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ANCA-associated vasculitis: On clinical management and renal outcome

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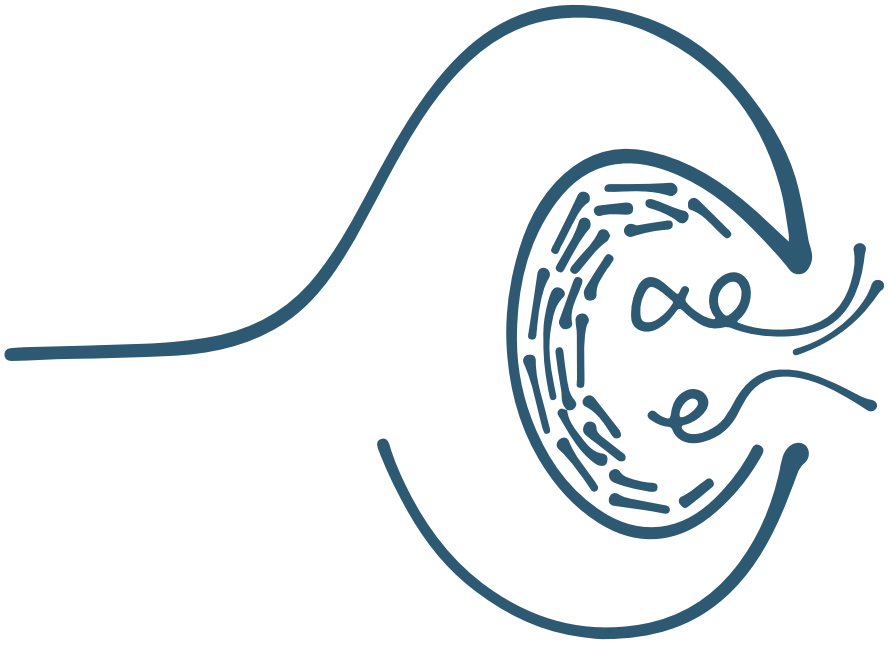
1

GENERAL INTRODUCTION



BRIEF INTRODUCTION

Antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) is a necrotizing small vessel vasculitis with few or no immune deposits. Vasculitis means inflammation of the blood vessel wall. Small vessel vasculitis predominantly affects small vessels; these are defined as small intraparenchymal arteries, arterioles, capillaries, and venules. However, in AAV medium-sized arteries and veins, i.e. the main visceral arteries and their branches, may also be affected.[1] AAV comprises a group of diseases consisting of granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome). Some consider renal limited vasculitis (RLV) to be a separate subtype of AAV. GPA, MPA and EGPA are associated with circulating ANCA specific for antigens located within neutrophil granules and monocyte lysosomes. The classical antigens in AAV are proteinase 3 (PR3)[2-5] and myeloperoxidase (MPO).[6] Up to approximately 10% of patients diagnosed with AAV are ANCA-negative using currently available indirect immunofluorescence (IIF) and Enzyme-Linked Immuno Sorbent Assays (ELISA).[7-12]

AAV is a rare disease. The overall incidence of AAV in Europe, Northern America and Japan is approximately 20 per million/year. The incidence of GPA is higher in Northern Europe, whereas that of MPA is higher in Southern Europe and Japan.[13-15] AAV has a peak incidence of 65 per million/year between the ages of 65 and 74 years, although it can occur at any age.[16,17] Data from the United Kingdom (UK) show prevalences of 148 per million for GPA and 65 per million for MPA.[13,15-17] The prevalence is generally higher in men; women more often develop the disease at a younger age.[17,18] The overall prevalence of AAV is highest in Caucasians.[18,19] A study in France showed that the prevalence of AAV was twice as high in Europeans (105 per million) compared to non-Europeans (53 per million, mainly African and Asian descent).[20] PR3-ANCA is more common than MPO-ANCA in the UK and Northern Europe, while MPO-ANCA is more prevalent than PR3-ANCA in Southern Europe and Asia.[13,16,21] These data show that AAV is quite diverse regarding the incidence and prevalence in different regions, different races and over age.

Patients typically present with prodromal "flu-like" symptoms, such as fever, polymyalgia, polyarthralgia, headache, malaise, anorexia, and unintended weight loss during several weeks or months.[22,23] These symptoms are non-specific and overlap with symptoms of many other non-vasculitic diseases. Some patients may initially present with focal vasculitic disease such as cutaneous vasculitis, bloody-purulent rhinitis, scleritis, or arthritis. During the disease course more disease manifestations

may occur and various organs may become involved. Virtually every organ can be affected by the disease,[24-27] but organs commonly involved are: ear, nose and throat (ENT), kidneys, lungs, skin, eyes and the nervous system.[22,23,28,29] The organs involved in the various ANCA-associated vasculitides overlap, but some organs are involved more commonly in specific disease entities. For example, ENT involvement occurs in about 90% of patients with GPA and in 35% of patients with MPA.[22,23] ANCA-associated glomerulonephritis (AAGN) is commonly seen in GPA and MPA and is characterized by a pauci-immune necrotizing crescentic glomerulonephritis.[23,30,31]

It is challenging for physicians to recognize AAV at an early stage. In a large survey study of 701 GPA patients, time between disease onset and diagnosis was 3-12 months for most patients. Of these patients, 44% visited one to three physicians, 45% visited four to eight physicians, and 11% visited nine or more physicians before the final diagnosis was made. Only 7% of the patients received a diagnosis of GPA upon their first visit to a physician.[18] The diagnosis of AAV is based on the clinicopathologic disease manifestations and ANCA-serology. The gold standard for diagnosing AAV is histology of a lesion from an affected organ.

