ANCA-associated vasculitis: On clinical management and renal outcome

Goceroglu, A.

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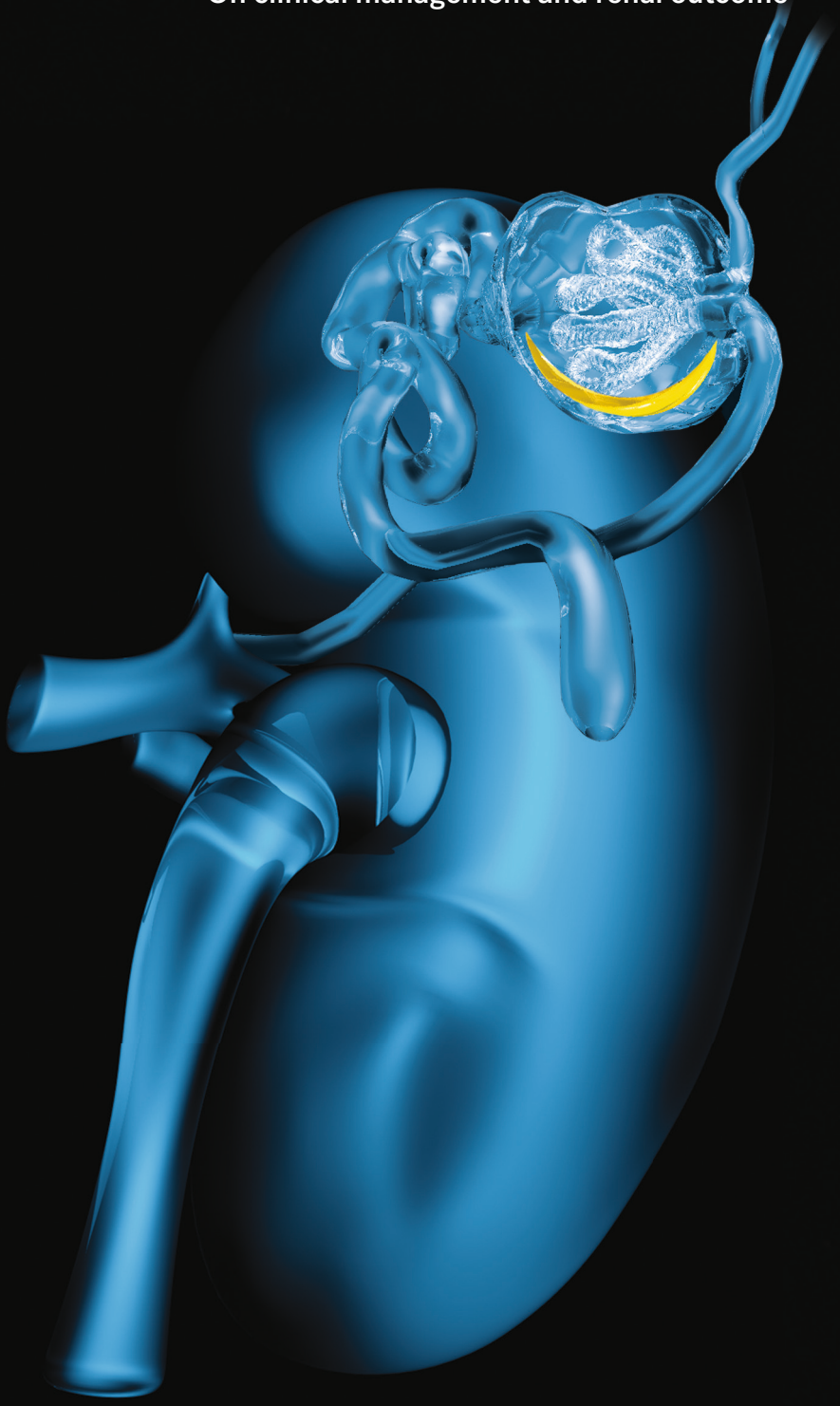

Arda Göçeroğlu

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ANCA-associated vasculitis

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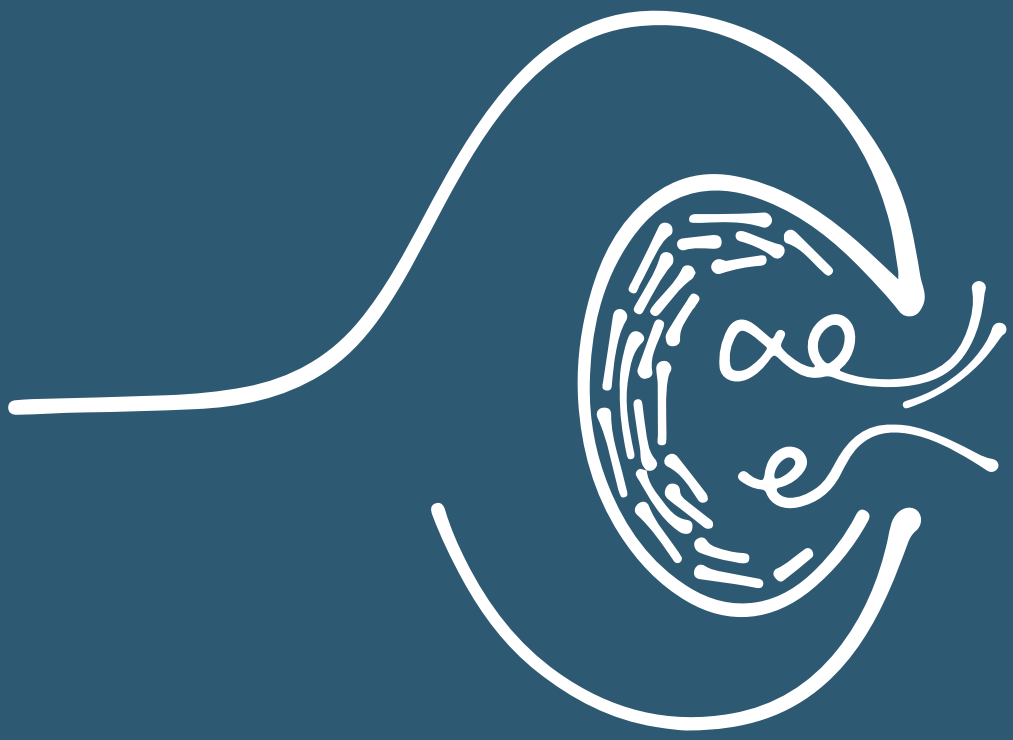
Güzel şeylerin zamanı geçmez.

“De tijd van mooie dingen gaat nooit voorbij”

— A Turkish dictum

Aan mijn ouders, (over)grootouders en geliefde



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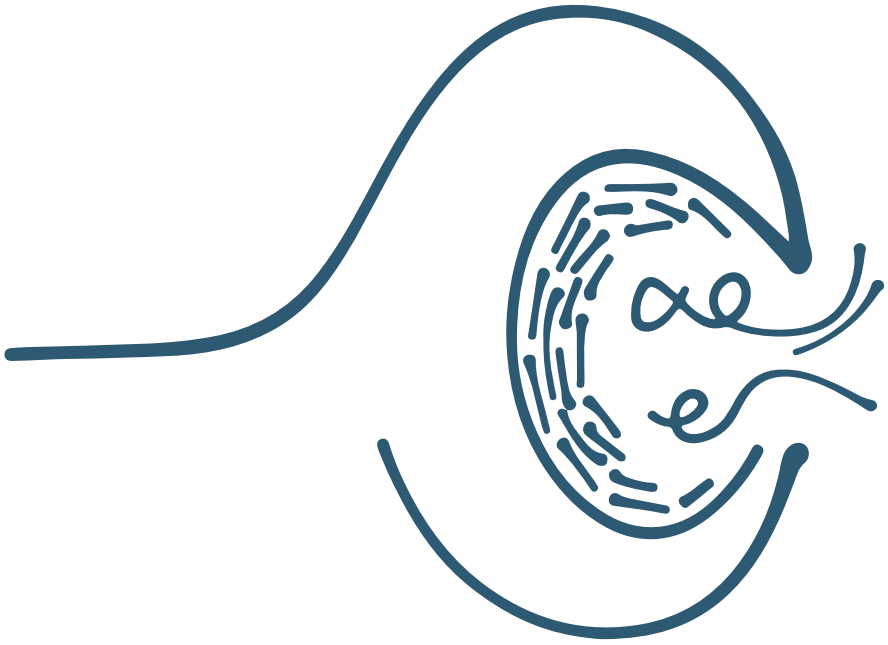
GENERAL INTRODUCTION



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.

