Proteinase 3-ANCA Vasculitis versus Myeloperoxidase-ANCA Vasculitis

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ABSTRACT
In patients with GN or vasculitis, ANCAs are directed against proteinase 3 (PR3) or myeloperoxidase (MPO). The differences between PR3-ANCA-associated vasculitis (AAV) and MPO-AAV described in the past have been supplemented during the last decade. In this review, we discuss the differences between these two small-vessel vasculitides, focusing especially on possible etiologic and pathophysiologic differences. PR3-AAV is more common in northern parts of the world, whereas MPO-AAV is more common in southern regions of Europe, Asia, and the Pacific, with the exception of New Zealand and Australia. A genetic contribution has been extensively studied, and there is a high prevalence of the HLA-DPB1*04:01 allele in patients with PR3-AAV as opposed to patients with MPO-AAV and/or healthy controls. Histologically, MPO-AAV and PR3-AAV are similar but show qualitative differences when analyzed carefully. Clinically, both serotypes are difficult to distinguish, but quantitative differences are present. More organs are affected in PR3-AAV, whereas renal limited vasculitis occurs more often in patients with MPO-AAV. For future clinical trials, we advocate classifying patients by ANCA serotype as opposed to the traditional disease type classification.


ANCA were first described more than 50 years ago. Later, these antibodies were discovered in the context of GN and vasculitis. The discovery of these antibodies in granulomatosis with polyangiitis (GPA; formerly known as Wegener’s granulomatosis) marked a breakthrough in diagnostics, since GPA had been known for at least half a century without a serologic marker. Subsequently, ANCA were found in two other forms of small-to-medium-vessel vasculitis, i.e., microscopic polyangiitis (MPA) and eosinophilic GPA (formerly known as Churg–Strauss syndrome). ANCA were traditionally tested with an immunofluorescence test on fixed neutrophils. Three patterns can be found: cytoplasmic (cANCA), perinuclear (pANCA), and atypical. ANCA, as detected by immunofluorescence techniques, have been found in many different diseases.

In addition to detecting cANCA or pANCA by immunofluorescence, a test to detect proteinase 3 (PR3)-ANCA or myeloperoxidase (MPO)-ANCA is mandatory for the diagnosis of ANCA-associated vasculitis (AAV). In the case of GN and/or vasculitis, ANCA are almost always directed against PR3 or MPO, and PR3-ANCA and MPO-ANCA are specific for GPA and MPA. The distinction between GPA and MPA in the context of ANCA serotype is far from perfect and underlines the discordance between disease categorization and ANCA serotype categorization. Most patients are single-positive, meaning that only one ANCA serotype can be detected. Many differences exist between patients with PR3-AAV and MPO-AAV, which were highlighted in a review by Franssen et al. more than a decade ago. Since then, more differences between PR3-AAV and MPO-AAV have been discovered.

The aim of the current review is to discuss differences between PR3-AAV and MPO-AAV in the light of the discoveries that took place during the last decade.

CONCISE METHODS
Medline and Embase were searched for combinations of the following indexed subject headings [MeSH]: vasculitis, antibodies, antineutrophil cytoplasmic, anti-neutrophil cytoplasmic antibody-associated vasculitis, “ANCA [text word]”, granulomatosis with polyangiitis, Wegener’s granulomatosis, microscopic polyangiitis, “pauci-immune [text word]”. Eosinophilic GPA was not included in this review. The rating of the quality of evidence as depicted in Table 1 is based on

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Epidemiology

The AAV form a group of rare autoimmune diseases with an increasing annual incidence,19 currently reported as 20 per million/year.20 Several studies have shown that the male to female ratio is higher in PR3-AAV (1.3–1.9) than in MPO-AAV (0.3–0.8).12,16 In our cohort of patients with renal involvement the male to female ratio was 3.0 in PR3-AAV patients and 1.9 in MPO-AAV patients.21