Without treatment, average patient survival is approximately five months: 82% of patients die within the first year after diagnosis and more than 90% die within two years. The most common causes of death are rapidly progressive renal failure and respiratory failure.[32] Modern immunosuppressive treatment has changed the fulminant disease course of AAV into a more chronic course, characterized by remission and relapses. Standard treatment consists of remission induction with high dose glucocorticoids and high dose oral or intravenous pulse cyclophosphamide or rituximab for three to six months, followed by remission maintenance treatment with azathioprine or methotrexate while glucocorticoids are slowly reduced and withdrawn.[10,33] Rituximab – a monoclonal anti-B cell agent – has been thoroughly investigated in recent years and is used more and more to substitute cyclophosphamide as induction treatment.[12,34] Intravenous methylprednisolone or plasma exchange can be added as induction therapy in case of severe or life-threatening vasculitic disease at presentation, such as pulmonary hemorrhage.[11,35]

This introduction provides a general overview of the two major ANCA-associated vasculitides, namely GPA and MPA, with a special focus on renal involvement. EGPA is beyond the scope of this thesis. Below, an overview of three perspectives is given: the patient's perspective, the physician's perspective and the researcher's perspective.

The last part of the general introduction lists the incentives for and aims of the studies described in this thesis, and provides a thesis outline.

I PATIENT'S PERSPECTIVE

Assessment tools in vasculitis have always been based on consensus of expert physicians and what they consider relevant in terms of disease activity, disease extent and tissue damage. Examples are the Birmingham Vasculitis Activity Score (BVAS),[36-38] the Disease Extent Index[39] and the Vasculitis Damage Index.[40] The patient's perspective is missing. Studies showed that patients and physicians rate disease manifestations and impact differently in AAV with no or very low correlation between both.[41-47]

In recent years, attention for the patient's perspective has increased in the research field of AAV, following a trend in medicine in general. Patient care should not only be focused on curing the patient, but also on maintaining or improving quality of life (QoL). Patient-reported outcome (PRO) is a way to measure the QoL.

Qualitiy of Life in AAV

Studies in AAV have reported reduced QoL compared to the general population.[41,43,46-50] Most of these studies used the Short Form 36 (SF-36), which is a generic measure combining a physical component summary (PCS) score and a mental component summary (MCS) score.[51] A higher score indicates a better QoL. In two studies, both including approximately 400 patients, the PCS and MCS were lower compared to the general UK population. Physical components scored worse than mental components (figure 1).[48,50] Treatments aimed at the physical consequences of AAV will probably give most improvement in QoL.

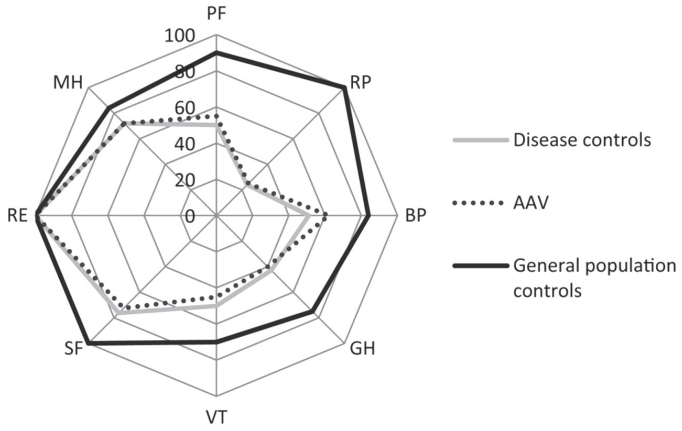
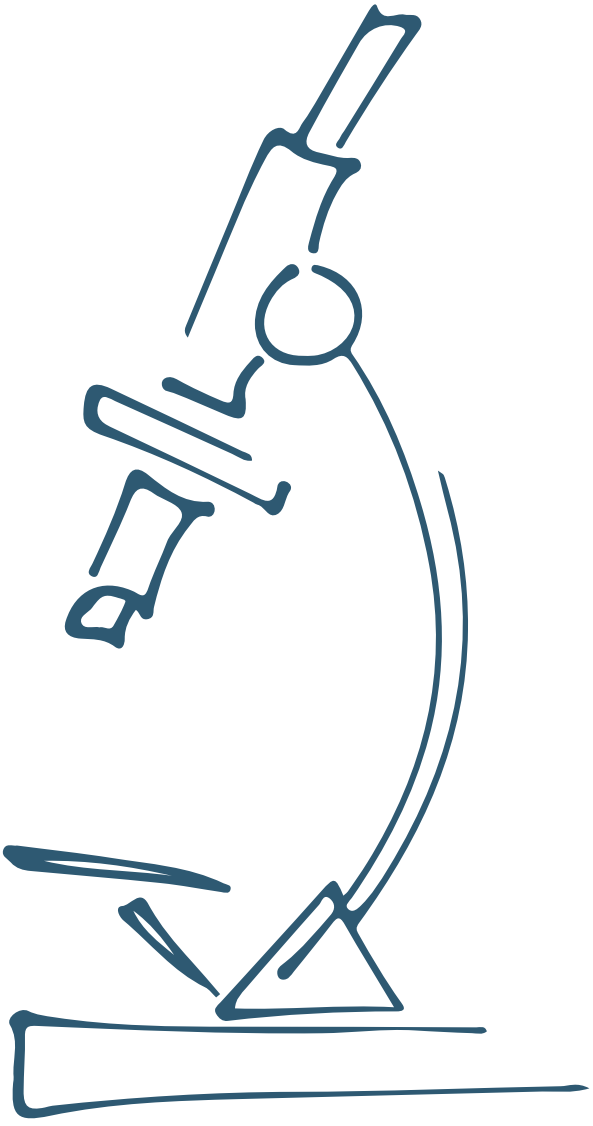


