Towards precision medicine in ANCA-associated vasculitis

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Abstract
ANCA-associated vasculitis (AAV) is characterized by inflammation and destruction of small and medium-sized vessels. Current management strategies for AAV have been validated in large groups of patients. However, recent insights indicate that distinct patient subsets may actually exist within AAV, thereby justifying the development of more personalized treatment strategies. In this review, we discuss current evidence for a better classification of AAV based on ANCA type. We describe how thus defined categories of AAV patients may differ in genetic background, clinical presentation, immune pathology, response to treatment and disease outcome. We also explore how these insights may provide a rationale for targeted treatments in different categories of AAV patients. Finally, we provide recommendations on how to further establish precision medicine in AAV.

Key words: disease subsets, vasculitis, ANCA, precision medicine, personalized medicine

Rheumatology key messages
- ANCA type identifies distinct prognostic subsets of ANCA vasculitis patients.
- Dedicated trials are needed before implementing distinct treatment strategies in MPO-ANCA and PR3-ANCA patients.
- The efficacy of various targeted, and eventually ANCA-directed, treatments should be further investigated in ANCA-associated vasculitis.

Introduction

ANCA-associated vasculitis (AAV) is one of the most widely studied forms of autoimmune vasculitis. In AAV, small and medium-sized blood vessels are infiltrated by immune cells and eventually destroyed. Small vessels in the kidneys and respiratory tract are frequently involved in AAV. ANCAs are detected in the majority of AAV patients and are directed to proteins present on the membrane of activated neutrophils and monocytes. Currently, AAV patients are managed with standard immunosuppressive treatment regimens.

In recent years, insight into the immune pathology and clinical spectrum of AAV has increased considerably. It has been reported that immunological markers may identify distinct patient categories within AAV. These AAV categories are associated with a particular genetic background, immune pathology, clinical presentation, response to treatment and prognosis [1, 2]. Insight into the immune pathology of distinct AAV categories may provide a rationale for targeted treatments and could help to eventually implement precision medicine for AAV patients. Precision medicine in AAV is timely and relevant, given the increasing number of targeted treatments available [3, 4]. Furthermore, it might help to maximize clinical outcomes while minimizing the risk of unnecessary drug toxicity and costs [5].

In this review we discuss the current evidence for disease subsets within AAV. We discuss how ANCA type identifies distinct categories of AAV patients. We evaluate...
Precision medicine in AAV

Table 1 Immunological categorization of AAV patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PR3-ANCA vasculitis</th>
<th>MPO-ANCA vasculitis</th>
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<tr>
<td>Associated genes</td>
<td>HLA-DP, α1-antitrypsin, PR3</td>
<td>HLA-DQ</td>
</tr>
<tr>
<td>Pathology</td>
<td>Necrotizing vasculitis often with destructive granulomatous inflammation</td>
<td>Necrotizing vasculitis often with chronic damage, that is, fibrosis/sclerosis</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>On average four organs involved</td>
<td>On average two organs involved</td>
</tr>
<tr>
<td>Therapy and prognosis</td>
<td>High relapse risk with CYC/AZA</td>
<td>Low relapse risk with CYC/AZA</td>
</tr>
<tr>
<td></td>
<td>Possibly better remission induction and maintenance with RTX than CYC/AZA</td>
<td>Possibly similar remission induction and maintenance with RTX vs CYC/AZA</td>
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</table>

ANCA type identifies AAV patients with distinct genetic background, immunopathology, clinical presentation, response to treatment and prognosis.

Current classification and management of AAV

The diagnosis of AAV is based on a variable combination of clinical symptoms, the presence of PR3- or MPO-ANCA, and biopsy findings consistent with AAV [6]. Traditionally AAV has been divided into three disease groups [7]: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic GPA (EGPA). EGPA shows eosinophilic necrotizing vasculitis in combination with asthma and eosinophilia. Given its strong association with eosinophilic inflammation and unique therapeutic targets, EGPA is considered a disease entity different from GPA and MPA [8, 9], hence EGPA will not be further discussed in this review. GPA is characterized by necrotizing vasculitis with granulomatous inflammation of the upper and lower respiratory tract, often together with glomerulonephritis. MPA presents necrotizing vasculitis without granulomatous inflammation, often involving glomerulonephritis and pulmonary capillaritis. Although GPA and MPA patients show some clinical differences, the GPA/MPA classification is not robust [10]. ACR classification criteria have been described for GPA but not for MPA [11]. Although two alternative classification systems do recognize both GPA and MPA, an individual patient may be classified as having GPA by one system but MPA by the other [12]. Moreover, GPA/MPA classification may change over time in an individual patient. A therapeutically more relevant classification of AAV is based on disease severity [13]: that is, localized disease (one organ affected, no systemic involvement), early systemic disease (at least one organ affected, systemic features, no threatened vital organ), generalized disease (at least one organ affected, systemic features, threatened vital organ function) and severe disease (at least one organ affected, systemic features and vital organ failure).

