ANCA Associated Vasculitis: A Personal Story of 35 Years of Research

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North America
all happy songs:
Happy - Pharrell Williams
Good Vibrations - The Beach Boys
Hallelujah - Leonard Cohen

Europe
all sad songs:
Paint it black - Rolling Stones
While my guitar gently weeps - The Beatles
Cold coffee - Ed Sheeran
1919 women’s suffrage
1919 Nobel prize medicine
Jules Bordet
for the discovery of complement
# My personal (research) voyage

<table>
<thead>
<tr>
<th>Company</th>
<th>Nature of Affiliation</th>
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<tbody>
<tr>
<td>* InflaRx</td>
<td>Chairman of the DSB</td>
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InflaRx has started a phase II study with a complement blocker

**STOP BACTERIA!**

**MYELOPEROXIDASE**

**YES**

**NOT**
Case report
(personal motivation)

• 1984
• 34-yr old female severely ill
• Arthralgias, dyspnea
• Renal insufficiency
• 3 weeks before ‘hair dye’ allergy
Red blood cell casts
Local focal necrotising crescentic glomerulonephritis;
IF: *pauci-immune*
AUTOANTIBODIES AGAINST NEUTROPHILS AND MONOCYTES: TOOL FOR DIAGNOSIS AND MARKER OF DISEASE ACTIVITY IN WEGENER’S GRANULOMATOSIS

F. J. van der Woude
S. Loratto
N. Rasmussen
A. Wink
Case report
(personal motivation)

• 1984: 34-yr old female severely ill
• Arthralgias, dyspnea, renal insufficiency
• 3 weeks before hair dye allergy
• **Histology:** pauci-immune NCGN
• **C-ANCA:** negative
History

Immune Complex Small Vessel Vasculitis
  Cryoglobulinemic Vasculitis
  IgA Vasculitis (Henoch-Schönlein)
  Hypocomplementemic Urticarial Vasculitis
    (Anti-C1q Vasculitis)

Medium Vessel Vasculitis
  Polyarteritis Nodosa
  Kawasaki Disease

Anti-GBM Disease

ANCA-Associated Small Vessel Vasculitis
  Microscopic Polyangiitis
  Granulomatosis with Polyangiitis
    (Wegener’s)
  Eosinophilic Granulomatosis with Polyangiitis
    (Churg-Strauss)

Large Vessel Vasculitis
  Takayasu Arteritis
  Giant Cell Arteritis

Jennette et al. Arthritis Rheum 2013
History


Matani (1761): probably first description of a vasculitis patient (Pisa)

Rokitansky (1852): nodular lesions in arteries throughout the body

Eppinger (1887): Rokitansky’s patient had histologic evidence of arteritis
1866: Periarteritis Nodosa
Adolf Kussmaul and Rudolf Maier

- 27 yr old man with fever, weakness, profound muscle weakness, neuritis, abdominal pain, cutaneous nodules, and oliguria

- Gross pathology: numerous nodules in small and medium-sized arteries in many tissues

- Histology: inflammation and necrosis in arteries
History


Gabriel Godman

First descriptions of GPA:
* Klinger H. Frankf Z Path 1931
* Wegener F. Beitr Path Anat 1939

Godman and Churg concluded in 1954 that GPA, EGPA and MPA are related and differ from Classic Periarteritis Nodosa

Arch Pathol 1954;58:533-553
According to the Chapel Hill Nomenclature System
pauci-immune CGN:

- GPA
- EGPA
- MPA

Falk RJ et al. *Arthritis Rheum* 2011
C-ANCA negative....but in the ANCA (IFT) test we found a cloudy pattern of staining

2nd year resident in Internal Medicine

Research in vasculitis
Myeloperoxidase
1941 isolated as “verdoperoxidase” by Kjell Agner

- Ellen van der Schoot:
Characterization of myeloid leukemia by monoclonal antibodies, with an emphasis on antibodies against myeloperoxidase

- stored in azurophilic granules of neutrophils and lysosomes of monocytes

\[ \text{H}_2\text{O}_2 + \text{Cl}^- \xrightarrow{\text{MPO}} \text{H}_2\text{O} + \text{OCl}^- \]
P-ANCA

Staining of alcohol-fixed neutrophils

Cytoplasmic

C-ANCA

Staining of formalin-fixed neutrophils

Cytoplasmic

Perinuclear

P-ANCA

Staining of formalin-fixed neutrophils

Cytoplasmic

Immunoassay antigen specificity

Proteinase 3 (PR3-ANCA), Myeloperoxidase (MPO-ANCA), elastase

In the beginning.....