Figure 1 | Median SF-36 scores of the study by Basu *et al.* (410 AAV patients, 318 chronic disease controls, 470 general population controls).
This figure shows that QoL is scored lower by AAV patients compared to the general population controls. QoL is scored similar between AAV patients and chronic disease controls, i.e. inflammatory arthritis or chronic kidney disease. Physical components scored worse than mental components in the AAV group. PF, Physical Function; RP, Role Physical; BP, Bodily Pain; GH, General Health; VT, Vitality; SF, Social Functioning; RE, Role Emotional; MH, Mental Health; AAV, ANCA-associated vasculitis; QoL, Quality of Life. Reproduced from Basu *et al.* (Ann Rheum Dis 2014;73:207-211) with permission.

One major limitation of the SF-36 is that the scoring system is not disease-specific. Therefore, a vasculitis-specific PRO tool is being developed. This tool is based on ranked vasculitis-related burdens.[52] Patients (n=264) from 3 countries (Germany, United States, United Kingdom) ranked 40 vasculitis-related items and listed the five most important aspects of the disease in their daily live. Eighty-one percent of these patients had AAV, the remaining 19% had other vasculitides. On a scale of 0-10 (0 = no impact on QoL, 10 = extremely negative impact on QoL), the impact of vasculitis on QoL was scored as 4.6 ± SD 2.4. Symptoms with the highest burden were fatigue, loss of energy, weight gain, joint pain, and sinusitis. Most commonly mentioned in the free text section were fatigue and energy loss, pain and musculoskeletal symptoms.[42]

Due to the differences in perception of “illness” by patients and physicians it is important to incorporate disease-specific PRO instruments in the clinical management of the disease in order to tailor clinical management. Treatment goals should be extended beyond induction of remission and its maintenance, which, in itself, also can partly recover QoL.



II RESEARCHER'S PERSPECTIVE

This part focuses on important aspects of the pathogenesis of AAV in fundamental research. Research on clinical management will be discussed in the next part: Physician's perspective.

Pathogenesis

The exact pathogenesis of AAV is unknown, but neutrophils are considered to play a key role. Due to currently unknown triggers, cytokines are released that stimulate priming and accumulation of circulating neutrophils. Potential triggers are infections (particularly infections with Staphylococcus aureus)[53-55] and silica exposure,[56] combined with a genetic predisposition.[57-59] After neutrophil priming, PR3 and MPO are exposed on the neutrophil cell membrane, where they become accessible to ANCA produced by B-cells. When ANCA bind to their specific antigens, the neutrophil becomes activated and degranulates leading to the production of reactive oxygen species and release of proteolytic enzymes. Neutrophils that are activated by ANCA can also directly interact with endothelial cells through β2-integrin and other adhesion molecules expressed by activated endothelial cells. Both mechanisms are cytotoxic for the endothelial cells.[60] In addition, neutrophils release factors that activate the alternative pathway of the complement system. This pathway generates C5a, which recruits and primes more neutrophils.[61-66] The role of the complement system was long underestimated, probably because of the characteristic pauci-immune immunofluorescence pattern seen in renal biopsies of AAGN. The importance of the complement system is supported by the presence of activation components of the alternative pathway in plasma and tissue of AAV patients.[61,62,65,66]

These different processes, which occur simultaneously, amplify the inflammatory process resulting in acute necrotic injury to blood vessel walls. There is evidence that during this inflammatory process neutrophil extracellular traps (NETs) are formed through NETosis. NETosis is a specific form of cell death, which is characterized by the release of decondensed chromatin threads containing cytoplasmic proteins including PR3 and MPO. NETs have been demonstrated to have a beneficial role in the defense against infections, but have also been implicated in autoimmune diseases, in which they trigger and promote a vasculitic response by presenting PR3 and MPO in their decondensed chromatin threads (figure 2 and 3).[67,68] In addition, abnormalities in cellular immunity seem to play an important role in the pathogenesis of AAV demonstrated by evidence of altered T cell characteristics with an increase in activated and memory T cells. The homeostasis of CD4+ T cells seems disturbed in AAV patients with an amplification of the auto-immune response due to activated CD4+ cells.[69-72]