As described in current treatment recommendations [6, 14], the management of AAV is primarily based on disease severity and organ involvement. Traditionally, glucocorticoids in combination with cyclophosphamide (CYC) have been applied as first-line treatment for induction of remission in AAV patients [15]. Methotrexate (MTX), which is inferior to CYC for induction of remission, is sometimes used in patients with non-severe disease and sufficient renal function [16]. Similarly, mycophenolate mofetil (MMF) may be used in patients with non-severe AAV [17]. More recently, anti-CD20 therapy [i.e. rituximab (RTX)] has become an important first-line treatment in AAV, as this targeted treatment is non-inferior to CYC for induction of remission [3, 18]. In contrast, TNF-blocker therapy has no benefit when given along with standard induction therapy [19]. Plasma exchange is added to the standard induction therapy of patients with severe or refractory disease, in particular those with severe renal involvement (serum creatinine >500 μmol/l) [20, 21]. Subsequent to induction of remission, azathioprine (AZA) or MTX have been applied to maintain remission [22, 23]. Interestingly, strong evidence now indicates that RTX is superior to azathioprine (AZA) in maintaining CYC-induced remission [4]. However, the optimal dosing strategy and duration of this anti-CD20 treatment remains to be established. Overall, current treatment in AAV is primarily based on disease severity and organ involvement.

PR3-ANCA and MPO-ANCA define distinct subsets of AAV patients

Given the strong association of GPA with PR3-ANCA and MPA with MPO-ANCA [24], it is not surprising that both the traditional GPA/MPA classification and ANCA type may identify categories of AAV patients with distinct organ involvement. Besides necrotizing vasculitis occurring in both conditions, PR3-ANCA vasculitis is often associated with destructive granulomatous inflammation, whereas signs of chronic damage, such as lung fibrosis and glomerular sclerosis, are more frequently observed in MPO-ANCA patients (Table 1) [12, 25–27]. Renal involvement is more common in MPO-ANCA patients, whereas upper airway involvement is more often seen in PR3-ANCA patients [12, 25, 28]. More organs are typically affected in PR3-ANCA vasculitis than MPO-ANCA.
vasculitis [29]. With respect to organ involvement, PR3-ANCA vasculitis and MPO-ANCA vasculitis mostly overlap with GPA and MPA, respectively [25].

However, accumulating evidence indicates that ANCA type better identifies AAV patients with distinct genetic associations and prognosis than the GPA/MPA classification (Table 1) [1, 2]. PR3-ANCA vasculitis is associated with genetic variants of HLA-DP, z1-antitrypsin (i.e. the natural inhibitor of PR3) and PR3 itself, whereas MPO-ANCA vasculitis is associated with genetic variants of HLA-DQ [24]. In contrast, these genetic associations were substantially weaker when patients were grouped according to the GPA/MPA classification. Interestingly, the increased prevalence of HLA-DP1*04 in PR3-ANCA patients has been linked to a higher relapse risk in these patients [30]. Although few studies have indicated that ANCA subtype does not impact disease outcome [31, 32], the majority of studies indeed conclude that PR3-ANCA patients are more prone to relapse than MPO-ANCA patients [12, 23, 29, 33-36]. In most of these studies, ANCA type predicted relapses better than the classic GPA/MPA classification [12, 23, 25, 33, 34]. Furthermore, a post hoc analysis of a large multicentre study has suggested that PR3-ANCA patients may respond better to RTX for induction and maintenance of remission than CYC/ZA, whereas these treatments were equally effective in MPO-ANCA patients [25, 36]. When these AAV patients were classified as having GPA or MPA, no differential response to RTX and CYC/ZA was observed in either group [25]. However, findings from this post hoc analysis remain to be confirmed in a dedicated randomized controlled trial (RCT). Overall, classification of AAV patients by ANCA type may have more clinical and therapeutic consequences than the traditional GPA/MPA classification.