The incidence of PR3-AAV and MPO-AAV varies worldwide, possibly as the result of a combination of genetic pools and certain environmental factors. For example, the incidence of PR3-AAV in the United Kingdom has been reported as 11.3 per million/year and 3.0 per million/year in Spain, whereas the incidence of MPO-AAV has been reported as 5.9 and 7.9 per million/year, respectively.22,23 In Japan, the incidence of AAV is 22.6 per million/year with 84% of patients being MPO-ANCA positive.24 Although population-based data from China are lacking, the large percentage of patients with PR3-AAV but not with MPO-AAV. Xie et al.25 had an odds ratio that was significantly increased compared with MPO-AAV. The association between this particular single nucleotide polymorphism and PR3-ANCA was stronger than with the clinical diagnosis of GPA.26 A weaker association was found between MPO-AAV and HLA-DQ. Polymorphisms in the genes SERPINA1 and PRTN3 were included.39 However, none of these findings had an odds ratio that was significant genome-wide (P value <5×10⁻⁸). Lyons et al.39 found a higher prevalence of SERPINA1 polymorphisms in patients with PR3-AAV compared with those with MPO-AAV. The association between SERPINA1 and GPA was confirmed by Xie et al.38 Although not significant genome-wide, PRTN3 in the study by Lyons et al.39 was associated with PR3-AAV but not with MPO-AAV. Xie et al.38

<table>
<thead>
<tr>
<th>Name</th>
<th>Treatment Studied</th>
<th>Patients</th>
<th>Primary Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYCAZAREM168</td>
<td>Azathioprine versus cyclophosphamide in remission maintenance</td>
<td>GPA and MPA</td>
<td>Azathioprine is as effective as cyclophosphamide and reduces cumulative cyclophosphamide doses for maintenance of remission</td>
</tr>
<tr>
<td>CYCLOS169</td>
<td>IV cyclophosphamide versus oral cyclophosphamide in remission induction</td>
<td>GPA, MPA and RLV</td>
<td>IV cyclophosphamide is as effective as oral cyclophosphamide and reduces cumulative cyclophosphamide doses for induction therapy</td>
</tr>
<tr>
<td>IMPROVE170</td>
<td>Mycophenolate mofetil versus azathioprine in remission maintenance</td>
<td>GPA and MPA</td>
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</tr>
<tr>
<td>MAINRITSAN154</td>
<td>Rituximab versus azathioprine in remission maintenance</td>
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<td>Rituximab is more effective to prevent relapse compared with azathioprine whereas adverse events are similarly frequent</td>
</tr>
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</tr>
<tr>
<td>MTX versus LEF172</td>
<td>Oral methotrexate versus leflunomide for remission maintenance</td>
<td>GPA</td>
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</tr>
<tr>
<td>NORMAM173</td>
<td>Methotrexate versus cyclophosphamide for remission induction</td>
<td>GPA and MPA</td>
<td>Methotrexate is less effective than cyclophosphamide in patients with non-renal AAV for disease control</td>
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<tr>
<td>RAVE135,153</td>
<td>Rituximab versus cyclophosphamide in remission induction</td>
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<td>A single course of rituximab is as effective and as safe as treatment with cyclophosphamide followed by azathioprine</td>
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<tr>
<td>RITUXVAS152</td>
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<tr>
<td>WEGENT174</td>
<td>Methotrexate versus azathioprine in remission maintenance</td>
<td>GPA and MPA</td>
<td>Methotrexate is as effective and as safe for remission maintenance as azathioprine</td>
</tr>
<tr>
<td>WGET175</td>
<td>Etanercept with standard therapy versus standard therapy in remission induction and maintenance</td>
<td>GPA</td>
<td>Etanercept is not effective for maintenance of remission and when combined with standard therapy results in a high rate of treatment related complications (e.g., malignancies)</td>
</tr>
</tbody>
</table>

IV, intravenous; RLV, renal-limited vasculitis.
did not include PRTN3 in their analysis. The findings of Lyons et al. support a pathogenic role for ANCA—which implicates the MHC, the PR3 antigen itself, and α1-antitrypsin—so further diminishing the possibility of ANCA being an epiphenomenon. Although CTLA4 polymorphisms are more prevalent in patients compared with healthy controls, no difference can be found between ANCA serotypes.39 Our group, however, has found that patients with MPO-AAV tend to more often be G-allele carriers of a polymorphism in CTLA4 compared with patients with PR3-AAV.34 Recently, we extended this study and found that 76.4% of the patients with MPO-AAV (n=72) were G-allele carriers compared with 58.8% of patients with PR3-AAV (n=80) and 55.1% of healthy controls (n=185; P=0.01; M. Hilhorst et al., unpublished data).