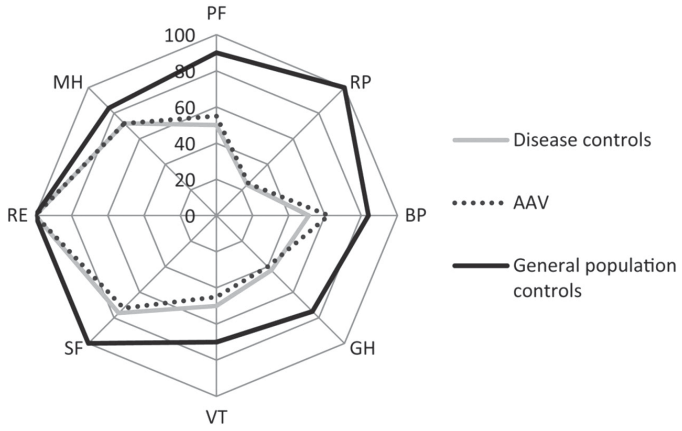
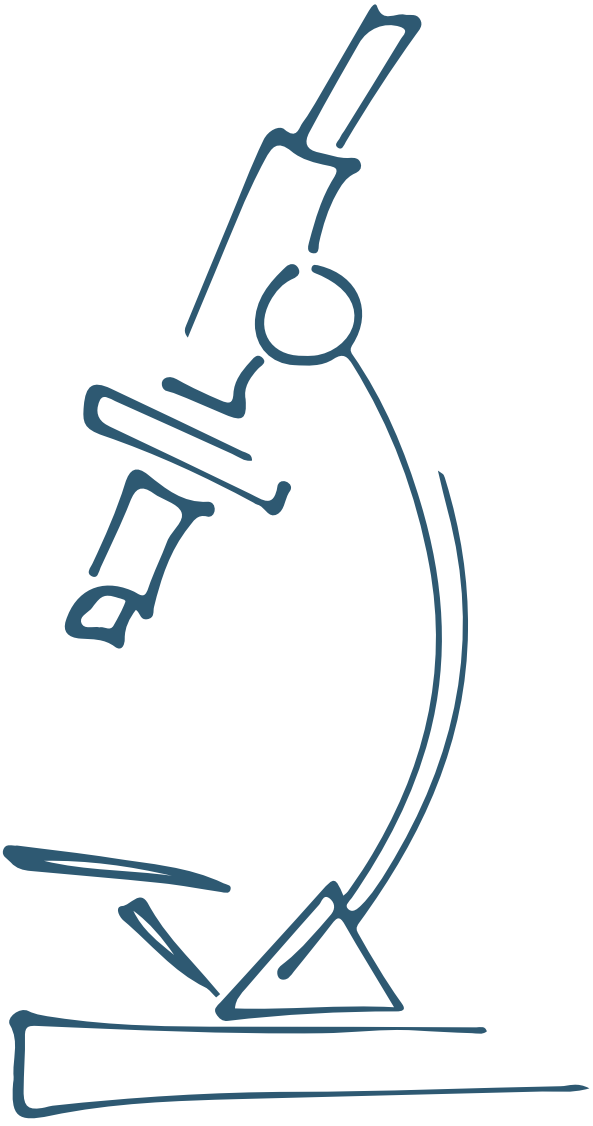


Figure 1 | Median SF-36 scores of the study by Basu *et al.* (410 AAV patients, 318 chronic disease controls, 470 general population controls).
This figure shows that QoL is scored lower by AAV patients compared to the general population controls. QoL is scored similar between AAV patients and chronic 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]

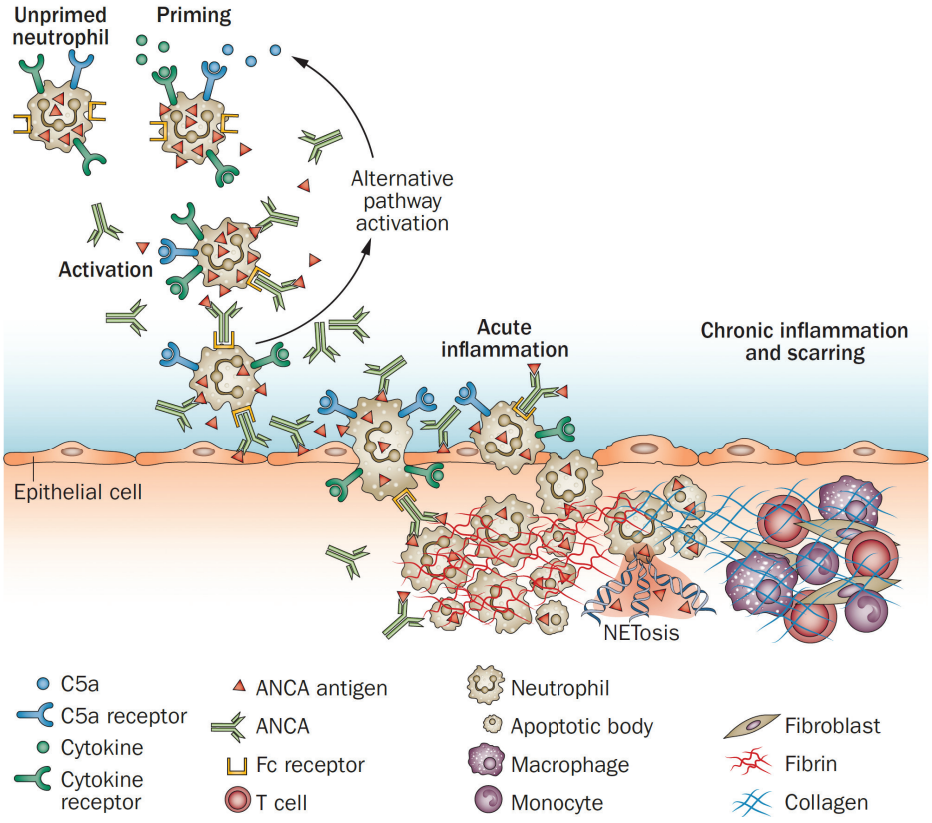


Figure 2 | Current hypothesized pathogenesis of ANCA-associated vasculitis.
A trigger activates the release of cytokines, which stimulate priming and accumulation of circulating neutrophils. PR3 and MPO are exposed on the neutrophil, making them accessible to ANCA produced by B-cells. ANCA binding to 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.

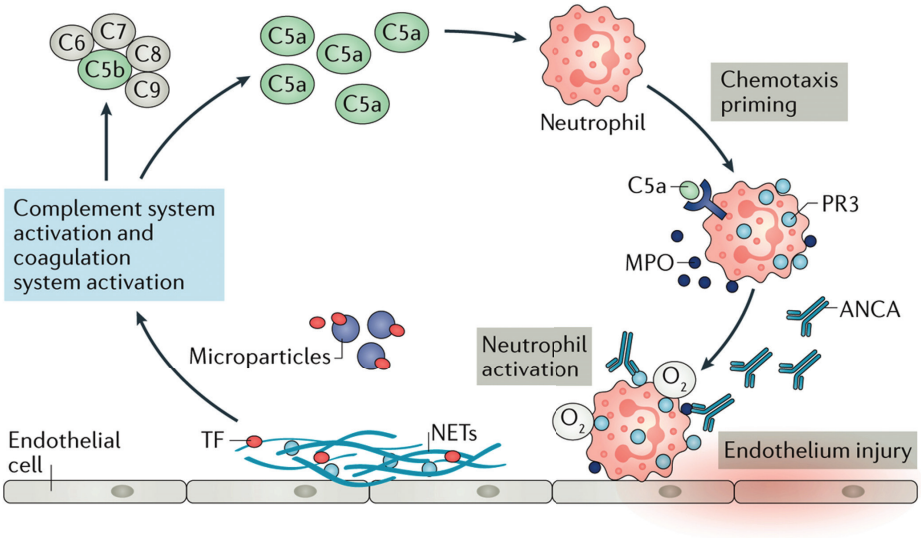


Figure 3 | Current hypothesized pathogenesis of ANCA-associated vasculitis focusing more on complement system acitvation.
Activated neutrophils stimulate the complement system, i.e. alternative pathway. C5a is produced which recruits and primes more neutrophils. NETs are formed by NETosis of neutrophils. 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