**Immunological targets in PR3-ANCA and MPO-ANCA patients**

Although PR3-ANCA patients show more granulomatous inflammation, the overall immune pathology appears rather similar in PR3-ANCA and MPO-ANCA patients. Many targeted treatments might therefore be potentially useful in both AAV categories (Figure 1 and Table 2). Although direct proof for ANCA pathogenicity in humans is limited [37], these autoantibodies likely promote vascular inflammation during the effector phase of the disease [38]. In vitro studies have shown that PR3-ANCA and MPO-ANCA exert activating effects on pre-activated neutrophils and monocytes that express PR3 and MPO on their membrane surface. These neutrophils and monocytes transfer cytoplasmic PR3 and MPO to their surface membrane upon stimulation by microbial products or pro-inflammatory cytokines, such as TNF-a [39-44]. ANCA promote the release of reactive oxygen species and pro-inflammatory cytokines from neutrophils and monocytes [39, 40, 45-48]. ANCA also stimulate the release of complement activating factors and neutrophil extracellular traps from neutrophils [39, 49]. These ANCA-induced effects may eventually result in endothelial inflammation and damage. Anti-CD20 therapy (i.e. RTX) reduces ANCA levels in AAV patients and has shown remarkable efficacy for inducing and maintaining remission in AAV [3, 4, 50]. Pathogenic B cell activation in AAV could potentially be targeted by inhibitors of Bruton’s tyrosine kinase. The alternative complement system may further amplify the ANCA-mediated inflammatory response in AAV patients [40, 49]. In animal models of AAV, deficiencies in C5 or its receptor (i.e. C5aR) completely protect against glomerulonephritis development, whereas anti-C5 treatment markedly attenuates glomerulonephritis [51-53]. Following the promising results of a C5aR inhibitor (avacopan) in a small trial with AAV patients [54], the efficacy of this agent is now being evaluated in a larger study (ClinicalTrials.gov, NCT02994927).

As not all ANCs are equally pathogenic [55, 56], it might be interesting to develop treatments that selectively modulate the pathogenic potential of ANCs themselves. Very low levels of PR3-ANCA and MPO-ANCA, not detected by routine measurements, are present in the sera of healthy humans [55, 56]. However, these natural ANCs bind differently to PR3 or MPO than ANCs in AAV patients, either with less affinity (PR3-ANCA) or by recognizing different epitopes (MPO-ANCA) [55, 57]. In addition to affinity maturation and epitope spreading, aberrant antibody glycosylation may enhance the pathogenic effects of ANCs [58, 59]. In order to achieve affinity maturation, epitope spreading and altered antibody glycosylation, the PR3-specific and MPO-specific B cells of AAV patients likely require T cell help. In particular, IL-21 and IL-17 producing T helper (Th) 17 cells, which may develop in part through IL-23, appear to help autoreactive B cells in patients with systemic autoimmune diseases [60]. AAV patients also show increased serum levels of IL-23 and marked expansion of IL-21 and IL-17 producing Th17 cells [61-63]. These pro-inflammatory cytokines might be potentially targeted by anti-IL-12/IL-23, anti-IL-17 and anti-IL-17R treatments. Furthermore, Th17 activation may be inhibited by Janus kinase inhibitors and the co-stimulation blocker CTLA4-Ig (abatacept), which is currently under investigation in AAV (ClinicalTrials.gov, NCT02108860). Aberrant glycosylation of ANCs might be corrected by agents removing N-linked glycans from these autoantibodies (i.e. EndoS) [64]. B cell activating factor (BAFF; or B lymphocyte stimulator (BLyS)) might be another target of treatment in AAV, as this cytokine promotes differentiation of PR3- and MPO-specific B cells into antibody-secreting cells [65]. Recently a trial in which AAV patients received anti-BAFF therapy (i.e. belimumab) has been completed and its results are currently awaited (ClinicalTrials.gov, NCT01663623).

It has been suggested that a strong Th1 response may promote development of granulomatous lesions in AAV patients [66]. Organs affected by AAV are infiltrated by large numbers of Th cells with an effector memory phenotype [67, 68]. In granulomatous lesions, many of these cells are IFN-gamma-producing Th1 cells [69-71]. It has been proposed that IFN-gamma directly promotes development of
granulomatous inflammation in AAV [66]. Overproduction of IL-12 and IL-18 may partly underlie this aberrant Th1 cell response [72-74]. Dendritic cells are also present at the site of inflammation and cluster in T cell rich areas [75]. PR3 may directly promote maturation of dendritic cells with a robust Th1-polarizing potential [76]. However, the effect of anti-IFN-γ therapy (i.e. fontolizumab) in PR3-ANCA patients remains to be investigated.