In the North American genome-wide studies, a link between SEMA6A and GPA was found,38 but this was not confirmed in an independent cohort of European patients.43 Interestingly, the allele HLA-DRB1*15 has been associated with PR3-AAV in African Americans, but not with MPO-AAV in these patients or with Caucasian patients in general.44 Such findings further support a strong genetic background of AAV, which may support categorization of patients based on ANCA serotypes rather than on clinical diagnosis.

Etiology
Incidence and prevalence of AAV are different at different latitudes.27 The geographical pattern can be explained by factors such as genetic background and/or environmental factors.45 Especially in MPO-AAV, silica exposure is thought to play a role.46,47 Macrophages phagocytosing silica, e.g., in the lung, produce proinflammatory cytokines which attract neutrophils. The silica particles are then transported to regional lymph nodes, resulting in chronic activation of T cells and triggering autoimmune reactions in susceptible patients.48–50 A higher incidence of MPO-AAV was reported after the Kobe earthquake in Japan, possibly related to increased silica exposure.51 After closure of the last coal mine in the region of Maastricht, the Netherlands, MPO-AAV diagnoses appeared to decrease compared with the number of PR3-AAV diagnoses.52 Whether this observation supports the etiologic contribution of silica exposure is, however, speculative.

Bacterial infections have been thought to be an important factor in the initiation of AAV.53–56 It is known that PR3-ANCA-positive patients who carry nasal Staphylococcus aureus chronically are at higher risk for disease relapse.54 While MPO-AAV in association with S. aureus infection has been mentioned,57,58 so far no studies on the role of nasal S. aureus carriage in MPO-AAV have been performed. A sequence homology between PR3 and parts of the S. aureus genome has been suggested, but evidence that such interaction is operative in vivo is lacking.59 Furthermore, a homology between complementary PR3 and S. aureus was found.60 Various possible mechanisms by which S. aureus contributes to the pathophysiology of AAV have been postulated.55 These mechanisms, however, fail to explain a difference between PR3-AAV and MPO-AAV. Importantly, ANCA directed against human lysosome-associated membrane protein 2, present in both PR3-ANCA- and MPO-ANCA-associated GN,61 were found to react with the bacterial adhesin FimH, supporting an autoimmune model of molecular mimicry in ANCA-associated GN.62

Pathophysiology: In Vitro Data and Animal Models
In vitro data
While neutrophils express low levels of PR3 and MPO on their cell surface, PR3 and MPO are expressed extensively upon stimulation (e.g., with TNF-α),63–65 making it possible for ANCA to bind. It has been shown that PR3 and MPO levels are aberrantly increased in patients with AAV compared with healthy controls,66 possibly because of epigenetic factors.67 Both ANCA serotypes are able to activate neutrophils via Fab as well as Fcγ engagement,68 resulting in the release of inflammatory mediators.69 Subsequently, PR3 and MPO are both released into the circulation and bind to endothelial cells.70 Both enzymes can be internalized by endothelial cells, yet exert different effects. PR3 induces apoptosis of the endothelial cell whereas MPO induces the production of intracellular oxidants.71 It is currently unknown whether PR3-ANCA and MPO-ANCA induce different pathways of inflammation. During active disease, an increase in the antiangiogenic factor sFlt1 (also known as sVEGFR-1) can be observed in patients with PR3-AAV and to a much lesser extent in those with MPO-AAV. Monocytes were shown to be the main source of sFlt1, releasing this factor upon stimulation with serum from patients with PR3-AAV but not after stimulation with serum from patients with MPO-AAV.72 It has been suggested that C5a is a major driver of sFlt1 release by monocytes.73 Complement activation may therefore differ between PR3-ANCA and MPO-ANCA.74

Animal Models
To demonstrate pathogenicity of ANCA in vivo, several animal models have been developed.75 The development of an animal model for AAV was most successful for MPO-AAV. Initially, Brown-Norway rats were immunized with MPO and their kidneys were perfused with H2O2 and a lysosomal extract containing MPO and elastinolytic enzymes. This resulted in a severe pauci-immune necrotizing crescentic GN (NCGN) with up to 80% fibrinoid necrosis and 70% crescentic lesions, and localization of MPO along the glomerular basement membrane. This proves that anti-MPO antibodies cause severe damage when initial immune complex formation occurs.76 Injecting mice with anti-MPO antibodies (raised in Mpo−/− mice) results in a mild form of NCGN77 whereas an additional injection with LPS causes a more severe NCGN.78 Finally, it has been demonstrated that human MPO immunization in WKY rats results in anti-MPO antibody binding to rat leucocytes, causing vasculitis and GN.79