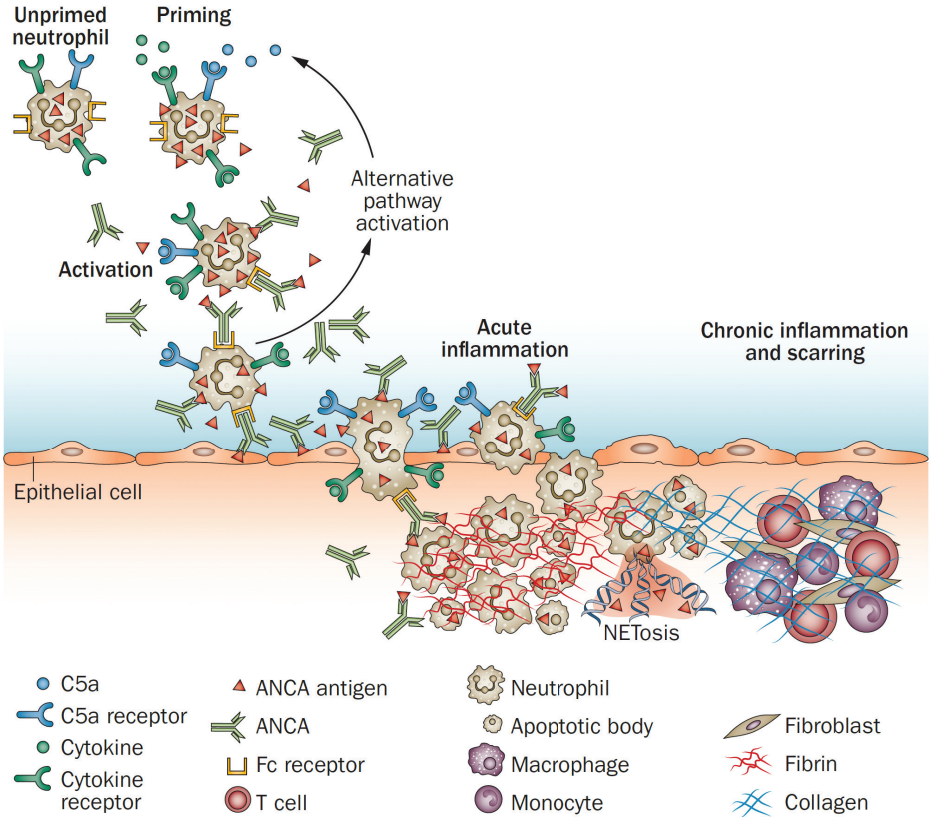


Figure 2 | Current hypothesized pathogenesis of ANCA-associated vasculitis.
A trigger activates the release of cytokines, which stimulate priming and accumulation of circulating neutrophils. PR3 and MPO are exposed on the neutrophil, making them accessible to ANCA produced by B-cells. ANCA binding to the exposed antigens activate the neutrophil: release of proteolytic enzymes, interaction with endothelial cells, activate alternative pathway of the complement system. The processes amplify the inflammatory process resulting in vasculitis. In addition, NETs are formed by neutrophil cell death (NETosis), which promote the vasculitic inflammation process. ANCA, Antineutrophil cytoplasmic autoantibody; PR3, proteinase 3; MPO, myeloperoxidase; NET, neutrophil extracellular traps. Reproduced from Jennette *et al.* (Nat Rev Rheumatol 2014;10:463-473) with permission.

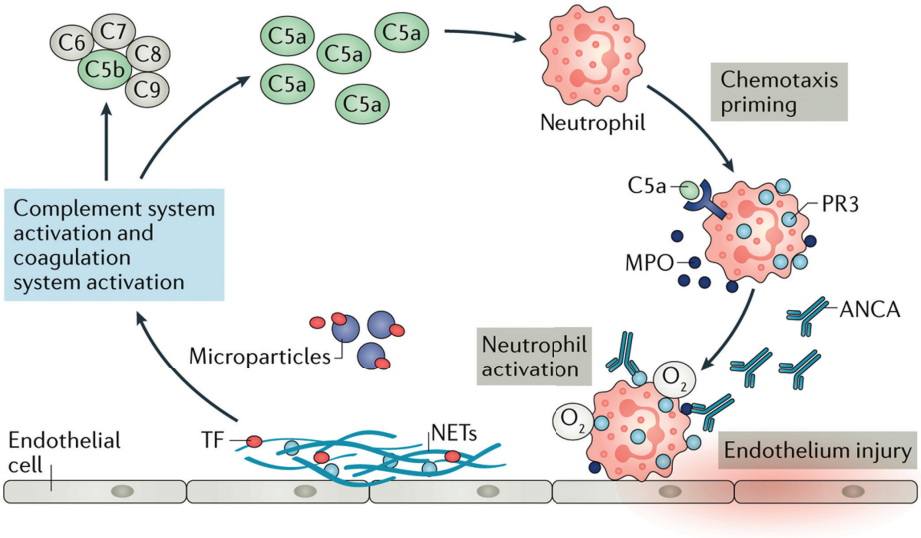


Figure 3 | Current hypothesized pathogenesis of ANCA-associated vasculitis focusing more on complement system acitvation.
Activated neutrophils stimulate the complement system, i.e. alternative pathway. C5a is produced which recruits and primes more neutrophils. NETs are formed by NETosis of neutrophils. This is an amplifying process resulting in vasculitis. ANCA, Antineutrophil cytoplasmic autoantibody; PR3, proteinase 3; MPO, myeloperoxidase; NET, neutrophil extracellular traps; TF, Tissue Factor. Reproduced from Chen *et al.* (Nat Rev Nephrol 2017;13:359-367) with permission.

ANCA and other autoantibodies

MPO-ANCA and PR3-ANCA are defining features of AAV. Their presence is used to establish the diagnosis of AAV and there is increasing evidence that they play an important role in its pathogenesis.[73] Low levels of circulating MPO-ANCA and PR3-ANCA have been detected in healthy individuals and it has been demonstrated that epitope specificity of MPO-ANCA differs between AAV patients and healthy individuals.[74,75] Varying epitope-specificity of ANCA will influence their effects and their potential for pathogenicity.