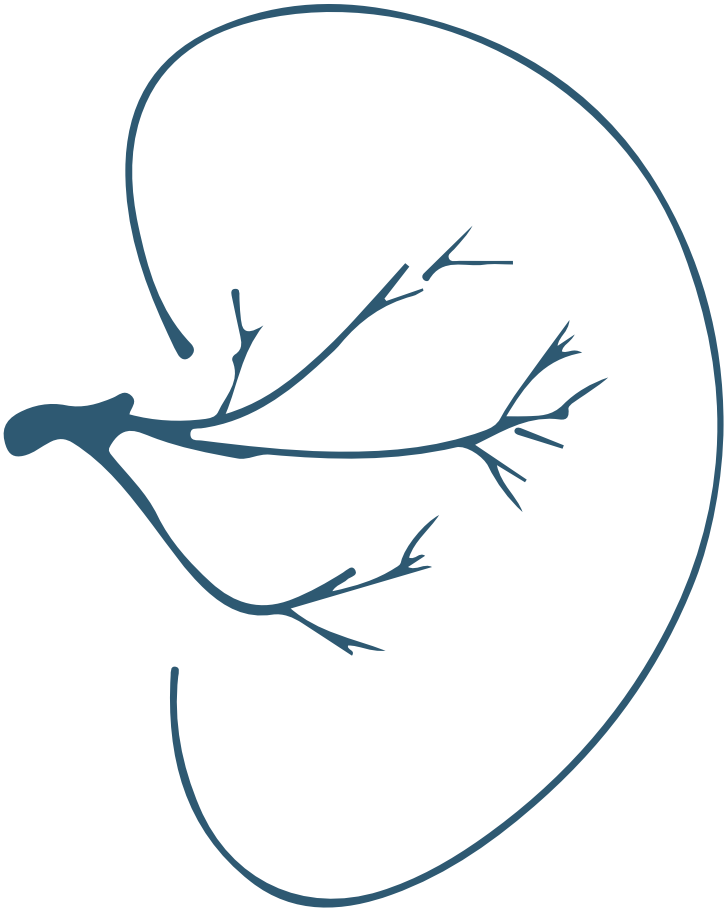
The first animal model in AAV was described in 2002 and was a MPO-AAV mouse model. They developed hematuria and proteinuria, and the kidneys showed comparable histopathologic lesions as seen in human AAGN.[92] Hereafter, a rat model was developed for MPO-AAV in which rats were immunized with human MPO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

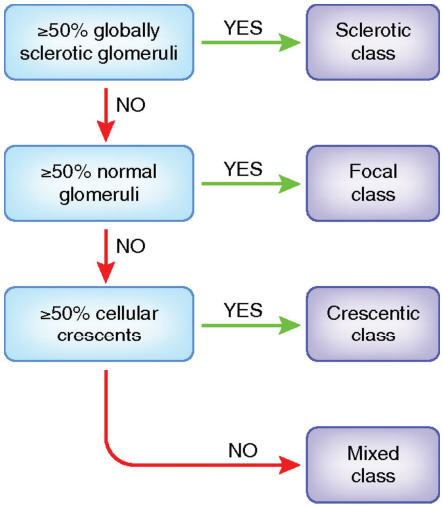


Figure 4 | Histopathologic classification of ANCA-associated glomerulonephritis.
This classification classifies each renal biopsy with AAGN into a class based on the predominant glomerular phenotype. AAGN, ANCA-associated glomerulonephritis. Reproduced from Berden *et al.* (J Am Soc Nephrol 2010;21:1628-1636)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

REFERENCES

1. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*, 2013;65(1): 1-11.
2. Goldschmeding R, van der Schoot CE, ten Bokkel HD, et al. Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest*, 1989;84(5): 1577-1587.
3. Jennette JC, Hoidal JR, Falk RJ. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood*, 1990;75(11): 2263-2264.
4. Ludemann J, Utecht B, Gross WL. Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinolytic enzyme. *J Exp Med,* 1990;171(1): 357-362.
5. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA. Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. *Blood,* 1989;74(6): 1888-1893.
6. Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med,* 1988;318(25): 1651-1657.
7. de Groot K, Harper L, Jayne DR, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. *Ann Intern Med,* 2009;150(10): 670-680.
8. de Groot K, Rasmussen N, Bacon PA, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum,* 2005;52(8): 2461-2469.
9. Hiemstra TF, Walsh M, Mahr A, et al. Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. *JAMA,* 2010;304(21): 2381-2388.
10. Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med,* 2003;349(1): 36-44.
11. Jayne DR, Gaskin G, Rasmussen N, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol,* 2007;18(7): 2180-2188.
12. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med,* 2010;363(3): 221-232.
13. Fujimoto S, Watts RA, Kobayashi S, et al. Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the U.K. *Rheumatology (Oxford),* 2011;50(10): 1916-1920.
14. Watts RA, Gonzalez-Gay MA, Lane SE, Garcia-Porrua C, Bentham G, Scott DG. Geoepidemiology of systemic vasculitis: comparison of the incidence in two regions of Europe. *Ann Rheum Dis,* 2001;60(2): 170-172.
15. Watts RA, Scott DG, Jayne DR, et al. Renal vasculitis in Japan and the UK--are there differences in epidemiology and clinical phenotype? *Nephrol Dial Transplant,* 2008;23(12): 3928-3931.
16. Scott DG, Watts RA. Epidemiology and clinical features of systemic vasculitis. *Clin Exp Nephrol,* 2013;17: 607-610.
17. Watts RA, Lane SE, Bentham G, Scott DG. Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. *Arthritis Rheum,* 2000;43(2): 414-419.

18. Abdou NI, Kullman GJ, Hoffman GS, et al. Wegener's granulomatosis: survey of 701 patients in North America. Changes in outcome in the 1990s. *J Rheumatol,* 2002;29(2): 309-316.
19. Cao Y, Schmitz JL, Yang J, et al. DRB1*15 allele is a risk factor for PR3-ANCA disease in African Americans. *J Am Soc Nephrol,* 2011;22(6): 1161-1167.
20. Mahr A, Guillevin L, Poissonnet M, Ayme S. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum,* 2004;51(1): 92-99.
21. Liu LJ, Chen M, Yu F, Zhao MH, Wang HY. Evaluation of a new algorithm in classification of systemic vasculitis. *Rheumatology (Oxford),* 2008;47(5): 708-712.
22. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med,* 1992;116(6): 488-498.
23. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med,* 1997;337(21): 1512-1523.
24. Klinger H. Grenzformen der Periarteriitis Nodosa. *Frankf Z Path,* 1931;42: 455-480.
25. Wegener F. Über generalisierte, septische Gefässerkrankungen. *Verh Dtsch Pathol Ges,* 1936;29: 202-209.
26. Wegener F. Über eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystems und der Nieren. *Beitr Pathol Anat Allg Pathol,* 1939;102: 36.
27. Wohlwill F. Über die nur mikroskopisch erkennbare Form der Paeriarteriitis nodosa. *Virchows Archiv,* 1923;246: 377-411.
28. Reinhold-Keller E, Beuge N, Latza U, et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. *Arthritis Rheum,* 2000;43(5): 1021-1032.
29. Stone JH. Limited versus severe Wegener's granulomatosis: baseline data on patients in the Wegener's granulomatosis etanercept trial. *Arthritis Rheum,* 2003;48(8): 2299-2309.
30. Bajema IM, Hagen EC, van der Woude FJ, Bruijn JA. Wegener's granulomatosis: a meta-analysis of 349 literary case reports. *J Lab Clin Med,* 1997;129(1): 17-22.
31. Falk RJ, Jennette JC. ANCA small-vessel vasculitis. *J Am Soc Nephrol,* 1997;8(2): 314-322.
32. Walton EW. Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *BMJ,* 1958;2(5091): 265-270.
33. Pagnoux C, Mahr A, Hamidou MA, et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med,* 2008;359(26): 2790-2803.
34. Jones RB, Cohen Tervaert JW, Hauser T, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med,* 2010;363(3): 211-220.
35. Gallagher H, Kwan JT, Jayne DR. Pulmonary renal syndrome: a 4-year, single-center experience. *Am J Kidney Dis,* 2002;39(1): 42-47.
36. Luqmani RA, Bacon PA, Moots RJ, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM,* 1994;87(11): 671-678.
37. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis,* 2009;68(12): 1827-1832.

38. Stone JH, Hoffman GS, Merkel PA, et al. A disease-specific activity index for Wegener's granulomatosis: modification of the Birmingham Vasculitis Activity Score. International Network for the Study of the Systemic Vasculitides (INSSYS). *Arthritis Rheum,* 2001;44(4): 912-920.
39. de Groot K, Gross WL, Herlyn K, Reinhold-Keller E. Development and validation of a disease extent index for Wegener's granulomatosis. *Clin Nephrol,* 2001;55(1): 31-38.
40. Exley AR, Bacon PA, Luqmani RA, et al. Development and initial validation of the Vasculitis Damage Index for the standardized clinical assessment of damage in the systemic vasculitides. *Arthritis Rheum,* 1997;40(2): 371-380.
41. Faurschou M, Sigaard L, Bjorner JB, Baslund B. Impaired health-related quality of life in patients treated for Wegener's granulomatosis. *J Rheumatol,* 2010;37(10): 2081-2085.
42. Herlyn K, Hellmich B, Seo P, Merkel PA. Patient-reported outcome assessment in vasculitis may provide important data and a unique perspective. *Arthritis Care Res (Hoboken),* 2010;62(11): 1639-1645.
43. Koutantji M, Harrold E, Lane SE, Pearce S, Watts RA, Scott DG. Investigation of quality of life, mood, pain, disability, and disease status in primary systemic vasculitis. *Arthritis Rheum,* 2003;49(6): 826-837.
44. Seo P, Jayne D, Luqmani R, Merkel PA. Assessment of damage in vasculitis: expert ratings of damage. *Rheumatology (Oxford),* 2009;48(7): 823-827.
45. Seo P, Min YI, Holbrook JT, et al. Damage caused by Wegener's granulomatosis and its treatment: prospective data from the Wegener's Granulomatosis Etanercept Trial (WGET). *Arthritis Rheum,* 2005;52(7): 2168-2178.
46. Suka M, Hayashi T, Kobayashi S, Ito S, Yumura W, Ozaki S. Improvement in health-related quality of life in MPO-ANCA-associated vasculitis patients treated with cyclophosphamide plus prednisolone: an analysis of 18 months of follow-up data from the JMAAV study. *Mod Rheumatol,* 2012;22(6): 877-884.
47. Tomasson G, Boers M, Walsh M, et al. Assessment of health-related quality of life as an outcome measure in granulomatosis with polyangiitis (Wegener's). *Arthritis Care Res (Hoboken),* 2012;64(2): 273-279.
48. Basu N, McClean A, Harper L, et al. The characterisation and determinants of quality of life in ANCA associated vasculitis. *Ann Rheum Dis,* 2014;73(1): 207-211.
49. Brezinova P, Englbrecht M, Lovric S, et al. Coping strategies and depressiveness in primary systemic vasculitis--what is their impact on health-related quality of life? *Rheumatology (Oxford),* 2013;52(10): 1856-1864.
50. Walsh M, Mukhtyar C, Mahr A, et al. Health-related quality of life in patients with newly diagnosed antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Care Res (Hoboken),* 2011;63(7): 1055-1061.
51. McHorney CA, Ware Jr JE, Raczek AE. The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs. *Med Care,* 1993;31(3): 247-263.
52. Merkel PA, Aydin SZ, Boers M, et al. The OMERACT core set of outcome measures for use in clinical trials of ANCA-associated vasculitis. *J Rheumatol,* 2011;38(7): 1480-1486.
53. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med,* 1983;98(1): 76-85.