Similar approaches were used to develop an animal model for PR3-AAV but these proved unsuccessful. Immunization of mice and rats with chimeric human/mouse PR3 induced anti-PR3 antibodies, but no disease.80 Similarly, nonobese diabetic (NOD) mice immunized with recombinant mouse PR3 developed anti-PR3 antibodies but no signs of autoimmune disease. In fact, vasculitis could only be induced when splenocytes from these immunized NOD mice were transferred into NOD-SCID mice.81 A more recent study using humanized NOD-SCID-IL-2Rγ−/− mice injected with LPS and human IgG containing anti-PR3 antibodies showed the development of lung hemorrhage and mild kidney disease, characterized by
mesangial hypercellularity and leukocyte influx. This supports the pathogenicity of PR3-ANCA.62 Induction of an inflammatory reaction with granulomatous features in a PR3-AAV animal model has, however, been unsuccessful to date. One hypothesis on why PR3-AAV animal models are more difficult to develop than MPO-AAV animal models is that mouse PR3 is undetectable on isolated mouse neutrophils, probably as the result of a different interaction between PR3 and NB1 (CD177) in mice.83 This makes the antigen unavailable to the circulating antibodies.84

The differences in animal models for PR3-AAV and MPO-AAV could indicate a difference between mice and men and may not necessarily reflect a difference in pathophysiology.

**Histopathologic Features**

A biopsy showing necrotizing vasculitis confirms the presence of AAV. A hallmark of GPA is granulomatous inflammation. Granuloma formation is thought to be initiated by small aggregates of neutrophils surrounding necrotic areas (microabscesses).85–87 In GPA, granulomatous inflammation can be found in several affected organs, a feature not found in MPA.88 Granuloma formation can be found in both PR3-ANCA–positive patients and MPO-ANCA–positive patients diagnosed with GPA.11 Therefore, granuloma formation—including periglomerularly (Figure 1)—does not discriminate PR3-AAV from MPO-AAV (Figure 2). In our study, for those patients with a renal biopsy, 23 of 92 PR3-AAV patients (25.0%) and 17 of 89 MPO-AAV patients (19.1%) had periglomerular granulomas. Whether granulomas in PR3-AAV differ from granulomas in MPO-AAV is at present unknown.

Renal histology in PR3-AAV and MPO-AAV does not discriminate, because similar abnormalities are recorded: NCNG with fibrinoid necrosis upon light microscopy in combination with pauci-immunity (<2+ on a scale of 0 to 4+) upon immunofluorescence.89 The ANCA-associated GN classification90 classifies renal biopsies based on percentage of glomeruli involved. A distinction between four groups can be made: when >50% of glomeruli are normal, the renal biopsy is classified as focal; when >50% of glomeruli contain cellular crescents, the biopsy is termed crescentic; when >50% of glomeruli are sclerosed, the biopsy is classified as sclerotic; and when the biopsy is not classified in the other groups, it is classified as mixed. This classification has prognostic value91 with the best renal survival in the focal group, followed by the mixed and crescentic groups and the worst renal survival in the sclerotic group.21 Although renal histology between PR3-AAV and MPO-AAV is similar, significant quantitative differences can be observed.92 An important difference is the presence of more normal glomeruli in PR3-AAV compared with MPO-AAV (31%–40% and 26%–28%, respectively), with a comparable eGFR between these groups at the time of renal biopsy.52,90,93 In contrast, more fibrotic changes are recorded in MPO-AAV,93,94 reported as interstitial fibrosis in addition to fibrous crescents (19% in MPO-AAV versus 10% in PR3-AAV)52 and obliterated glomeruli (25% in MPO-AAV versus 15% in PR3-AAV).95 Since chronic features are known to be associated with bad renal outcome52,95 patients with MPO-AAV may have worse renal survival than patients with PR3-AAV. Data on this matter remain, however, inconclusive.92,96–98 In addition to renal fibrosis, an association between lung fibrosis and MPO-ANCA has been observed.99–101 Pulmonary fibrosis in PR3-AAV seems to occur less often.102 It has been postulated that fibrotic changes in MPO-AAV are either due to a delayed diagnosis93 or due to a more profibrotic pathogenesis.103,104

Although ANCA-associated GN is by definition pauci-immune, a subset of renal biopsies were found to have complement and immunoglobulin depositions, as well as electron-dense deposits ultrastructurally.105 Electron-dense deposits were found as frequently in PR3-AAV as in MPO-AAV.105,106 In the Limburg Renal Registry,107 we found C3 deposition in more than half of renal biopsies. C3d depositions were more prevalent in patients with MPO-AAV compared with patients with PR3-AAV (M. Hilhorst et al, unpublished data).

