containing staphylococcus organisms [15] that promotes relapse in a completely different fashion. In sum, the differences between various forms of ANCA vasculitis have much to do with genetics and in particular with MHC, with epigenetic control of specific MPO and PR3 expression or the encounter of a mimic or a complementary mimic of these epitopes, and with environmental encounters including infection and other environmental immunologic stimuli such as silica exposure [16].

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L27. Antibodies versus phenotypes: A clinician’s view

Introduction
Prior to 1985, several authors had pointed to associations of anti-neutrophil autoantibodies and vasculitis [1,2]. However, the report by Fokko van der Woude and colleagues of auto-antibodies to neutrophil cytoplasmic antigens in the sera of patients with Wegener’s granulomatosis (now granulomatosis with polyangiitis, GPA) that appeared to correlate with disease activity attracted more attention [3]. It is hard to overestimate the effect these discoveries have had in advancing understanding of vasculitis as evidenced by the sequence of International ANCA vasculitis workshops that began in 1988. Shortly after their association with GPA, ANCA were found in the sera of patients with microscopic polyangiitis (MPA), and the antigenic targets, proteinase 3 and myeloperoxidase were identified in 1988 and 1990 [4,5]. Efforts to standardize ANCA testing in a European Union (EU) funded initiative failed to achieve satisfactory reproducibility with indirect immunofluorescence (IF), even in expert laboratories, but demonstrated that variability could be reduced to acceptable levels by antigen specific ELISA’s provided there was a common source of pre-coated ELISA plates and careful adherence to an agreed protocol [6]. Unfortunately, despite the use of international...
standard sera, IIF remains unstandardised and antigen specific
assays vary widely in their sensitivity and specificity with
consequent impact on the variability of ANCA performance
in clinical studies [7].

Antibodies and classification
The core phenotype of GPA, with the triad of ENT, lung and renal
involvement was well understood prior to 1985, but the term
MPA was not consistently used. The 1990 American College of
Rheumatology (ACR) classification of vasculitis did not use the
term MPA, such presentations being mostly included in poly-
arteritis nodosa (PAN). Nephrologists were familiar with ‘pauci-
immune’ crescentic nephritis, either as an isolated entity,
then called idiopathic rapidly progressive glomerulonephritis
(RPGN), or in the context of MPA/PAN, or indeed GPA. Between
1985 and 1990, important concepts emerged: firstly, as most
patients with a pauci-immune RPGN were ANCA positive, there
might be a common pathogenesis and the syndromes of GPA,
MPA and, now, renal-limited vasculitis, could be combined as
ANCA-associated vasculitis (AAV); and secondly, that MPA was
an entity that could be clearly differentiated from PAN on the
basis of ANCA positivity and the size of blood vessel involve-
ment. The observation of serological overlap with 20% of GPA
patients being MPO-ANCA and 50% of MPA patients being
PR3-ANCA positive also supported a common terminology. These
concepts were articulated in the first Chapel Hill Consen-
sus Conference, drove the definition of the study population
in EU clinical trials, and were further supported by in vitro
studies of neutrophil activation.

Difficulties with the term ANCA-associated vasculitis have arisen
from ANCA negativity in GPA presentations limited to the head
and neck, some MPA presentations without nephritis, such as
vasculitic peripheral neuropathy, and single organ vasculitis,
for example involving the brain or skin. There are also important
differences in epidemiology and outcome between PR3 and
MPO-ANCA subgroups. MPO-ANCA patients are older, more likely
to have renal disease, more frequent in Southern EU, China and
Japan, more likely to have an underlying cause for their vasculitis,
less likely to relapse, and have worse patient and renal survival
[8–10]. Pulmonary fibrosis occurring prior to or concurrent with
AAV is associated with MPO-ANCA and is a prominent pulmonary
feature of MPA in Japan [11]. A genome-wide association study
from Northern EU found striking differences in genetic associa-
tions between PR3 and MPO-ANCA subgroups, and an unsuper-
vised cluster analysis of baseline demographic variables in AAV
found ANCA to be a key discriminator that predicted outcome
[12,13]. The value of the term ‘ANCA associated vasculitis’ was
reaffirmed in the 2012 Chapel Hill Consensus update, but it seems
likely that PR3 and MPO-ANCA vasculitis and ANCA negative
vasculitis will be terms increasingly used in the future [14].
Churg-Strauss syndrome (now eosinophilic GPA, EGPA) presents
a particular problem with 30–50% being ANCA positive, and
such patients have more of a vasculitic phenotype. Should
ANCA positive EGPA patients be moved into PR3 and MPO-
ANCA vasculitis categories, leaving ANCA negative EGPA, when
eosinophilic manifestations predominate, grouped as an eosi-
nophilic ANCA negative vasculitis?