54. Pinching AJ, Rees AJ, Pussell BA, Lockwood CM, Mitchison RS, Peters DK. Relapses in Wegener's granulomatosis: the role of infection. *BMJ,* 1980;281(6244): 836-838.
55. Stegeman CA, Cohen Tervaert JW, de Jong PE, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N Engl J Med,* 1996;335(1): 16-20.
56. Hogan SL, Cooper GS, Savitz DA, et al. Association of silica exposure with anti-neutrophil cytoplasmic autoantibody small-vessel vasculitis: a population-based, case-control study. *Clin J Am Soc Nephrol,* 2007;2(2): 290-299.
57. de Lind van Wijngaarden RA, van Rijn L, Hagen EC, et al. Hypotheses on the etiology of antineutrophil cytoplasmic autoantibody associated vasculitis: the cause is hidden, but the result is known. *Clin J Am Soc Nephrol,* 2008;3(1): 237-252.
58. Lyons PA, Rayner TF, Trivedi S, et al. Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med,* 2012;367(3): 214-223.
59. Rahmattulla C, Mooyaart AL, van Hooven D, et al. Genetic variants in ANCA-associated vasculitis: a meta-analysis. *Ann Rheum Dis,* 2015;75: 1687-1692.
60. Savage CO, Pottinger BE, Gaskin G, Pusey CD, Pearson JD. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol,* 1992;141(2): 335-342.
61. Chen M, Xing GQ, Yu F, Liu G, Zhao MH. Complement deposition in renal histopathology of patients with ANCA-associated pauci-immune glomerulonephritis. *Nephrol Dial Transplant,* 2009;24(4): 1247-1252.
62. Gou SJ, Yuan J, Chen M, Yu F, Zhao MH. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int,* 2013;83(1): 129-137.
63. Huugen D, van Esch A, Xiao H, et al. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int,* 2007;71(7): 646-654.
64. Schreiber A, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol,* 2009;20(2): 289-298.
65. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol,* 2007;170(1): 52-64.
66. Xing GQ, Chen M, Liu G, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. *J Clin Immunol,* 2009;29(3): 282-291.
67. Kessenbrock K, Krumbholz M, Schonermarck U, et al. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med,* 2009;15(6): 623-625.
68. Sangaletti S, Tripodo C, Chiodoni C, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood,* 2012;120(15): 3007-3018.
69. Abdulahad WH, van der Geld YM, Stegeman CA, Kallenberg CG. Persistent expansion of CD4+ effector memory T cells in Wegener's granulomatosis. *Kidney Int,* 2006;70(5): 938-947.

70. Berden AE, Kallenberg CG, Savage CO, et al. Cellular immunity in Wegener's granulomatosis: characterizing T lymphocytes. *Arthritis Rheum,* 2009;60(6): 1578-1587
71. Gan PY, Steinmetz OM, Tan DS, et al. Th17 cells promote autoimmune anti-myeloperoxidase glomerulonephritis. *J Am Soc Nephrol,* 2010;21(6): 925-931.
72. Ruth AJ, Kitching AR, Kwan RY, et al. Anti-neutrophil cytoplasmic antibodies and effector CD4+ cells play nonredundant roles in anti-myeloperoxidase crescentic glomerulonephritis. *J Am Soc Nephrol,* 2006;17(7): 1940-1949.
73. Jennette JC, Falk RJ, Hu P, Xiao H. Pathogenesis of antineutrophil cytoplasmic autoantibody-associated small-vessel vasculitis. *Annu Rev Pathol,* 2013;8: 139-160.
74. Cui Z, Zhao MH, Segelmark M, Hellmark T. Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int,* 2010;78(6): 590-597.
75. Roth AJ, Ooi JD, Hess JJ, et al. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. *J Clin Invest,* 2013;123(4): 1773-1783.
76. Ludemann G, Gross WL. Autoantibodies against cytoplasmic structures of neutrophil granulocytes in Wegener's granulomatosis. *Clin Exp Immunol,* 1987;69(2): 350-357.
77. Tomasson G, Grayson PC, Mahr AD, LaValley M, Merkel PA. Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis--a meta-analysis. *Rheumatology (Oxford),* 2012;51(1): 100-109.
78. Savige J, Dimech W, Fritzler M, et al. Addendum to the International Consensus Statement on testing and reporting of antineutrophil cytoplasmic antibodies. Quality control guidelines, comments, and recommendations for testing in other autoimmune diseases. *Am J Clin Pathol,* 2003;120(3): 312-318.
79. Savige J, Gillis D, Benson E, et al. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am J Clin Pathol,* 1999;111(4): 507-513.
80. Damoiseaux J, Csernok E, Rasmussen N, et al. Detection of antineutrophil cytoplasmic antibodies (ANCAs): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. *Ann Rheum Dis,* 2016;76(4): 647-653.
81. Csernok E, Moosig F. Current and emerging techniques for ANCA detection in vasculitis. *Nat Rev Rheumatol,* 2014;10(8): 494-501.
82. Bautz DJ, Preston GA, Lionaki S, et al. Antibodies with dual reactivity to plasminogen and complementary PR3 in PR3-ANCA vasculitis. *J Am Soc Nephrol,* 2008;19(12): 2421-2429.
83. Berden AE, Nolan SL, Morris HL, et al. Anti-plasminogen antibodies compromise fibrinolysis and associate with renal histology in ANCA-associated vasculitis. *J Am Soc Nephrol,* 2010;21(12): 2169-2179.
84. Hao J, Wang C, Gou SJ, Zhao MH, Chen M. The association between anti-plasminogen antibodies and disease activity in ANCA-associated vasculitis. *Rheumatology (Oxford),* 2014;53(2): 300-306.
85. Pendergraft III WF, Pressler BM, Jennette JC, Falk RJ, Preston GA. Autoantigen complementarity: a new theory implicating complementary proteins as initiators of autoimmune disease. *J Mol Med (Berlin),* 2005;83(1): 12-25.

86. Pendergraft III WF, Preston GA, Shah RR, et al. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med,* 2004;10(1): 72-79.
87. Kain R, Exner M, Brandes R, et al. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med,* 2008;14(10): 1088-1096.
88. Kain R, Matsui K, Exner M, et al. A novel class of autoantigens of anti-neutrophil cytoplasmic antibodies in necrotizing and crescentic glomerulonephritis: the lysosomal membrane glycoprotein h-lamp-2 in neutrophil granulocytes and a related membrane protein in glomerular endothelial cells. *J Exp Med,* 1995;181(2): 585-597.
89. Kain R, Tadema H, McKinney EF, et al. High prevalence of autoantibodies to hLAMP-2 in anti-neutrophil cytoplasmic antibody-associated vasculitis. *J Am Soc Nephrol,* 2012;23(3): 556-566.
90. Gough NR, Fambrough DM. Different steady state subcellular distributions of the three splice variants of lysosome-associated membrane protein LAMP-2 are determined largely by the COOH-terminal amino acid residue. *J Cell Biol,* 1997;137(5): 1161-1169.
91. Roth AJ, Brown MC, Smith RN, et al. Anti-LAMP-2 antibodies are not prevalent in patients with antineutrophil cytoplasmic autoantibody glomerulonephritis. *J Am Soc Nephrol,* 2012;23(3): 545-555.
92. Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest,* 2002;110(7): 955-963.
93. Little MA, Smyth CL, Yadav R, et al. Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood,* 2005;106(6): 2050-2058.
94. Pfister H, Ollert M, Frohlich LF, et al. Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood,* 2004;104(5): 1411-1418.
95. Little MA, Al-Ani B, Ren S, et al. Anti-proteinase 3 anti-neutrophil cytoplasm autoantibodies recapitulate systemic vasculitis in mice with a humanized immune system. *PLoS One,* 2012;7(1): e28626.
96. Jagiello P, Gencik M, Arning L, et al. New genomic region for Wegener's granulomatosis as revealed by an extended association screen with 202 apoptosis-related genes. *Hum Genet,* 2004;114(5): 468-477.
97. Monach PA, Merkel PA. Genetics of vasculitis. *Curr Opin Rheumatol,* 2010;22(2): 157-163.
98. Willcocks LC, Lyons PA, Rees AJ, Smith KG. The contribution of genetic variation and infection to the pathogenesis of ANCA-associated systemic vasculitis. *Arthritis Res Ther,* 2010;12(1): 202.
99. Jennette JC, Wilkman AS, Falk RJ. Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol,* 1989;135(5): 921-930.
100. Berden AE, Jones RB, Erasmus DD, et al. Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. *J Am Soc Nephrol,* 2012;23(2): 313-321.