There is some evidence to support a different role for complement between the two ANCA serotypes108–110 (vide supra).

**Clinical Features**

Most AAV patients are diagnosed between ages 50 and 70 years, without clear differences between ANCA serotypes. AAV is a systemic disease that involves the ear, nose, and throat, the lungs, the kidneys, the heart, the digestive
Ophthalmologic manifestations such as orbital inflammatory disease are observed more often in PR3-AAV than in MPO-AAV.126,127

Outcome
Outcome differs according to ANCA serotype. Patients with PR3-AAV relapse significantly more often (hazard ratio 1.6–3.2) than patients with MPO-AAV.52,98,128–130 Risk factors for relapse other than ANCA serotype are age, nasal carriage of S. aureus in PR3-AAV, cardiovascular involvement and a rise in ANCA titers in patients with renal involvement.54,131 Worse renal involvement has been reported to be associated with a lower risk for relapse.54,129 Most studies have not analyzed risk factors for relapse stratifying by ANCA serotype.

Patient survival in AAV has improved substantially over the last three decades,52 with mortality rates currently at 2%–30% at 1 year and 19%–50% at 5 years, strongly depending on renal involvement and/or age at onset.96,132–135 A systematic review demonstrated worse patient survival in patients with MPA compared with patients with GPA.136 In our patients with AAV who had renal involvement, patient survival was worse in patients with MPO-AAV (n=92) compared with patients with PR3-AAV (n=89), whereas renal survival was similar (P=0.28). Various studies, however, could not find a clear difference in patient survival between PR3-AAV and MPO-AAV patients.98,137 whereas one study reported a worse patient survival in patients with PR3-AAV.138 Three studies found no difference in renal survival between ANCA serotypes,92,96,139 whereas several recent studies reported a worse renal survival in patients with MPO-AAV.98,140–142 Survival in patients with PR3-AAV may be largely determined by diffuse alveolar hemorrhage at presentation and renal relapses in follow-up.98,143 whereas renal and/or patient survival in MPO-AAV is probably related to smoldering disease44 causing end-stage renal disease.

With improving treatment and survival, long-term morbidity plays an increasingly important role in AAV. Within the first year after diagnosis, infection forms the most important risk of death.144,145 Thereafter,
cardiovascular disease, malignancies and infections are the main causes of death.\textsuperscript{145} The occurrence rate of infection does not seem to differ between PR3-AAV or MPO-AAV.\textsuperscript{146}

Cardiovascular disease in AAV has been described as being increased, occurring in 14\% of patients within 5 years of diagnosis,\textsuperscript{146} with patients with MPO-AAV at a higher risk.\textsuperscript{146,147}

Patients with AAV tend to develop more venous thromboembolic events compared with healthy controls,\textsuperscript{148–150} with no clear distinction between patients with PR3-AAV and MPO-AAV.

No difference in the occurrence of malignancies has been shown between MPO-AAV and PR3-AAV patients,\textsuperscript{151} but more long-term data are needed.

### Treatment

The treatment of AAV has been an important research topic during the last two decades and has shown continuous improvement in outcome. The introduction of B-cell targeting therapy for remission induction marked an important milestone. The RITUXVAS trial showed that in newly diagnosed patients with severe renal involvement, rituximab in combination with two pulses of cyclophosphamide induced remission as effectively as a standard regimen of cyclophosphamide pulses, with no significant difference in adverse events.\textsuperscript{152} In the RAVE trial, rituximab was shown to be noninferior to cyclophosphamide in newly diagnosed patients and superior to cyclophosphamide in relapsing patients\textsuperscript{153} after 6 months follow-up, whereas at 18 months follow-up it became apparent that a single course of rituximab was as effective for inducing sustained remission as conventional immunosuppressive treatment with cyclophosphamide followed by azathioprine.\textsuperscript{135} The MAINRITSAN trial showed that patients in remission (after induction