35 patients with pauci-immune necrotizing and/or crescentic glomerulonephritis:
9 pts biopsy proves GPA: all PR3-ANCA
26 pts MPA:
12 x PR3-ANCA; 11 x MPO-ANCA
3 x infectious GN; no ANCA

(Cohen Tervaert et al. Kidney Int 1990)
## ANCA-associated vasculitis:

A third group in GPA, MPA and EGPA: **ANCA negative patients**

<table>
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<tr>
<th></th>
<th>PR3</th>
<th>MPO</th>
<th>Neg</th>
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<tbody>
<tr>
<td>GPA</td>
<td>80%</td>
<td>15%</td>
<td>5%</td>
</tr>
<tr>
<td>MPA</td>
<td>20%</td>
<td>70%</td>
<td>10%</td>
</tr>
<tr>
<td>EGPA</td>
<td>5%</td>
<td>35%</td>
<td>60%</td>
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Loco-regional GPA  
Non-renal MPA  
Non-renal EGPA

*Cohen Tervaert et al Arch Int Med 1989;  
Cohen Tervaert et al Kidney Int 1990;  
Cohen Tervaert et al Arthritis Rheum 1990;  
Clinical findings in small vessel vasculitis

- Glomerulonephritis
- Cutaneous vasculitis
- Pulmonary hemorrhage
- Multiple lung nodules
- Chronic destructive disease of the upper airways
- Long-standing sinusitis or otitis
- Mononeuritis multiplex or peripheral neuropathy
- (Epi)scleritis
In the beginning, part II ....

**PR3-ANCA versus MPO-ANCA:**
- PR3-ANCA more *generalized* disease
  - (5.10 organs versus 3.16 in MPO-ANCA)
- PR3-ANCA More *respiratory tract involvement*
  - (96% versus 67%)
- Granulomatous *inflammation* more often in PR3-ANCA than in MPO-ANCA
  - (53% versus 4%)
- MPO-ANCA pts more often *nasal polyps, asthma and/or eosinophilia* suggesting EGPA
- MPO-ANCA pts more often *renal-limited* disease

*Cohen Tervaert et al. Arthritis Rheum 1990*
*Goldschmeding et al. Neth J Med 1990*
PR3-ANCA: ALH

MPO-ANCA: Lung Fibrosis
Disease-free survival in 128 patients with ANCA-associated vasculitis according to ANCA specificity

Follow-up since diagnosis (years)

Disease-free survival (%)

MPO-ANCA

PR3-ANCA

p = 0.0021
RR 3.2 (1.4 - 4.4)

Genetics differ between PR3-ANCA and MPO-ANCA associated vasculitis

*Lyons et al New Engl J Med 2012*

**PR3-ANCA:**
- HLA-DP
- Alpha-1-antitrypsin (SERPINA1)
- PR3 (PRTN3)

**MPO-ANCA:**
- HLA-DQ
It is time for a change

Classification based on ANCA serotype:

PR3-ANCA GPA versus MPO-ANCA GPA

PR3-ANCA MPA versus MPO-ANCA MPA

MPO-ANCA EGPA versus ANCA negative EGPA

Hilhorst et al. JASN 2015
Cohen Tervaert JW. NDT:2019
ANCA is also a biomarker

Biomarker for relapse: C-ANCA measurement:
29 patients with ANCA rises; 26 followed by a relapse

