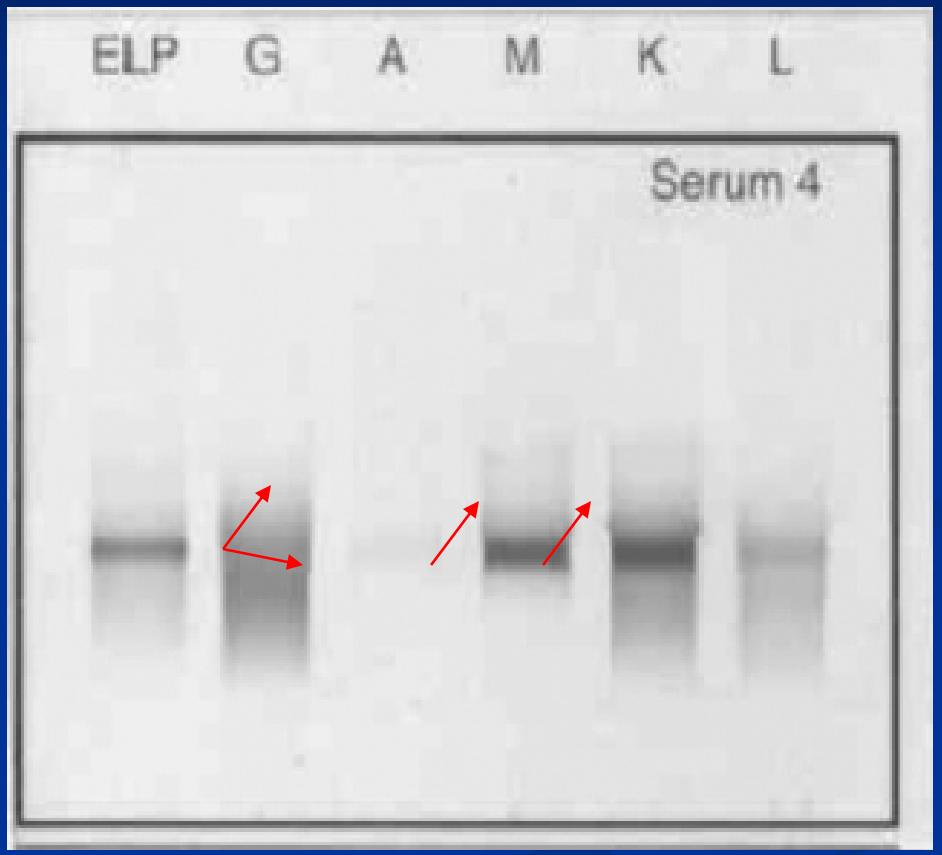


Cryoglobulinemic vasculitis



Cryoglobulin precipitate in a cryocrit tube

Type II (IgG - IgM-κ) cryoglobulinemia



Serum protein electrophoresis

Kawasaki disease

Laboratory Evaluation

- Markers of systemic inflammation
  - Elevated CRP, ESR, leukocytosis with left shift, **reactive thrombocytosis** (up to 1 million)
- Anemia (normocytic, normochromic)
- Sterile pyuria (urethral origin, don't do a cath)
- Transaminase elevation (mild to moderate)
- CSF findings
  - Mononuclear pleocytosis, hypoglycorrhachia, elevated protein
- Synovial fluid inflammation

Urgently need good biomarkers

- To distinguish active disease from damage or infection
- To predict relapse, treatment response, and prognosis.
- In the prototypic small-vessel vasculitis, the autoantibodies for which it is named are unsuitable as biomarkers of disease activity.
- A recent meta-analysis concluded that the data is insufficient to support using persistently positive or rising ANCA titer alone to guide treatment decisions.
- Also, traditional acute-phase reactants such as erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) lack sufficient sensitivity and specificity to predict relapse in a clinically significant manner.

New biomarkers not ready for primetime

- **Subsets of B and T cells**
- **Activated (CD19+/CD38+) B cells** vs **CD25+ regulatory B cells**
- For some patients taking rituximab the combination of B cell reconstitution and PR3 level can serve as a biomarker for relapsing disease.
- Activated T cells are detectable in the sera of AAV patients with both active and quiescent disease, but transcriptome analysis has identified CD8+ T cell expression profiles that divided patients into two distinct subgroups differing only in their risk of relapse.

New biomarkers not ready for primetime

- **Activation of the alternative complement pathway** is implicated in AAV pathogenesis.
- Patients with active renal AAV show higher circulating plasma levels of **C5a**, a common pathway component, and of **fragment Bb**,
- 3 proteins **CXCL13 (BCA-1), (MMP-3) and tissue inhibitor of metalloproteinases-1 (TIMP-1)** that may distinguish active AAV from remission better than ESR and CRP.

large vessel vasculitides, Giant Cell Arteritis (GCA) and Takayasu Arteritis (TA),

- Elevations of traditional inflammatory markers are not specific for active vasculitis (and may be affected by therapy).
- **IL-6** is elevated in the arterial lesions and the peripheral circulation in GCA and TA.
- IL-6 levels in the serum correlate with disease activity.[13] This observation may inform therapy:
- A large study using IL-6 blockade with tocilizumab is under way for the treatment of new and relapsing GCA.[14]

- Levels of **another pro-inflammatory cytokine, IL-17**, are also increased in the inflammatory lesions in active GCA. They may predict response to glucocorticoid treatment.
- Certain circulating proteins including several **matrix metalloproteinases** have been suggested to be useful biomarkers for TA disease activity.

Very recent biomarkers:

- Pentraxin-3 in giant cell arteritis and Takayasu's arteritis;
- von Willebrand factor antigen in childhood central nervous system vasculitis;
- eotaxin-3 and other markers related to eosinophils or Th2 immune responses in eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome);

- Curr Opin Rheumatol. 2014 Jan;26(1):24-30. doi: 10.1097/BOR.0000000000000009.


Thanks for attention