### Table 2. Currently planned or ongoing clinical trials in ANCA-associated vasculitis

<table>
<thead>
<tr>
<th>Name</th>
<th>Treatment Studied</th>
<th>Status</th>
<th>Patients</th>
<th>Primary Outcomes</th>
<th>Clinical Trial no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABROGATE</td>
<td>Abatacept (CTLA-4 immunoglobulin)</td>
<td>Not yet recruiting</td>
<td>GPA</td>
<td>Treatment failure after 12 months of treatment</td>
<td>NCT02108860</td>
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<tr>
<td>Evaluate the efficacy of achieving glucocorticoid-free remission in patients with relapsing non-severe GPA</td>
<td>ALEVIATE</td>
<td>Alectumab (monoclonal anti-CD52)</td>
<td>Unknown</td>
<td>GPA and MPA Complete or partial remission after 6 months; adverse event</td>
<td>NCT01405807</td>
</tr>
<tr>
<td>Evaluating whether alectumab induces sustained remission in refractory patients</td>
<td>BIANCA-SC</td>
<td>Belisibimod (selective antagonist of BAFF)</td>
<td>Not yet recruiting</td>
<td>GPA and MPA Induction of clinical remission</td>
<td>NCT01598857</td>
</tr>
<tr>
<td>Evaluate efficacy of belisibimod when taken with methotrexate to induce remission</td>
<td>BREVAS</td>
<td>Belimumab (human monoclonal anti-BAFF)</td>
<td>Recruiting</td>
<td>GPA and MPA Time to first relapse</td>
<td>NCT01663623</td>
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<tr>
<td>Assessing efficacy of belimumab in maintenance of remission following a standard induction regimen</td>
<td>CLASSIC</td>
<td>CCX168 (CSa inhibitor)</td>
<td>Recruiting</td>
<td>GPA and MPA Safety and efficacy of two-dose regimen</td>
<td>NCT02222155</td>
</tr>
<tr>
<td>Studying the safety and efficacy of CCX168 for induction therapy (low versus high dose) with conventional therapy</td>
<td>CLEAR</td>
<td>CCX168 (CSa inhibitor)</td>
<td>Recruiting</td>
<td>GPA and MPA Safety (adverse events) and efficacy (BVAS)</td>
<td>NCT01363388</td>
</tr>
<tr>
<td>Studying the safety and efficacy of CCX168 versus placebo for induction therapy on a background of conventional therapy</td>
<td>LoVAS</td>
<td>Rituximab with glucocorticoids</td>
<td>Recruiting</td>
<td>GPA and MPA Remission induction</td>
<td>NCT02198248</td>
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<tr>
<td>Comparing rituximab with low-dose glucocorticoids versus rituximab with high-dose glucocorticoids</td>
<td>MAINRITSAN-2</td>
<td>Rituximab (two strategies)</td>
<td>Active, not recruiting</td>
<td>GPA and MPA Number of minor and major relapses</td>
<td>NCT01731561</td>
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<tr>
<td>Assess efficacy of a rituximab regimen based on rate of ANCA and CD19 lymphocytes for maintenance of remission</td>
<td>PEXIVAS</td>
<td>Plasma exchange</td>
<td>Recruiting</td>
<td>GPA and MPA All-cause mortality; end-stage renal disease</td>
<td>NCT00987389</td>
</tr>
<tr>
<td>Determining whether plasma exchange with immunosuppressive therapy are effective in reducing death and ESRD</td>
<td>RAVELOS</td>
<td>Rituximab (chimeric monoclonal anti-CD20)</td>
<td>Recruiting by invitation</td>
<td>GPA and MPA Long-term safety and effects of rituximab</td>
<td>NCT01586858</td>
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<tr>
<td>Following the patients included in the RAVE study to study long-term outcome after rituximab treatment</td>
<td>RITAZAREM</td>
<td>Rituximab</td>
<td>Recruiting</td>
<td>GPA and MPA Time to first relapse</td>
<td>NCT01697267</td>
</tr>
<tr>
<td>Evaluating whether repeated rituximab will maintain remission</td>
<td>SCOUT</td>
<td>Glucocorticoids and rituximab</td>
<td>Recruiting</td>
<td>GPA and MPA Complete remission</td>
<td>NCT02169219</td>
</tr>
<tr>
<td>Studying whether an 8-week course of glucocorticoids with rituximab is effective as remission induction</td>
<td>TAPIR</td>
<td>Glucocorticoids</td>
<td>Recruiting</td>
<td>GPA Increase of the glucocorticoid dose for disease relapse</td>
<td>NCT01933724–NCT01940094</td>
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<td>Evaluating the effects of low-dose glucocorticoids (5 mg/day) versus stopping completely in GPA in remission</td>
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therapy with cyclophosphamide and corticosteroids) treated with rituximab for maintenance of remission had a lower risk for relapse compared with the conventional maintenance therapy of azathioprine (Table 1). Whether rituximab-based responses differ between patients with PR3-AAV and patients with MPO-AAV is at present unknown.