MPO-ANCA also a biomarker for disease activity

Cohen Tervaert et al Arthritis Rheum 1990
Boomsma et al Arthritis Rheum 2000
The predictive value of an ANCA rise in the entire cohort and subgroups of patients with AAV

ANCA rises predictive in renal patients

MPO-ANCA = PR3-ANCA

Kemna et al. JASN 2015;26:537-542
ANCA also a biomarker to predict relapsing disease activity

ANCA pathogenic?
Superoxide release measured by ferricytochrome c reduction using tumor necrosis factor-α (TNF-α)-primed neutrophils from a healthy donor. Neutrophils stimulated with IgG fractions from PR3-ANCA and MPO-ANCA positive patients.

*Franssen et al. JASN 1999*

*In vitro* studies demonstrate that ANCA activates primed neutrophils and monocytes.
ANCA: a pathogenetic antibody?

MPO/anti-MPO associated crescentic glomerulonephritis

- Intracapillary thrombosis
- Capillary wall necrosis
- Influx of inflammatory cells
- Proliferative crescentic glomerulonephritis

Day 1
Day 4
Day 10

Human MPO + Neutrophil extract + H₂O₂ 2 weeks

Rat models for anti-MPO associated vasculitis/glomerulonephritis

Summarized in: Heeringa et al. 
Kidney Int 1998
A new era: MPO knock-out
Passive transfer of MPO-ANCA

<table>
<thead>
<tr>
<th>Type of IgG</th>
<th>Recipient mouse</th>
<th>% crescents</th>
<th>% necrosis</th>
<th>BUN (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-MPO</td>
<td>C57Bl/6j</td>
<td>3.3 (2-6)</td>
<td>4.7 (3-7)</td>
<td>23.3 (21-27)</td>
</tr>
<tr>
<td>Anti-BSA</td>
<td>C57Bl/6j</td>
<td>0</td>
<td>0</td>
<td>25.7 (22-29)</td>
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MPO-ANCA+ LPS: more severe renal lesions than MPO-ANCA without LPS:

*the second hit*
Human mice injected with human PR3-ANCA and LPS

Little MA et al. PLOS One 2012 -the Irish contribution-
3 major factors in AAV

ANCA

Neutrophils

Activating trigger

Inflammation of the vessel wall (vasculitis) caused by white blood cells that have been stimulated by ANCA
Up-to-date: How to treat vasculitis?
Rituximab comprises human IgG and $\kappa$-constant regions. The variable region is from the anti-CD20 murine antibody fragment IDEC-2B8.

Rituximab vs. Cyclophosphamide in ANCA Vasculitis

• RITUXVAS
  - Rituximab + IV cyclophosphamide vs. IV cyclophosphamide

• RAVE
  - Rituximab vs. oral cyclophosphamide
RITUXVAS: Trial Overview

Control

Rituximab

CYC & MP

Azathioprine

Rituximab

CYC & MP

All patients

Steroid taper
Rituximab severe AAV results
*NEJM 2010*

- Median age 68 yrs; median GFR 18
- Sustained remission: 76% versus 82%
- Death: 18% versus 18%

*Rituximab is not superior to standard iv cyclophosphamide and not associated with less SAEs*
Treatment Effect According to Prednisone Dose at 6 Months

OR for CR (t=6 months) in PR3-ANCA: 2.11; MPO-ANCA: NS

Relapsers: more often CR when treated with Rituximab

Adverse events similar frequency in both groups

Rituximab: the first proven alternative of CYC induction for ANCA-associated vasculitis

Q: For severe cases: add cyclophosphamide infusions in the beginning?
What about the neutrophil?
Pauci-immune GN but.....
C3C found in kidney biopsies of AAV patients

Brouwer et al. Kidney Int 1994
Hilhorst et al. NDT 2017
Therapy using antibodies to C5 ameliorate disease

(Huugen et al. Kidney Int 2007)
Complement in MPO-ANCA mouse model

- C5 -/-: no disease
- Classical pathway -/-: disease
- Alternative Pathway -/-: no disease
- C5b-C9 pathway -/-: disease

Xiao et al. AJP 2007; Xiao et al JASN 2014
Small study: Avacopan; no prednisone: Non-inferior

Jayne et al. 2017
High dose steroids needed?