Presentations with dual positivity for anti-glomerular basement
membrane (GBM) antibodies and ANCA, usually MPO-ANCA,
sit as an overlap between AAV and anti-GBM disease, their
phenotype and outcome is more severe than AAV. Overlap
presentations also occur between MPO-ANCA and SLE, that
associate with vasculitic features [15].

Autoantibodies to plasminogen and tissue plasminogen activator
have been described in US and EU AAV cohorts that associate
with thromboembolic risk and renal histopathology [16,17].
Although confirmatory studies are required, this subgrouping
will have prognostic and therapeutic significance.

Antibodies and diagnosis
The outcome of generalized AAV is most strongly influenced by
renal function at diagnosis and diagnostic delay is reducing,
evidenced by lower serum creatinine at diagnosis in recent
cohorts [18]. Whether earlier diagnosis is simply due to ANCA
testing or a wider increased sensitivity to vasculitis is unclear.
For a patient with suspected nephritis, without other cause on a
conventional immunological work-up, PR3 or MPO-ANCA posi-
tivity confers over a 95% chance of a necrotizing, crescentic
glomerulonephritis on biopsy.
The diagnostic value of ANCA is lower for non-renal presenta-
tions due to lower sensitivity, for example in ENT and neuro-
logical disease, and lower specificity in pulmonary disease.
Tuberculosis, endocarditis and other infections can cause a
positive ANCA, and rarely a secondary vasculitis. Consensus
diagnostic criteria do not exist in vasculitis but are an aim of the
ongoing ‘Diagnostic and Classification Criteria in Vasculitis Study
(DCVAS)’ [19]. Clinical studies have used the 1990 ACR classi-
fication criteria for GPA or its modified variant that adds ANCA
as a fifth criterion [20]. Studies of the European Vasculitis Study
group (EUVAS) have defined a compatible clinical presentation
for GPA and MPA and then required either a positive ANCA or
confirmatory biopsy along with the exclusion of other possible
causes. This approach has had a specificity of over 99% in
expert centres but the sensitivity has not been assessed.

Other neutrophil autoantigens, including lactoferrin, elastase,
cathepsin G and bacterial permeability increasing protein are
recognized but do not feature in routine assays. Cocaine and
other drug-induced vasculitides have been associated with
elastase, and cocaine contamination with levamisole causes
vasculitis with PR3 or MPO-ANCA positivity [21]. Anti-bacterial
permeability increasing protein antibodies, causing a C-ANCA
IIF pattern, are found in pulmonary infections, including
tuberculosis, bronchiectasis and cystic fibrosis, where their
levels correlate with progressive lung damage but are not
closely linked with increased rates of vasculitis in these disorders. Five to 10% of patients with pauci-immune renal vasculitis or pulmonary capillaritis are ANCA negative; in some this is a ‘false’ negative with ANCA detectable by repeated testing or by more sensitive assays, or can be due to ANCA restricted to the IgM isotype not detected in IgG ANCA assays. Neutrophil infiltration is more intense in the renal histology of ANCA negative vasculitis, but the cause of this pathology is unknown [22]. ‘Natural’ MPO-ANCA occurring in healthy individuals differ from disease associated MPO-ANCA by their affinity and isotype restriction, and differences in MPO-ANCA epitope reactivity reflect their correlation with vasculitis [23].