ANCA-specific and autoantigen-specific treatment of AAV patients

An ultimate form of patient-tailored therapy in PR3-ANCA and MPO-ANCA patients would be to apply either ANCA-specific or autoantigen-specific treatment (Fig. 1). Therapeutic trials with tolerogenic dendritic cells are ongoing in patients with other autoimmune diseases [77].
AAV, tolerogenic dendritic cells could be loaded with PR3 or MPO and transferred into PR3-ANCA or MPO-ANCA patients, respectively. Another strategy would be to treat AAV patients with PR3- or MPO-specific regulatory T cells [78]. However, the inflammatory environment in AAV patients may compromise the function of these regulatory T cells [79]. A recently reported treatment of interesting potential would be to apply cytotoxic T cells carrying a chimeric autoantibody receptor recognizing anti-PR3 or anti-MPO immunoglobulins on B cells of AAV patients. Once transferred into an AAV patient, such cytotoxic T cells would selectively kill PR3- or MPO-specific B cells while leaving other B cells unaffected [80]. Although these treatments hold great promise for future precision medicine in AAV, important issues regarding their feasibility, safety and efficacy remain to be investigated.

**Concluding remarks and future perspectives**

Identification of distinct patient subsets may be a first step towards development of precision medicine for AAV. Accumulating evidence indicates that ANCA type better defines distinct subsets of AAV patients than the classic GPA/MPA classification. Identification of distinct prognostic subsets may help to better individualize the treatment of AAV patients in the future. ANCA-specific or autoantigen-specific treatment holds great promise for the future management of AAV but is only in its infancy. In the near future, the therapeutic arsenal for AAV is likely to expand with already existing targeted treatments (Table 2).

Before ANCA-based precision medicine is implemented in daily care, this immunological classification of AAV should be further validated. In an early stage, protocolized cohort studies with well-characterized vasculitis patients may be helpful (Fig. 2A). Subsequently, post hoc analyses of RCTs may further validate the immunological classification systems (Fig. 2B). Ideally, dedicated RCTs should eventually evaluate the effects of targeted treatments in MPO-ANCA and PR3-ANCA patients (Fig. 2C). For example, a post hoc analysis of a large multicentre study suggested that PR3-ANCA patients are more likely to obtain and maintain remission with RTX than CYC/AZA

**Table 2 Targeted treatments in AAV**

<table>
<thead>
<tr>
<th>Immune target</th>
<th>Agents</th>
<th>Trials in AAV</th>
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<tbody>
<tr>
<td>BAFF (BLyS)</td>
<td>Belimumab (x-BAFF)</td>
<td>Belimumab&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Tabalumab (x-BAFF)</td>
<td></td>
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<tr>
<td></td>
<td>Blisibimod (BAFF antagonist)</td>
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<tr>
<td>CD80/CD86</td>
<td>Abatacept (CTLA4-Ig)</td>
<td>Abatacept&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD20</td>
<td>Rituximab (x-CD20)</td>
<td>Rituximab&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Ofatumumab (x-CD20)</td>
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<tr>
<td></td>
<td>Ocrelizumab (x-CD20)</td>
<td></td>
</tr>
<tr>
<td>C5a or C5aR</td>
<td>Avacopan (C5aR inhibitor)</td>
<td>Avacopan&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup>Currently investigated in a RCT, as registered at ClinicalTrials.gov. <sup>b</sup>Efficacy proven in an RCT.

**Fig. 2 Study design to implement precision medicine in AAV**

Post hoc analyses of (A) protocolized cohort studies and (B) randomized controlled trials (RCTs) may help to validate the ANCA-based disease categories with distinct response to treatment and prognosis. (C) Eventually, dedicated RCTs may be performed to test whether certain treatments show different efficacy in MPO-ANCA and PR3-ANCA patients. The latter type of studies will justify precision medicine based on ANCA type.
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Finally, precision medicine as proposed in the current review may only be a first step towards fully individualized treatment of AAV patients. With the dawn of systems biology, we anticipate that eventually a multitude of biologic markers will help to classify these vasculitis patients and guide the selection of targeted treatments. We expect that implementation of precision medicine in AAV will require extensive local and global collaboration efforts between vasculitis expertise centres.

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