Different new treatments in AAV are currently being studied. Importantly, in patients with more limited forms of AAV (mainly limited GPA) remissions can be achieved with methotrexate and possibly abatacept (CTLA4-immunoglobulins). Also, for patients with refractory forms of AAV, plasma exchange, intravenous immunoglobulins, deoxyspergualin, mycophenolate mofetil, alemtuzumab and/or mizoribine may be effective. Furthermore, blocking complement activation via C5a or its receptor, effective in animal models, may be an appealing option.

Besides exploring new treatment strategies, many questions remain to be answered with regard to the traditional treatment regimens. One example is whether glucocorticoids need to be maintained at low dose when patients attain remission or whether they need to be stopped completely. Another question is how patients who never had a relapse should be treated in the long-term. Currently ongoing trials may provide answers to these essential questions (Table 2).

Despite the differences between PR3-AAV and MPO-AAV, treatment protocols are the same (Figure 3). Nevertheless, because patients with PR3-AAV relapse more frequently, retreatment in these patients is more common. In agreement with Furuta and Jayne, we advocate segregating AAV by ANCA serotype in future clinical trials as opposed to by the disease type classification.

**Figure 3.** Flow diagram for treatment in AAV. Evidence for every step is given as follows, linked to the symbols in the diagram. (A) Silva et al. and Stassen et al. (B) NORAM trial; (C) MEFEX trial; (D) PEXIVAS trial (table 2); (E) RAVE trial and RITUXVAS trial; (F) LoVAS trial and RAVELOS study (table 2); (G) CYCLOPS trial; (H) WEGENT trial; (I) CYCAZAREM trial; (J) IMPROVE trial; (K) MAINRITSAN (table 1), RITAZAREM and SCOUT trials (table 2); (L) TAPIR trial (table 2).

**New Classification**

The differences in presentation with regard to PR3-AAV and MPO-AAV aided the development of a cluster analysis of 673 patients with AAV. This cluster analysis showed that GPA and MPA are difficult to distinguish and that their distinction is more subjective than the distinction based on ANCA serotype. Based on clinical and outcome data, patients with AAV can be clustered as nonrenal AAV patients, renal PR3-ANCA-positive patients and renal PR3-ANCA-negative patients. This cluster analysis supports a break-up into PR3 and MPO-ANCA, rather than into GPA and MPA. Also, based on the clear genetic distinctions between ANCA serotypes, categorization of patients based on ANCA serotype is appealing. At present, however, patients are still categorized according to a clinical diagnosis. The difference of diagnosing GPA or MPA is based on, sometimes subtle, clinical features and is less objective.
when compared with a distinction by ANCA serotype. With some evidence for a worse renal and patient outcome for MPO-AAV compared with PR3-AAV, there is increasing support to categorize patients based on their ANCA serotype. Finally, categorizing patients by ANCA serotype instead of clinical diagnosis may decrease a selection bias for including patients in clinical trials.

Table 3. Similarities and differences between PR3-AAV and MPO-AAV with quality of evidence