114 patients
52 MP; 62 no MP
Outcome no difference;
More infections (HR 2.7) and more DM (HR 6.33)
Chaouzas et al. BMC Nephrol 2019

49 patients:
RTX + RTX + 1 - 2 weeks Pred
At 6 months 47/49 remission
Pepper et al. Rheumatol 2019
LET'S HAVE ONE MORE
AND THEN WE'LL GO!!
AAV pathomechanisms: it takes two to tango

What about the triggers?
ANCA rise in the fall:
HR 4.37 (1.60 – 11.90)

Time to relapse after an ANCA rise according to season

Kemna et al. J Rheumatol 2017
ANCA and Neutrophil-Mediated Cytotoxicity

∗ neutrophils express surface ANCA antigens in response to cytokine stimulation
Cytokine production due to an infection

The second hit
ANCAs bind to surface ANCA antigens and stimulate neutrophil degranulation.

Diagram:
- Antibody binding
- Degranulation
- Endothelial damage
Triggers

**PR3-ANCA**

*PD-1 blockers* *(Cohen Tervaert et al. NEJM 2018)*
*St. Aureus* *(Stegeman et al NEJM 1994)*
*Vitamin D* *(Kemna et al. J Rheumatol 2017)*

**MPO-ANCA**

*Silica* *(Cohen Tervaert et al. 1998)*
*Smoking* *(Cohen Tervaert 2019)*
*Silicones* *(Cohen Tervaert et al. 2013)*
*Mesh* *(Cohen Tervaert 2019)*
The two-hit hypothesis: ANCA and infection

B. Generalized AAV

Time (months)

Proportion of patients without relapse

0 2 4 6 8 10 12 14 16 18

HR 4.10 (1.37-12.25; p=0.01)

Chronic SA carriers vs.
Intermittent carriers / non-carriers

Time (months)

Sarmela et al. Rheumatology 2017

NORAM and CYCAZAREM study:
At diagnosis few patients
S. Aureus carrier
(mainly PR3-ANCA)
High Mobility Group Box 1 protein

Stress
Injury

Infection

Passive secretion
Necrotic

Active secretion
Inflammatory

Cohen Tervaert JW. NDT 2019
Infections act as adjuvants
The two-hit hypothesis
Both HMGB1 and ANCA stimulate NET generation

ANCA aetiology: triggers all inducers of NET formation

*S. Aureus*

*Other infections*

Silica

Smoking
Breakdown of immune tolerance

DC loaded with NET components induce ANCA in mice

Sangaletti et al. Blood 2012
In autoimmunity NETosis is pivotal.

**FMF**
- NET-mediated bioactive IL-1β exposure has been implicated in the pathophysiology of FMF.

**RA**
- NET-mediated bioactive IL-17 exposure triggers collagen production from myofibroblasts in pulmonary fibrosis, while in psoriasis it causes keratinocyte hyperplasia.

**SLE**
- NET-mediated bioactive PAD4 exposure citrullinates proteins that act as autoantigens in RA.

**VASCUlITIS**
- NET-mediated bioactive TF exposure triggers coagulation process and thrombus formation.
NETs activate complement

Wang et al 2015; Segelmark et al. 2016
Septra (2 x 960 mg) maintenance therapy
Efficacy in 81 patients with GPA in remission

63% relapse reduction
Take home message: ANCA vasculitis

Both MPO-ANCA and PR3-ANCA induce the disease: **RITUXIMAB**

Complement and NET formation play an important role in PMN activation:

Avacopan and other complement blockers (in trial)

A second hit is (often) crucial: **SEPTRA**
Outcome of AAV:
- More atherosclerotic and thrombotic events
- Fatigue
Acknowledgement vasculitis studies Maastricht, Groningen and Chapel Hill

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- Prof. Jan Weening
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NIERSTICHTING