ANCA can be detected in patient sera by IIF and ELISA. The cytoplasmic ANCA (cANCA) pattern seen on IIF is associated with the presence of PR3-ANCA, while the perinuclear ANCA (pANCA) pattern is associated with MPO-ANCA.[2-6,76] Up to approximately 10% of patients with AAV are currently ANCA-negative using these techniques.[7-12] Roth *et al.*, however, detected MPO-ANCA in 14/21 patients that were tested ANCA-negative with standard detection techniques, using purified IgG in a highly sensitive epitope excision/mass spectrometry approach. They demonstrated that serum ceruloplasmin masked the detection of the ANCA-epitope in these patients, resulting in negative

results on routine assays. Through purification of IgG, ceruloplasmin was eliminated from the assay allowing detection of MPO-ANCA. MPO-ANCAs against this specific, covered epitope were shown to have pathogenic properties: they were capable to activate neutrophils *in vitro* and to induce nephritis in mice.[75]

Although ANCA seem to play a role in the pathogenesis of AAV, a correlation between the titer of these antibodies and the level of disease activity or the prediction of a relapse by a rise of the titer, is not yet shown convincingly. A meta-analysis described that a rise in—or persistence of—ANCA has modest predictive value for future disease relapse in AAV patients. Therefore, the isolated use of serial ANCA measurements is insufficient for therapeutic decision-making.[77]

Currently described factors that influence the detection of ANCA and the assessment of their pathogenicity are epitope specificity, masking of (pathogenic) epitopes, modified antigens and technical limitations of current assays. The International Consensus Statement on Testing and Reporting ANCA advocated to screen for the presence of ANCA with IIF and confirm positive results on IIF with PR3-ANCA and MPO-ANCA specific ELISA.[78,79] Current clinical practice often consists of making a presumptive diagnosis of AAV based on the clinical presentation, ANCA positivity with ELISA and a low suspicion for another disease, with obtaining a biopsy as soon as possible to confirm the diagnosis. A recent study showed a large variability between different IIF methods and a high diagnostic performance of PR3-ANCA and MPO-ANCA by ELISA. Therefore, the use of both IIF and ELISA testing of each sample is not deemed necessary anymore for maximal diagnostic accuracy.[80] Different immunoassays have been developed for the detection of ANCA to improve their performance. Although these assays at the moment do not replace current methodologies, future research may change this.[81]

In addition to classical ANCA, the presence of other autoantibodies has also been reported in AAV. Recently, studies described the presence of anti-plasminogen autoantibodies (α-PLG) in 22%-43% of PR3-AAV and 6%-27% of MPO-AAV patients in different cohorts.[82-84] The presence of these antibodies disturbs the conversion of plasminogen into plasmin, thereby inhibiting fibrinolysis.[82,83] These antibodies were associated with a susceptibility for thrombosis in PR3-ANCA patients with 56% (5/9) of the PR3-AAV patients with a thrombotic event being α-PLG positive compared to 9% (5/57) of randomly selected disease controls with idiopathic thrombosis.[82] Their presence was also associated with significantly more (cellular) crescents and fibrinoid necrosis in renal biopsies accompanied by a worse renal function at diagnosis and

during follow-up.[83] The assays used in the different studies to detect α-PLG were, however, not clearly defined and showed some differences between each other. Therefore, we investigated different ELISA set-ups for detecting α-PLG in order to optimize the assay and present an assay to promote uniform reporting. **Chapter 2** presents an optimized ELISA and validates the presence of α-PLG in AAV using this new assay.

A few years ago, an unexpected finding introduced a new theory: in patients with AAV harboring PR3-ANCA, the presence of antibodies against complementary PR3 (cPR3, the peptide translated from the antisense DNA strand) was detected. These antibodies had an idiotypic relationship with antibodies against PR3. Immunizing mice with cPR3 resulted not only in the production of antibodies against cPR3, but also against PR3. In several microorganisms, such as *Staphylococcus aureus*, genetic sequences were identified with similarities to the antisense DNA of PR3. These genetic sequences encode proteins of *Staphylococcus aureus* that resemble human cPR3. When antibodies are made against the amino acid sequence of the epitope of *Staphylococcus aureus*, these antibodies will also bind to cPR3. This phenomenon is referred to as molecular mimicry. The concept of molecular mimicry is a theoretically important concept in thinking on the etiology of autoimmune diseases. Above findings led to the hypothesis that a foreign protein homologous to cPR3 could elicit an immune response by inducing the formation of antibodies. These antibodies would then cross-react with cPR3 and elicit an anti-antibody response causing the formation of anti-idiotypic antibodies. Following this hypothesis, the anti-idiotypic antibodies are the actual ANCA and are reactive against the PR3-antigen and in this way could cause AAV.[85,86] Interestingly, dual reactivity to cPR3 and plasminogen due to a homologue amino acid sequence has been described in PR3-AAV.[82]

Kain *et al*. described the presence of another type of autoantibodies, namely against human lysosomal-associated membrane protein-2 (hLAMP-2) in approximately 80-95% of European patients presenting with AAGN with or without other systemic manifestations of AAV.[87-89] hLAMP-2 is a protein integrated in the membranes of intracellular vesicles of neutrophils that also contain MPO and PR3. In contrast to MPO and PR3, this protein shuttles between lysosomes, endosomes and the cell membrane.[90] The availability of hLAMP-2 on the surface of neutrophils or endothelial cells is abundant and hLAMP-2 is therefore directly accessible to circulating antibodies. Antibodies directed against hLAMP-2 have been detected in PR3-ANCA, MPO-ANCA and ANCA-negative patients. *In vitro*, anti-hLAMP-2 antibodies activate neutrophils and directly kill microvascular endothelial cells. When 15 Wistar Kyoto (WKY) rats