101. de Lind van Wijngaarden RA, Hauer HA, Wolterbeek R, et al. Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: A prospective analysis of 100 patients with severe renal involvement. *J Am Soc Nephrol,* 2006;17(8): 2264-2274.
102. Hauer HA, Bajema IM, Van Houwelingen HC, et al. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int,* 2002;62(5): 1732-1742.
103. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol,* 2010;21(10): 1628-1636.
104. Chen YX, Xu J, Pan XX, et al. Histopathological Classification and Renal Outcome in Patients with Antineutrophil Cytoplasmic Antibodies-associated Renal Vasculitis: A Study of 186 Patients and Metaanalysis. *J Rheumatol,* 2017;44(3): 304-313.
105. Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis,* 2003;41(4): 776-784.
106. Little MA, Nazar L, Farrington K. Outcome in glomerulonephritis due to systemic small vessel vasculitis: effect of functional status and non-vasculitic co-morbidity. *Nephrol Dial Transplant,* 2004;19(2): 356-364.
107. Slot MC, Cohen Tervaert JW, Franssen CFM, Stegeman CA. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int,* 2003;63(2): 670-677.
108. Westman KWA, Bygren PG, Olsson H, Ranstam J, Wieslander J. Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J Am Soc Nephrol,* 1998;9(5): 842-852.
109. Allen A, Pusey C, Gaskin G. Outcome of renal replacement therapy in antineutrophil cytoplasmic antibody-associated systemic vasculitis. *J Am Soc Nephrol,* 1998;9(7): 1258-1263.
110. Geetha D, Eirin A, True K, et al. Renal transplantation in antineutrophil cytoplasmic antibody-associated vasculitis: a multicenter experience. *Transplantation,* 2011;91(12): 1370-1375.
111. Gera M, Griffin MD, Specks U, Leung N, Stegall MD, Fervenza FC. Recurrence of ANCA-associated vasculitis following renal transplantation in the modern era of immunosupression. *Kidney Int,* 2007;71(12): 1296-1301.
112. Little MA, Hassan B, Jacques S, et al. Renal transplantation in systemic vasculitis: when is it safe? *Nephrol Dial Transplant,* 2009;24(10): 3219-3225.
113. Marco H, Mirapeix E, Arcos E, et al. Long-term outcome of antineutrophil cytoplasmic antibody-associated small vessel vasculitis after renal transplantation. *Clin Transplant,* 2013;27: 338-347.
114. Fauci AS, Katz P, Haynes BF, Wolff SM. Cyclophosphamide therapy of severe systemic necrotizing vasculitis. *N Engl J Med,* 1979;301(5): 235-238.
115. Rasmussen N, Jayne DRW, Abramowicz D, et al. European therapeutic trials in ANCA-associated systemic vasculitis: disease scoring, consensus regimens and proposed clinical trials. European Community Study Group on Clinical Trials in Systemic Vasculitis ECSYSVASTRIAL. *Clin Exp Immunol,* 1995;101 Suppl 1: 29-34.
116. Merkel PA, Kaplan AA, Falk RJ. Initial immunosuppressive therapy in granulomatosis with polyangiitis and microscopic polyangiitis. *Up To Date,* 2017.

117. Walsh M, Faurschou M, Berden A, et al. Long-term follow-up of cyclophosphamide compared with azathioprine for initial maintenance therapy in ANCA-associated vasculitis. *Clin J Am Soc Nephrol,* 2014;9(9): 1571-1576.
118. Guillevin L, Cordier JF, Lhote F, et al. A prospective, multicenter, randomized trial comparing steroids and pulse cyclophosphamide versus steroids and oral cyclophosphamide in the treatment of generalized Wegener's granulomatosis. *Arthritis Rheum,* 1997;40(12): 2187-2198.
119. Harper L, Morgan MD, Walsh M, et al. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. *Ann Rheum Dis,* 2012;71(6): 955-960.
120. Walsh M, Casian A, Flossmann O, et al. Long-term follow-up of patients with severe ANCA-associated vasculitis comparing plasma exchange to intravenous methylprednisolone treatment is unclear. *Kidney Int,* 2013;84(2): 397-402.
121. Walsh M. Plasma exchange in antineutrophil cytoplasm antibody-associated vasculitis. *Curr Opin Nephrol Hypertens,* 2014;23(6): 555-559.
122. Jones RB, Hiemstra TF, Ballarin J, et al. Mycophenolate mofetil versus cyclophosphamide for remission induction in ANCA-associated vasculitis: a randomised, non-inferiority trial. *Ann Rheum Dis,* 2019;78(3): 399-405.
123. de Groot K, Adu D, Savage COS, (EUVAS) EVSG. The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. *Nephrol Dial Transplant,* 2001;16(10): 2018-2027.
124. Walsh M, Merkel PA, Mahr A, Jayne D. Effects of duration of glucocorticoid therapy on relapse rate in antineutrophil cytoplasmic antibody-associated vasculitis: a meta-analysis. *Arthritis Care Res (Hoboken),* 2010;62(8): 1166-1173.
125. Chambers SA, Isenberg D. Anti-B cell therapy (rituximab) in the treatment of autoimmune diseases. *Lupus,* 2005;14(3): 210-214.
126. Jones RB, Ferraro AJ, Chaudhry AN, et al. A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum,* 2009;60(7): 2156-2168.
127. Jones RB, Furuta S, Cohen Tervaert JW, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis: 2-year results of a randomised trial. *Ann Rheum Dis,* 2015;74(6): 1178-1182.
128. van Daalen EE, Rizzo R, Kronbichler A, et al. Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis. *Ann Rheum Dis,* 2017;76(6): 1064-1069.
129. Guillevin L, Pagnoux C, Karras A, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med,* 2014;371(19): 1771-1780.
130. Karras A, Pagnoux C, Haubitz M, et al. Randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis. *Ann Rheum Dis,* 2017;76(10): 1662-1668.
131. Goupil R, Brachemi S, Nadeau-Fredette AC, et al. Lymphopenia and treatment-related infectious complications in ANCA-associated vasculitis. *Clin J Am Soc Nephrol,* 2013;8(3): 416-423.
132. McGregor JG, Hogan SL, Hu Y, Jennette CE, Falk RJ, Nachman PH. Glucocorticoids and relapse and infection rates in anti-neutrophil cytoplasmic antibody disease. *Clin J Am Soc Nephrol,* 2012;7(2): 240-247.

133. Robson J, Doll H, Suppiah R, et al. Glucocorticoid treatment and damage in the anti-neutrophil cytoplasm antibody-associated vasculitides: long-term data from the European Vasculitis Study Group trials. *Rheumatology (Oxford)*, 2015;54(3): 471-481.
134. Jayne DRW, Bruchfeld AN, Harper L, et al. Randomized Trial of C5a Receptor Inhibitor Avacopan in ANCA-Associated Vasculitis. *J Am Soc Nephrol*, 2017;28(9): 2756-2767.
135. Walsh M, Merkel PA, Peh CA, et al. Plasma exchange and glucocorticoid dosing in the treatment of anti-neutrophil cytoplasm antibody associated vasculitis (PEXIVAS): protocol for a randomized controlled trial. *Trials*, 2013;14: 73.