**Antibodies and prognosis**

In a multivariate analysis controlling for age and renal impairment, MPO-ANCA was associated with worse patient and renal survival [8]. The renal histology shows more glomerular and tubulo-intestinal scarring and fewer acute cellular crescents and less tubular necrosis when compared to PR3-ANCA. Extraglomerular arteritis may also be more common in MPO-ANCA patients and is an adverse prognostic factor. An increased risk for cardiovascular events is also seen with MPO-ANCA as compared to PR3-ANCA but the reasons remain unexplained [24]. The ANCA binding level in a diagnostic sample does not carry prognostic significance with the exception of a PR3-ANCA ‘capture’ ELISA, when binding levels have reflected the severity of nephritis.

Relapse risk is consistently higher with PR3-ANCA, in part due to the association with respiratory tract disease in GPA [10]. A positive PR3-ANCA after 6 months induction therapy was associated with an 80% relapse rate at 4 years compared to 20% for those PR3-ANCA negative at 6 months [25]. Strong associations of ANCA binding level with disease activity have been difficult to demonstrate. Several studies have reported an increased relapse risk following a rise in ANCA, while others have found no association [26]. The differences in results have not been fully explained but patient population, study design, assay type and concomitant therapy are key factors.

Because glucocorticoid and/or immunosuppressive withdrawal increase relapse risk, concomitant therapy may protect patients from increases in ANCA level and associations of ANCA with disease activity become closer in the absence of therapy. A study of different PR3-ANCA assays on sera from patients in the EUVAS NORAM study found a wide variability in assay performance during remission monitoring and a closer association of changes in PR3-ANCA binding level with glucocorticoids and immunosuppression than with disease activity. Following rituximab treatment, and in the absence of other therapy, ANCA levels predictably fall for 6–12 months and a subsequent rise in ANCA, usually following B cell repopulation, is closely followed by relapse. Prospective studies basing treatment intensification with cyclophosphamide or azathioprine during the remission period on changes in ANCA level have demonstrated a reduction in flare rate with acceptable toxicity [27]. In the prediction of relapse, ANCA should be seen as one of a range of factors to be considered when managing maintenance therapy.

**Antibodies and treatment**

The pathogenic role of ANCA has led to treatment strategies aimed at ANCA removal. Plasma exchange removes other plasma constituents as well as ANCA and appears to improve the chances of renal recovery in severe renal vasculitis [28]. Selective strategies with double filtration apheresis, immunoabsorption and MPO antigen columns have also been used but not thoroughly evaluated. It is unclear whether ANCA negative patients also respond to plasma exchange or whether ANCA is a useful biomarker to guide the plasma exchange dose, as in anti-GBM disease. Intravenous immunoglobulin is another therapy with multiple mechanisms but it has been shown to inhibit binding and functional activity of ANCA in vitro, and to reduce ANCA levels and improve disease activity in vivo. The persistence of PR3-ANCA in a patient with refractory GPA was the rationale for the first use of rituximab, and B cell depletion reduces ANCA levels, however rituximab is effective in ANCA negative patients suggesting other mechanisms apart from control of ANCA directly.

**Newer directions**

Epitopes of MPO and PR3 and glycosylation status have defined antibodies associated with disease activity and may lead to assays of more value in diagnosis and the assessment of relapse risk [29]. Newer ANCA antibodies including complimentary PR3 and LAMP-2 occur in AAV patients that have given insights into pathogenesis but have not translated into clinical tools [30,31]. Anti-endothelial antibodies occur in AAV but have been hard to characterize; recently a subgroup of MPO-ANCA that cross-react with an endothelial antigen, moesin, have been described that may have clinical significance [32].

**Conclusions**

In addition to being a revolutionary tool in diagnosis and monitoring vasculitis, ANCA have driven revision of classification and the development of newer therapies. Newer antibody classes are being described in vasculitis of potential clinical value. The ANCA story elegantly demonstrates the transforming effect a biomarker can have on clinical practice and patient outcomes.

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