were intravenously injected with hLAMP-2 specific rabbit immunoglobulin G (IgG), which cross-reacts with rat LAMP-2, these rats developed hematuria, proteinuria, renal leukocyte infiltration, focal capillary necrosis and glomerular crescents. These anti-hLAMP-2 antibodies seem to be directed against 2 major epitopes, of which one shows homology with an amino acid sequence of mature FimH. FimH is a bacterial adhesin located at the tip of type 1 fimbriae that is crucial for attachment of Gram-negative pathogens, like *Escherichia coli*, to host epithelia. Due to this molecular mimicry - resembling amino acid sequence - between the epitopes of hLAMP-2 and FimH, an infection with *Escherichia coli* could theoretically cause the production of antibodies that also react with hLAMP-2. Injection of 10 WKY rats with recombinant FimH fusion protein resulted in antibodies to rat LAMP-2 and pauci-immune necrotizing glomerulonephritis similar to human disease in nine of them. Of the patients who tested positive for anti-hLAMP-2 autoantibodies, circa 70% turned out to have had an infection with FimH-expressing bacteria during the 12 weeks before presenting with AAGN.[87] After initializing immunosuppressive therapy, hLAMP-2 autoantibody titers became undetectable. During clinical relapse the autoantibodies became detectable again.[89] These findings suggest a pathogenicity of this novel ANCA. However, to date, the presence of anti-hLAMP-2 autoantibodies in patients with AAGN has not been confirmed by other research groups.[91]

Animal models

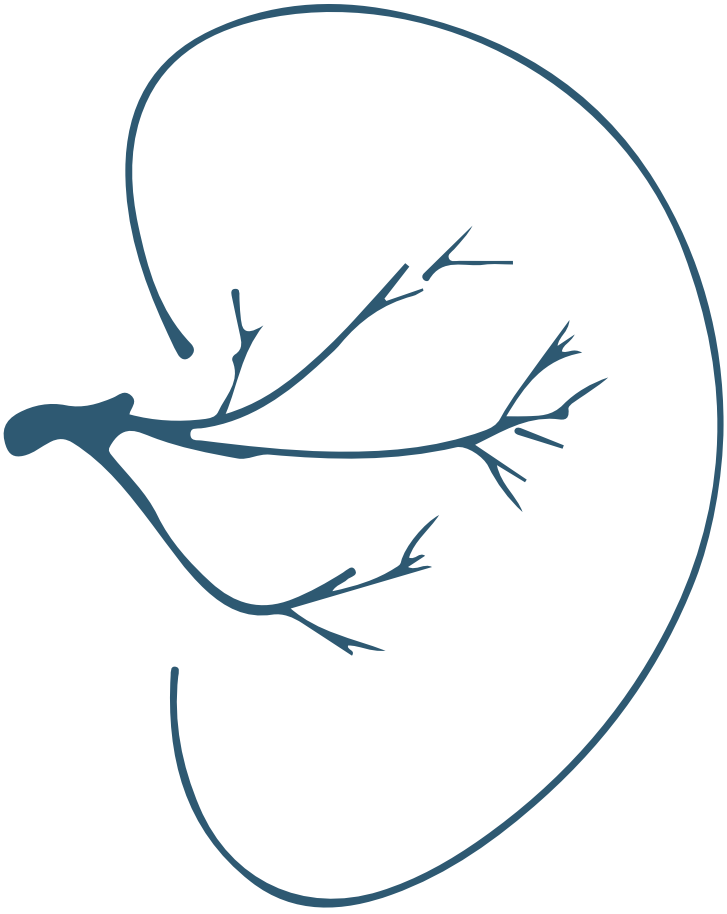
The first animal model in AAV was described in 2002 and was a MPO-AAV mouse model. They developed hematuria and proteinuria, and the kidneys showed comparable histopathologic lesions as seen in human AAGN.[92] Hereafter, a rat model was developed for MPO-AAV in which rats were immunized with human MPO and developed anti-human MPO-ANCA. These rats develop a disease similar to AAV.[93]

A PR3-AAV animal model is still lacking. The antibody transfer techniques used in the MPO-ANCA models were unsuccessful for PR3-ANCA.[94] In 2012, Little *et al*. published on a mouse model with a human immune system. This model has great potential, but also some limitations such as the chimeric nature of the immune response (human immune system in a mouse), and the model is technically challenging and expensive.[95]

Genetics

Evidence for an important role of genetics in the pathogenesis of AAV is growing.[96-98] A genome-wide association study in 2012 demonstrated several important genes that also made a distinction between ANCA serotypes.[58] A meta-analysis identified 33 genetic variants associated with AAV and confirmed the stronger genetic basis of

subdivisions based on ANCA-specificity compared to clinical diagnosis. Identified genes encode alpha-1-antitrypsin, are part of the major histocompatibility complex system or are involved in different distinct inflammatory processes.[59]



III PHYSICIAN'S PERSPECTIVE

The last perspective that will be introduced here is the physician's perspective. In this section, the focus is on the treatment options for AAV and disease relapse. The treatment of AAV is challenging and much research is performed to optimize treatment. One of the challenges in particular is disease relapse. **Chapter 6** of this thesis is a clinical review giving an overview of the diagnosis and management of AAV for general practitioners.