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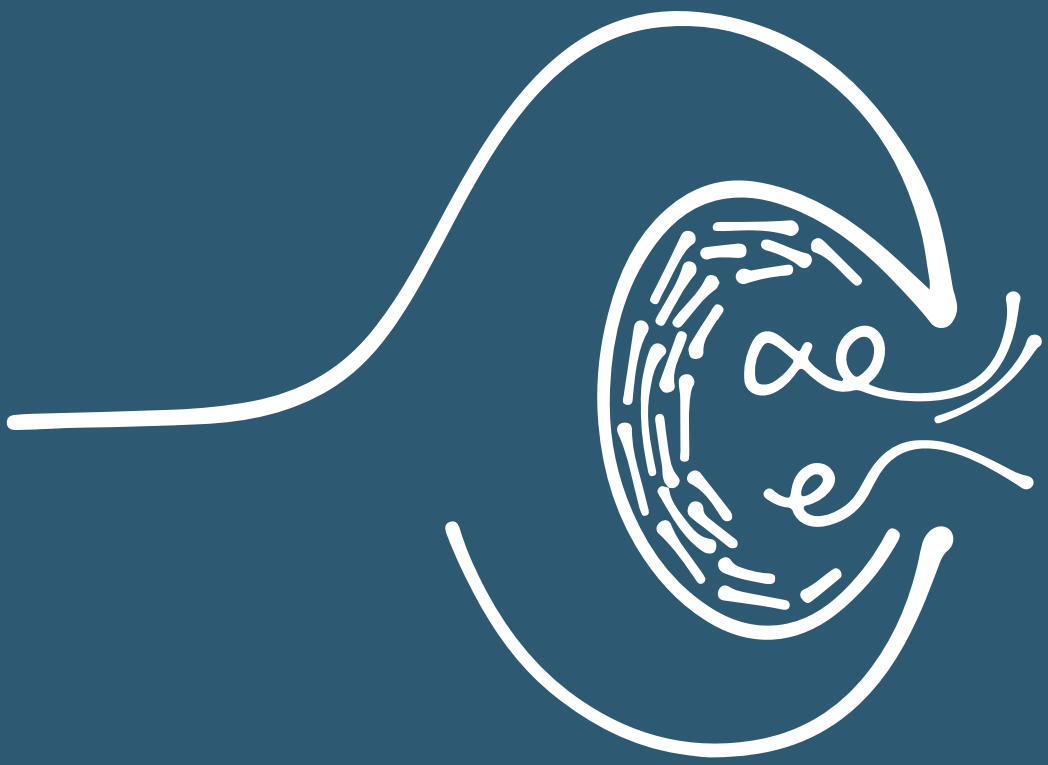

ANTI-PLASMINOGEN ANTIBODIES IN ANCA-ASSOCIATED VASCULITIS: AN OPTIMIZED ANTI-PLASMINOGEN ASSAY

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ABSTRACT

Introduction: Anti-plasminogen antibodies (α-PLG) were previously detected in a subpopulation of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients, showing a relation to renal lesions and outcome. Several studies showed different proportions of α-PLG positive AAV patients, possibly due to differences in the assays used. We here present a new, optimized α-PLG Enzyme-Linked Immuno Sorbent Assay (ELISA) and validate the presence of α-PLG in AAV.

Methods: Different ELISA set-ups were tested regarding plasminogen (PLG) antigen, concentrations, coating buffers, blocking agents, and environmental conditions.

Results: Purified lysine-PLG (lys-PLG) showed better differentiation between positive samples and negative samples than glutamic acid-PLG (glu-PLG). Therefore, lys-PLG was used as coating antigen. With the optimized α-PLG ELISA we found α-PLG in 14.3% of the myeloperoxidase (MPO)-ANCA patients, whereas all our proteinase-3 (PR3)-ANCA patients tested in our new assay were negative.

Conclusions: Concluding, in this study we have combined important technical findings and methods from previous studies to optimize the α-PLG assay, which can be used for future research purposes and will aid in uniform reporting of α-PLG status of patients.

INTRODUCTION

Recently, the presence of anti-plasminogen antibodies (α-PLG) in patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) received much attention, especially in relation to the nature and severity of renal lesions.[1–3] These antibodies inhibit fibrinolysis by disturbing the conversion of plasminogen (PLG) to plasmin.[1,2] A study on patients with AAV showed that patients with α-PLG had significantly more glomerular fibrinoid necrosis accompanied by worse renal function.[2] Evidently, the presence of α-PLG in AAV may be an important hallmark for a specific phenotype of the disease.[2,3] Three important studies on α-PLG in AAV reported differences in the proportion of α-PLG positive AAV patients ranging between 22%-43% for proteinase-3 (PR3)-AAV and 6%-27% for myeloperoxidase (MPO)-AAV.[1–3] It is possible that differences in α-PLG assays were to some extent responsible for these discrepant results. We therefore optimized the method for α-PLG Enzyme-Linked Immuno Sorbent Assay (ELISA) and with this new assay, we validated the presence of α-PLG in AAV.

METHODS

Positive controls

Eleven positive controls were derived from the studies of Bautz *et al.* and Berden *et al.*[1,2] These positive samples consisted of serum or plasma exchange (PEX) fluid. These patients had the following ANCA-specificities: 5 MPO-ANCA, 5 PR3-ANCA and 1 ANCA negative. These were detected with WIESLAB® MPO-ANCA / MPO IU, WIESLAB® Capture MPO-ANCA / CAP MPO IU, WIESLAB® PR3-ANCA / PR3 IU and WIESLAB® Capture PR3-ANCA / CAP PR3 IU (Euro Diagnostica, Malmö, Sweden). Patients had been diagnosed with AAV according to the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides.[4]

Healthy and disease controls

Samples from 220 healthy controls were used during the different steps for optimizing the assay. Samples of 157 disease controls were used. Of these samples 77 were anti-beta-2 glycoprotein 1 (β2GP1) positive, which is an autoantibody found in systemic lupus erythematosus and anti-phospholipid syndrome.[5] The remaining 80 samples were positive for anti-cyclic citrullinated peptides (CCP), which is an autoantibody found in rheumatoid arthritis.[6] Samples from healthy and disease controls were collected at Euro Diagnostica, Malmö, Sweden.

ANCA samples

For setting-up and optimizing the α-PLG assay 104 randomly selected samples of patients with ANCA positivity were used. Samples were not selected with respect to disease state. Of these samples 55 were PR3-ANCA positive and 49 were MPO-ANCA positive. These samples were collected at Euro Diagnostica, Malmö, Sweden. ANCA specificity of each patient was determined using WIESLAB® MPO-ANCA / MPO IU, WIESLAB® Capture MPO-ANCA / CAP MPO IU, WIESLAB® PR3-ANCA / PR3 IU and WIESLAB® Capture PR3-ANCA / CAP PR3 IU (Euro Diagnostica, Malmö, Sweden). The use of the samples in this study was approved by the Lund University ethics committee. All patients gave written informed consent to store samples for future development of analytical methods for the purpose of hospital care and treatment or similar activity. This study was conducted in accordance with the 1964 Declaration of Helsinki and subsequent amendments. This study was also performed according to the 'Netherlands Code of Conduct for Scientific Practice', an ethical code for performing observational studies with patient material approved by the Federatie van Medisch Wetenschappelijke Verenigingen (Federation of Medical Scientific Organisations) together with the legal and ethical committee of the Koninklijke Nederlandse Akademie van Wetenschappen (Royal Dutch Academy of Science) and the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (Dutch Organisation for Scientific Research). The data of the patients were analyzed anonymously.

Anti-plasminogen antibody assay

For developing an α-PLG assay we optimized each step in the assay by testing different alternatives for each step. These steps and their alternatives were:

- Coating material: glutamic acid plasminogen (glu-PLG), purified glu-PLG, lysine-plasminogen (lys-PLG) or purified lys-PLG obtained from Calbiochem and from Haematologic Technologies. Contaminating Immunoglobulin G (IgG) was removed from commercially obtained PLG by affinity chromatography on a Protein G HP SpinTrap column from GE Technologies using as binding buffer 0.01 phosphate, 0.15 M NaCl, 0.01 M EDTA pH 7.0.
- Coating buffer: 0,05 M Sodium Carbonate pH 9.5 or PBS.
- Blocking buffer: PBS with BSA 1%, gelatin 1% or Stabilcoat (Surmodics).
- Diluent: PBS with 0,05% Tween 20 and 2g/l BSA. Different serum dilutions (1:50, 1:100, 1:200, 1:400) and purified IgG from the serum samples were tested as primary antibodies.
- Wash: 0,15M NaCl with 0,05% Tween 20.

- Conjugated secondary antibodies: Sigma goat anti-human IgG HRP, Sigma goat anti-human IgG AP or Dako rabbit anti-human IgG HRP.
- Conjugate buffer: PBS with 0,05% Tween 20, 2g/l BSA and 1g/l bovine IgG.
- Substrate: para-Nitrophenylphosphate.

We tested different suppliers of antigen, different temperatures (room temperature and 37°C) and different time frames (30, 60 and 120 minutes) for each step. Box 1 describes the optimized assay.

Box 1 | Anti-plasminogen antibody ELISA assay
High binding microtiter plates were coated overnight with 3 µg/ml protein G purified lysine-plasminogen at room temperature. Carbonate buffer pH 9,6 was used as coating buffer. Plates were blocked for 60 minutes with 1% BSA in PBS at room temperature. After washing with PBS-Tween, serum and PEX fluid at 1/100 dilution were added in duplicates into appropriate microtiter wells and incubated at 37°C for 60 minutes. Wells were washed and incubated (30 minutes at 37°C) with a 1:5000 dilution of Sigma goat anti-human IgG-alkaline phosphatase. After washing, 4-nitrophenyl phosphate was used as the substrate at room temperature, and the 96-well microtiter plate was analyzed spectrophotometrically at 405 nm after 30 minutes.