<table>
<thead>
<tr>
<th>Feature</th>
<th>Evidence</th>
<th>PR3-AAV</th>
<th>MPO-AAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemiology</td>
<td></td>
<td>B</td>
<td>Higher</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PR3-AAV</td>
<td>MPO-AAV</td>
</tr>
<tr>
<td>Geographical distribution</td>
<td>A</td>
<td>More prevalent in northwestern Europe and North America</td>
<td>More prevalent in southern Europe, Asia and the Pacific</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incidence 11.3/million/yr in UK and 3/7.9/million/yr in Spain</td>
<td></td>
</tr>
<tr>
<td>Genetics</td>
<td></td>
<td>A</td>
<td>More prevalent</td>
</tr>
<tr>
<td>HLA-DPB1</td>
<td>B</td>
<td>Less prevalent</td>
<td>More prevalent</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td></td>
<td>PR3-AAV</td>
<td>MPO-AAV</td>
</tr>
<tr>
<td>PRTN3</td>
<td>B</td>
<td>More prevalent</td>
<td>Less prevalent</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>C</td>
<td>Less prevalent</td>
<td>More prevalent</td>
</tr>
<tr>
<td>HLA-DRB*15</td>
<td>C</td>
<td>More prevalent in African Americans</td>
<td>Not prevalent</td>
</tr>
<tr>
<td>Renal histopathology</td>
<td></td>
<td>C</td>
<td>In both serotypes granuloma formation can be seen</td>
</tr>
<tr>
<td>Normal glomeruli</td>
<td>A</td>
<td>More</td>
<td>Less</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>C</td>
<td>Less</td>
<td>More</td>
</tr>
<tr>
<td>Electron-dense deposits</td>
<td>D</td>
<td>Not different</td>
<td>More C3 deposition</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>C</td>
<td>Less C3 deposition</td>
<td>More C3 deposition</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td>A</td>
<td>Not different</td>
</tr>
<tr>
<td>Age at onset</td>
<td></td>
<td>B</td>
<td>More ear, nose, and throat involvement</td>
</tr>
<tr>
<td>Organ involvement in general</td>
<td></td>
<td>C</td>
<td>Cavitating lesions</td>
</tr>
<tr>
<td>Pulmonary</td>
<td></td>
<td>C</td>
<td>More</td>
</tr>
<tr>
<td>Alveolar lung hemorrhage</td>
<td></td>
<td>B</td>
<td>Necrotizing lesions</td>
</tr>
<tr>
<td>Ear, nose, and throat</td>
<td></td>
<td>C</td>
<td>Similar frequency</td>
</tr>
<tr>
<td>Nervous system</td>
<td></td>
<td>C</td>
<td>Not different</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>C</td>
<td>Not different</td>
</tr>
<tr>
<td>Cardiac</td>
<td></td>
<td>C</td>
<td>Not different</td>
</tr>
<tr>
<td>Ophthalmologic</td>
<td></td>
<td>C</td>
<td>More ophthalmologic involvement</td>
</tr>
<tr>
<td>Follow-up</td>
<td></td>
<td>A</td>
<td>Higher</td>
</tr>
<tr>
<td>Relapse rate</td>
<td></td>
<td>C</td>
<td>Similar/better</td>
</tr>
<tr>
<td>Patient survival</td>
<td></td>
<td>C</td>
<td>Similar/better</td>
</tr>
<tr>
<td>Renal survival</td>
<td></td>
<td>C</td>
<td>Similar/better</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td>D</td>
<td>Not different</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td>C</td>
<td>Fewer events</td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td></td>
<td>D</td>
<td>Not different</td>
</tr>
<tr>
<td>Malignancies</td>
<td></td>
<td>D</td>
<td>Not different</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td>C</td>
<td>Not different</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td>C</td>
<td>S. aureus*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low vitamin D levels*</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td></td>
<td>D</td>
<td>Not different</td>
</tr>
<tr>
<td>In vitro interaction of ANCA with neutrophils</td>
<td>D</td>
<td>PR3 induces apoptosis of endothelial cell</td>
<td>MPO induces production of intracellular oxidants</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td></td>
<td>D</td>
<td>PR3-ANCA induces release of sFlt1 by monocytes</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td>D</td>
<td>Less successful models, possibly due to different interaction of PR3 with NB1 in mice</td>
</tr>
</tbody>
</table>

Quality of evidence was based on the GRADE system as follows: A, Further research is very unlikely to change our confidence in the estimate of effect; B, Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; C, Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; D, Any estimate of effect is very uncertain). GRADE, Grading of Recommendations Assessment, Development and Evaluation.

*These etiologic factors remain speculative due to insufficient evidence.
CONCLUSION

Evidence that PR3-AAV and MPO-AAV are two distinct diseases of one entity has accumulated since our review in 2000 (Table 3). It remains, however, difficult to distinguish characteristic clinical differences, despite a clearly different genetic basis of both vasculitides. When considering genetic differences and cluster analyses data, it is appealing to break up the ANCA-associated vasculitides into PR3-ANCA vasculitis and MPO-ANCA vasculitis. This discrimination might prove of great significance in the scope of clinical trials and could aid the further improvement of treatment.

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DISCLOSURES

None.

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