Diagnosis of AAV and renal histopathology

Current practice is to make a presumptive diagnosis of AAV based on the clinical presentation, ANCA positivity with ELISA and a low suspicion for another disease, with obtaining a biopsy as soon as possible to confirm the diagnosis. The gold standard for establishing a diagnosis of renal involvement in AAV is a renal biopsy. Light microscopy shows necrotizing and crescentic glomerulonephritis. Immunofluorescence microscopy shows a pauci-immune pattern, which means a negative or subdued granular pattern for immunoglobulins and complement.[23,30,31,99] Renal histology of patients from the CYCAZAREM, MEPEX and RITUXVAS trials was evaluated with regard to renal outcome. These studies showed that, in addition to baseline renal function, also the amount of active (cellular crescents, fibrinoid necrosis and tubulitis) and chronic renal lesions (glomerulosclerosis, tubular atrophy and interstitial fibrosis) were associated with renal outcome. Chronic lesions and tubulitis were associated with adverse renal outcome, while cellular crescents and fibrinoid necrosis were associated with recovery of renal function. In addition, the percentage of normal glomeruli is strongly associated with renal outcome; a higher percentage of normal glomeruli at diagnosis is associated with a better renal function and dialysis-independency at one-year follow-up.[100-102] Based on these findings, Berden *et al.* proposed a histopathological classification of AAGN based on glomerular pathology as assessed by light microscopy (figure 4). This classification is based on four categories: focal, crescentic, sclerotic, and mixed class. Depending on the predominant glomerular phenotype, each renal biopsy with AAGN can be classified into one of these four classes. If the biopsy contains ≥50% normal glomeruli (not affected by the disease process), it will be classified as focal class; with ≥50% cellular crescentic glomeruli as crescentic class; and with ≥50% globally sclerotic glomeruli as sclerotic class. The mixed class includes biopsies wherein no glomerular feature predominates and all aforementioned glomerular phenotypes are present in varying degrees. Tubulointerstitial lesions are not included in the classification. This classification was shown to be associated with one- and five-year renal function and development of ESRF.[103] Validation studies and a meta-analysis confirmed the good prognosis for focal class and a bad prognosis for sclerotic class. In addition, these

studies showed a contradiction regarding the prognosis of crescentic and mixed class.[104] Due to this contradiction and the national characters of these validation studies, a large international validation study consisting of a worldwide cohort was called for. In addition to this, not much is known about the interobserver variability regarding this classification. **Chapter 4** describes a large international validation study with a worldwide cohort which also analyzed the interobserver variability of the histopathological classification of AAGN.

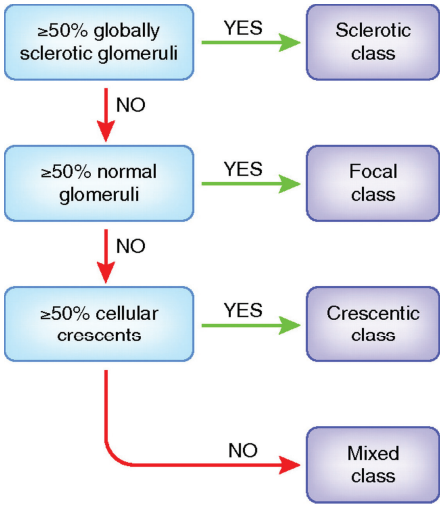


Figure 4 | Histopathologic classification of ANCA-associated glomerulonephritis.
This classification classifies each renal biopsy with AAGN into a class based on the predominant glomerular phenotype. AAGN, ANCA-associated glomerulonephritis. Reproduced from Berden *et al.* (J Am Soc Nephrol 2010;21:1628-1636) with permission.

Renal involvement in AAV (ANCA-associated glomerulonephritis)

Some patients present with ESRF due to AAGN and others develop it during follow-up despite treatment. Approximately 80% of patients with GPA and 90% of patients with MPA develop kidney involvement during the disease course.[23] AAGN progresses to ESRF requiring renal replacement therapy in approximately 20-40% of patients.[105-108] Data on outcome after renal transplantation in AAGN are based on relatively small cohorts. Reported one year graft survival rates are 86-100% and five year graft survival rates are 69-100%. Relapse rates ranged from 1.0-2.0% per patient year of follow-up.[109-113] Due to the small cohort studies a large cohort study was needed. In addition, data focusing on the impact of renal disease recurrence in the graft was lacking. Therefore, we performed a national study on the outcome of renal transplantation in a Dutch cohort of AAGN patients with special focus on the impact of renal disease recurrence on graft survival. This study is described in **chapter 5** of this thesis.

Treatment of AAV

Currently, standard treatment consists of inducing remission with high dose glucocorticoids and high dose oral or intravenous pulse cyclophosphamide or rituximab for three to six months, followed by maintenance therapy with azathioprine or methotrexate while glucocorticoids are slowly reduced and withdrawn.[10,33] In case of severe or life-threatening vasculitic disease, intravenous methylprednisolone or plasma exchange can be added to induction therapy.[11,35] Initially, patients were treated with cyclophosphamide for a longer period.[22,53,114,115] In mild AAV (serum creatinine <150 μmol/L and no critical organ manifestations) methotrexate can substitute cyclophosphamide in the induction regimen, but immunosuppressive therapy should not be stopped at 12 months, because of the higher chance for a relapse.[8] Azathioprine can be substituted by methotrexate as maintenance therapy in AAV patients with a creatinine <150 μmol/L.[33,116]

In patients with generalized AAV, cyclophosphamide can be substituted with azathioprine as remission maintenance therapy. This has no negative effect on disease relapse or severe adverse events.[10] This could decrease the toxic effect of cyclophosphamide. However, a long-term follow-up study (median follow-up of 8.5 years) showed that it remains uncertain whether it is beneficial to convert to azathioprine after three to six months of induction cyclophosphamide therapy instead of converting after 12 months, because on the long term there was no difference in the risk of relapse, end-stage renal failure (ESRF), developing malignancies and death.[117] In addition, intravenous pulse cyclophosphamide showed to cause lower cumulative cyclophosphamide dose compared to daily oral cyclophosphamide. This did not affect the remission rate at 18 months or adverse events with less leucopenia or mortality in case of pulse cyclophosphamide.[7,118] However, on the long term, pulse cyclophosphamide showed to have a higher relapse rate compared to the daily cyclophosphamide with no difference in mortality, morbidity or adverse events.[119]