IgG purification from serum or PEX fluid

IgG was purified from the sera or PEX fluid using the Melon Gel IgG Purification Kit (Pierce Protein Research Products; Thermo Scientific). SDS-PAGE was used to confirm the integrity of isolated IgG and Coomassie blue staining was used to visualize isolated IgG.

Testing different anti-human IgG conjugates

We tested different conjugates in varying concentrations to obtain the best signal of bound α-PLG on the plate coated with PLG. The conjugates tested were: Sigma goat anti-human IgG HRP 1/10000 and 1/20000, Sigma goat anti-human IgG AP 1/5000 and 1/10000, Dako rabbit anti-human IgG HRP 1/30000 and 1/50000.

Glu-plasminogen inhibition assays

Two inhibition assays were performed:

1. Glu-PLG 2 µg/ml was coated and incubated with strong positive control serum 1/200, healthy control serum 1/200 or Sigma goat anti-human IgG AP 1/10000. The sera were mixed with an increasing amount of soluble PLG (0-10µg/ml) and the conjugate was mixed with an increasing amount of human

IgG (0-10 µg/ml) for inhibition.

2. Soluble glu-PLG for inhibition (0-10 µg/ml) was pre-incubated overnight with polyclonal rabbit antibodies (α-PLG and anti-Chromogranin-A (CgA)), human sera (strong positive control and healthy control) or diluent. This was then added to a plate coated with glu-PLG 1 µg/ml. Swein anti-rabbit AP and goat anti-human AP were used for signal detection.

SDS-PAGE and Western blot

SDS-PAGE was performed with four proteins: unreduced glu-PLG, unreduced lys-PLG, reduced glu-PLG, reduced lys-PLG. Western blot was performed using rabbit α-PLG as specific antibody.

Titration curves plasminogen coating

Plates were coated with glu-PLG, purified glu-PLG, lys-PLG and purified lys-PLG in concentrations varying from 0 to 5 µg/ml. Samples from four positive controls and four negative controls in dilutions of 1/100 and 1/200 were tested. In addition, rabbit α-PLG, rabbit anti-CgA and diluent were tested. We performed the titrations with and without BSA block.

Statistical analysis

$\chi 2$-test was performed to compare the spectrophotometrical results of purfied glu-PLG and purified lys-PLG as coating antigens. A P-value <0.05 was considered statistically significant. Based on this, the cut-off value for α-PLG positivity was at the 97.5^{th} percentile (mean + 2 standard deviation) of the healthy controls. All statistical calculations were performed using SPSS (v25.0; IBM Corp, Armonk, NY).

RESULTS

Setting up the anti-plasminogen assay

We started with glu-PLG as antigen, and set up an ELISA using different serum dilutions (1:50, 1:100, 1:200, 1:400) and purified IgG from the samples. Results showed unspecific binding and therefore IgG was removed from the antigen samples by running the PLG antigens through a Protein G affinity column. Most of the background binding disappeared and protein G purified PLG was used in all experiments after this.

Unspecific background binding was still present after purification of the antigen and, therefore, different set-ups were tested to obtain differentiation between a strong positive control and negative controls (i.e. one healthy control and a sample with only a saline buffer). Different combinations of coating buffers, blocking agents and

diluents were tested. Optimal results distinguishing positive from negative controls were obtained with the combination carbonate buffer for coating and blocking with 1% BSA. Taking this set-up as baseline, we took further steps to optimize the assay. For the anti-human IgG conjugate we compared Sigma goat anti-human IgG HRP, Sigma goat anti-human IgG AP, and Dako rabbit anti-human IgG HRP. The Sigma goat anti-human IgG AP differentiated best between the positive and negative controls.

Specificity for glu-plasminogen

Minimal inhibition was obtained in a set-up where human PLG was added as an inhibitor to an ELISA with glu-PLG as the epitope, in contrast to a control condition in which rabbit α-PLG IgG was inhibited by soluble human PLG. Therefore, SDS-PAGE and Western blot were performed to test to which protein structure the α-PLG bind best. SDS-PAGE showed the presence of four proteins: unreduced glu-PLG, unreduced lys-PLG, reduced glu-PLG, reduced lys-PLG. No other protein was detected. When Western blot was performed with rabbit α-PLG, it showed strong binding for the unreduced PLG variants and weak binding for the reduced PLG variants (Figure 1). This indicates the importance of epitope conformation and this probably is true also for humans *in vivo*, explaining why different forms of the proteins react with different patient sera.

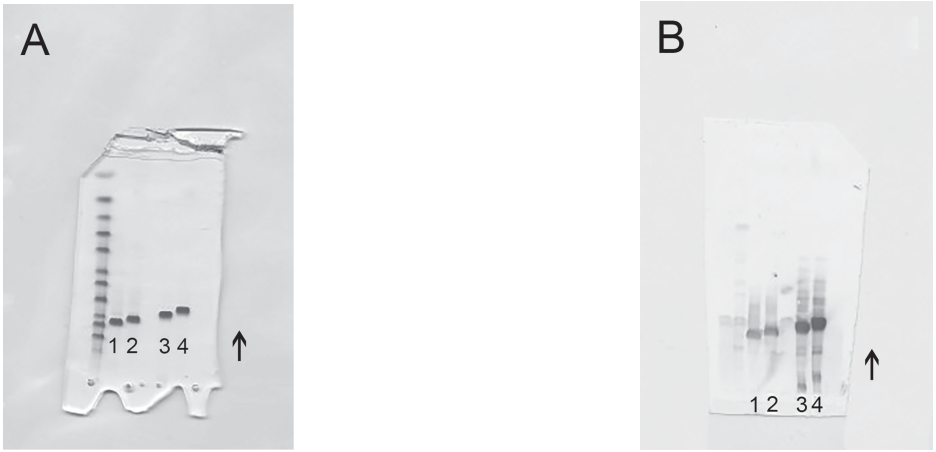

Figure 1 | SDS-PAGE and Western blot with rabbit anti-plasminogen antibodies.
(A) SDS-PAGE showed the presence of four 4 proteins. (B) Western blot with rabbit anti-plasminogen antibodies showing strong binding for the unreduced plasminogen variants and weak binding for the reduced plasminogen variants.
1, reduced glutamic acid plasminogen; 2, reduced lysine-plasminogen; 3, unreduced glutamic acid plasminogen (88 kDa); 4, unreduced lysine-plasminogen (83 kDa). The arrows show the direction of movement of the proteins.

Lys-plasminogen as antigen coating

Lys-PLG had a more saturated titration curve than glu-PLG with maximal saturation at lys-PLG 3 µg/ml. An assay with purified glu-PLG coating 2,5 µg/ml, 1/100 diluted samples, was performed on 8 positive controls, 22 ANCA samples and 13 healthy controls. Of the 8 positive controls, in this assay only two were positive. When performing the same assay with purified lys-PLG coating 2,5 µg/ml, the same two positive controls were positive, but in addition, there were three positive controls which were weakly positive. In both assays, none of the ANCA samples showed α-PLG positivity.

Using purified lys-PLG showed more spectrophotometrical differentiation between positive samples and negative samples compared to purified glu-PLG. In the case of using glu-PLG as coating antigen, the difference was not statistically significant (P=0.058). When using lys-PLG, the difference was statistically significant (P=0.001). Therefore, the assay was further optimized using lys-PLG as the coating antigen (Figure 2). We performed several assays with PLG antigens from different suppliers, different coating concentrations, serum dilutions and time periods at room temperature and at 37°C. All this was done in order to reduce unspecific binding and increase specific binding.