In case of severe renal involvement (serum creatinine >500 μmol/L or dialysis dependency at diagnosis), plasma exchange is superior to intravenous methylprednisolone as adjunctive therapy regarding dialysis-independency at three months and progression to ESRF at one year. There was no difference in patient survival and adverse events at one year.[11] The long-term follow-up study (four years median follow-up) showed no difference between both groups regarding developing ESRF or death.[120] The benefit of routine use of plasma exchange or use in case of specific organ manifestations (e.g. severe renal dysfunction, lung hemorrhage) remains unclear.[121]

Mycophenolate mofetil (MMF) showed to have comparable remission induction (at six months) and infection rates as cyclophosphamide, but with a higher relapse rate.[122] MMF is a less potent drug than azathioprine for maintenance therapy in AAV patients with renal involvement.[9]

Refractory disease and relapse

Refractory disease with current therapy modalities and disease relapse are challenges in the treatment of AAV. It would be helpful if we can identify patients on forehand who have a higher risk for a (renal) relapse so that the clinical management can be adapted to it. Therefore, studies on risk factors and predictors of (renal) relapse are needed for optimizing clinical management. **Chapter 3** of this thesis investigates risk factors and predictors for renal relapse. It is important to balance the risk of disease relapse and the risk of treatment related adverse effects, and identifying risk factors helps with balancing between these two.[123,124]

Due to the high relapse rates, despite treatment with immunosuppressive drugs, new treatment modalities are continuously being developed. One of the drugs under investigation is rituximab, a monoclonal antibody directed against CD20. Rituximab induces B cell depletion in peripheral blood, that is sustained for approximately 6-18 months, without affecting the plasma cell population.[125] In 2010, the RITUXVAS (newly diagnosed AAV with renal involvement; median glomerular filtration rate: 12-20 ml/min/1.73 m^2; interquartile range 5-44 ml/min/1.73 m^2) and RAVE trials (newly or relapsing severe AAV) compared rituximab with cyclophosphamide for remission induction. Noteworthy, patients in the RITUXVAS trial receiving rituximab also received two intravenous cyclophosphamide pulses. The rituximab group in the RAVE trial received placebo instead of azathioprine as maintenance therapy. There was no difference in remission induction and adverse events in patients with severe AAV. Rituximab showed superiority in treating patients with relapsing disease,[12,34] and is also the preferred agent for refractory disease.[126] At two year follow-up of RITUXVAS patients there was no difference regarding death, ESRF and relapse.[127] In another cohort the risk for malignancy was lower in patients treated with rituximab compared to cyclophosphamide.[128]

Rituximab was also compared with azathioprine as maintenance therapy in the MAINRITSAN trial in newly diagnosed and relapsing AAV. Patients receiving rituximab as maintenance therapy had more sustained disease remission (BVAS of zero) at 28 months compared to patients receiving azathioprine, especially in the case of PR3-ANCA specificity. There was no difference in severe adverse events.[129] Currently a new

trial (RITAZAREM, ClinicalTrials.gov Identifier: NCT01697267) compares rituximab with azathioprine as maintenance therapy after induction therapy in patients with disease relapse.

There is ongoing debate on the optimal duration of maintenance treatment. Current guidelines suggest at least 24 months to prevent disease relapse. Therefore, the REMAIN study compared relapse rates following 24 or 48 months of conventional remission maintenance therapy. This study showed that 48 months of maintenance therapy (azathioprine and prednisolone) had less relapses and an improved renal survival at 48 months compared to 24 months maintenance therapy. There was no difference in the incidence or severity of adverse events or patient survival between both groups.[130] Currently, there is still controversy about the duration of glucocorticoids use. It differs among trials and also among local practices at what rate corticosteroids are tapered and when they are completely stopped. A meta-analysis showed that longer courses of glucocorticoids are associated with fewer relapses.[124] On the contrary, this is also associated with many side-effects.[131-133] A randomized trial on a C5a receptor inhibitor (avacopan) showed that it was an effective treatment in replacing high-dose glucocorticoids in treating AAV patients with newly diagnosed or relapsing disease.[134]

Ongoing trials worth mentioning are PEXIVAS and CLASSIC. PEXIVAS (ClinicalTrials.gov Identifier: NCT00987389) is a double trial in which the role for adjuvant plasma exchange is investigated further and, in addition, low dose glucocorticoids are compared with standard dose glucocorticoids.[135] The CLASSIC (ClinicalTrials.gov identifier: NCT02222155) trials investigate the use of an oral C5a inhibitor (CCX168) as a novel induction approach.

THIS THESIS

Chapter 2 presents an optimized ELISA for the detection of α-PLG. With this new assay, we validated the presence of α-PLG in AAV.

Chapter 3 is a clinicopathologic study using biopsies from the CYCAZAREM and MEPEX trial. It investigates risk factors and predictors for renal relapse using two different statistical methods. This study has a special focus on the histopathologic classification of AAGN.

Chapter 4 is a worldwide validation study of the histopathologic classification of AAGN which also investigated the interobserver variability of the classification.

Chapter 5 describes the outcome of renal transplantation in a Dutch cohort of AAGN patients, focusing on renal disease recurrence and graft survival rates within five years of transplantation. The focus of the study was the impact of disease recurrence in the allograft on graft survival.

Chapter 6 of this thesis gives an overview of the clinical presentation, diagnosis and management of AAV.

Chapter 7 gives a summary of the results in this thesis and discusses them.

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