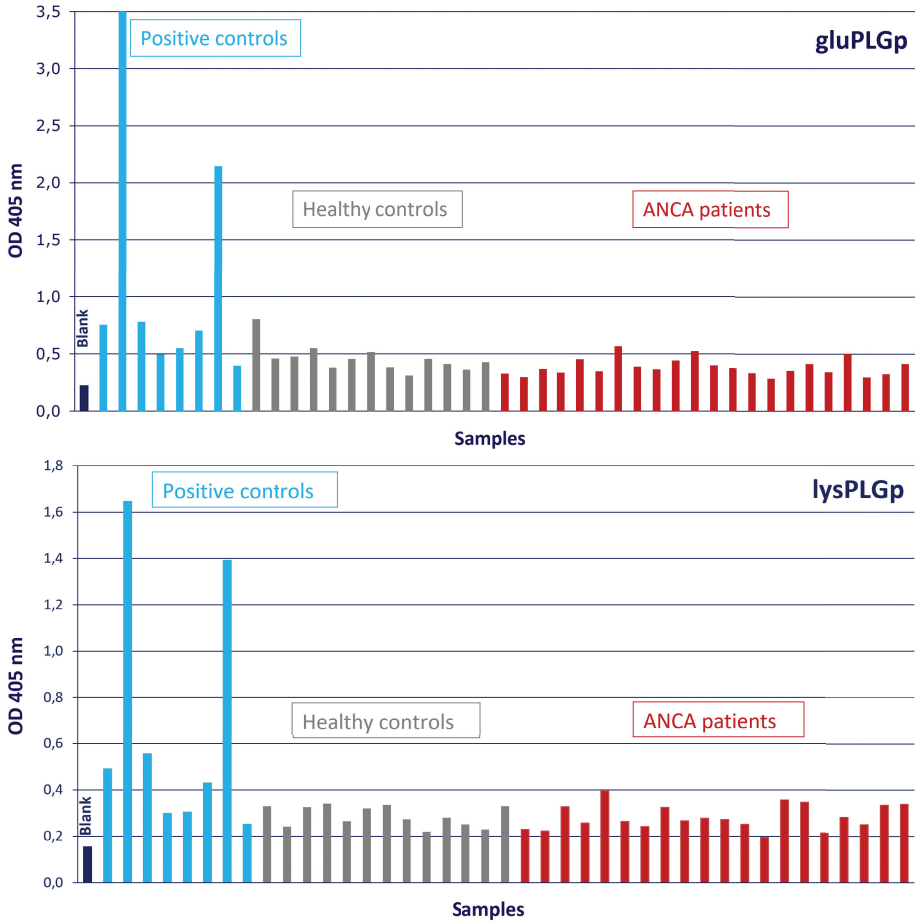

Figure 2 | Spectrophotometrical results.
(A) Spectrophotometrical results using purified glutamic acid plasminogen as an antigen in the assay. Optical density was measured with spectrometry using light of 405 nanometer for the detection of anti-plasminogen antibodies in the serum samples. (B) Spectrophotometrical results using purified lysine-plasminogen as an antigen in the assay. Optical density was measured with spectrometry using light of 405 nanometer for the detection of anti-plasminogen antibodies in the serum samples. Purified lysine-plasminogen showed more spectrophotometrical differentiation between positive samples and negative samples compared to purified glutamic acid plasminogen (Figure 2).
Abbreviations: gluPLGp, purified glutamic acid plasminogen; OD, optical density; nm, nanometer; lysPLGp, purified lysine-plasminogen.

Testing the current assay

With the current assay (Box 1), we tested a new cohort of samples. This cohort consisted of 75 ANCA positive patients (35 MPO-ANCA, 40 PR3-ANCA), 135 disease controls (anti- β2GP1 positive [n=55], anti-CCP positive [n=80]), 175 healthy controls, 11 positive controls (5 MPO-ANCA, 5 PR3-ANCA and 1 ANCA negative) and 1 negative

control (healthy control who was negative in all previous tests). The cut-off value for α-PLG positivity was 31 U/ml and higher based on the 97.5th percentile of 175 healthy controls (Figure 3). Table 1 shows the results.

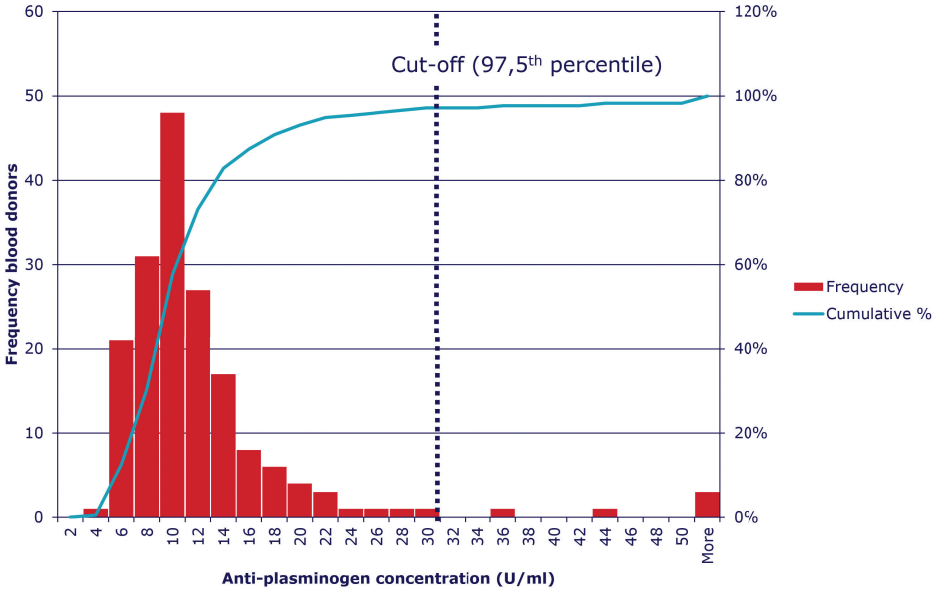

Figure 3 | Anti-plasminogen antibody concentrations in serum samples from 175 healthy controls.
The cut-off value for anti-plasminogen antibodies positivity was set to 31 U/ml and higher based on the 97.5th percentile of the healthy controls.

Table 1 | Overview of anti-plasminogen antibody positive patients using our optimized assay

Samples	α-PLG positive patients	α-PLG negative patients	Total patients	% α-PLG positive patients
MPO-ANCA	5	30	35	14.3
PR3-ANCA	0	40	40	0.0
Anti-β2GP1	11	44	55	20.0
Anti-CCP	10	70	80	12.5
Positive controls	7	4	11	63.6
Negative control	0	1	1	0.0

Abbreviations: α-PLG, anti-plasminogen antibodies; ANCA, anti-neutrophil cytoplasmic antibody; MPO, myeloperoxidase; PR3, proteinase-3; anti-β2GP1, anti-beta-2 glycoprotein 1; anti-CCP, anti-cyclic citrullinated peptides.

DISCUSSION

We developed an optimized assay for the detection of α-PLG, focusing on its usefulness in studies on AAV. We tested different assay set-ups with for example different types of PLG antigens and coating buffers. Importantly, we found that purified lys-PLG showed better spectrophotometrical differentiation between positive and negative samples than glu-PLG when used as a coating antigen (Figure 2).

Using our assay, we found that 14.3% of MPO-ANCA patients tested had α-PLG, whereas all our PR3-ANCA patients tested negative. However, α-PLG were detected in PR3-ANCA samples from our positive controls. Our results show discrepancies with previous studies. Bautz *et al.* described the presence of α-PLG in 22% (16/72) of patients with PR3-AAV and in 6% (2/34) of patients with MPO-AAV. In the PR3-AAV group, this proportion was significantly higher compared to healthy and disease controls. There was no difference between MPO-AAV and the controls.[1] Berden *et al.* found α-PLG in approximately 25% of patients with PR3-AAV and with MPO-AAV. Both were significantly higher than in healthy and disease controls.[2] Hao *et al.* detected α-PLG in 42.8% (3/7) of PR3-AAV patients and in 16.4% (16/97) of MPO-AAV patients. The proportion of AAV-patients with α-PLG was significantly higher than in healthy controls.[3] Although our positive control samples came from the studies of Bautz *et al.* and Berden *et al.*,[1,2] the discrepancy can be explained by the following: in the present study we have combined important technical findings/methods from the previous studies, which have led to the optimized α-PLG assay here presented. A known challenge in the α-PLG assay is the chance for false positive results. We optimized each step in the assay by testing different alternatives taking into account the assays used in the previous studies on α-PLG in AAV. Therefore, our assay is slightly different from the assays used previously, which could partly explain the discrepancy. The ultimate experimental set-up investigating what could have caused the discrepancies of the studies thus far conducted would entail that the optimized assay developed by us would be used on selected patients from the previous studies and in parallel with the assays used for these studies. Unfortunately, this set-up lay beyond the scope of the current experimental design.

In our assay we used purified PLG proteins as coating antigens. The PLG delivered by vendors is purified from blood, which means it can contain trace amounts of IgG. This will cause unspecific binding in the assay giving high background signals and therefore we decided to purify the PLG delivered by the vendor. This could also be a possible explanation for the discrepancies of our study with previous studies. Only Hao *et al.* described the purification of the PLG coating protein in their assay, but they did not

mention whether they purified it themselves or whether it was assumed to be purified.[3] This could be the most critical confounder that other studies encountered, if a protein was purchased and assumed to be purified, but in reality contained considerable amounts of contaminating IgG.

Originally, α-PLG were described in view of anti-complementary PR3 antibodies which were suggested to develop within an idiotypic antibody response.[1,7] This scenario assumed a typical combination of PR3-ANCA and α-PLG. With the repetitive finding of MPO-AAV patients having α-PLG in the absence of PR3-ANCA antibodies, this hypothesis became less likely than previously thought.

In previous studies, Bautz *et al.* and Hao *et al.* included diagnostic sera from patients with active disease at baseline, which is equal to the time of diagnosis.[1,3] In the study by Berden *et al.*, a few patients were included who were in clinical remission at baseline.[2] These studies showed data suggesting a transient nature of α-PLG. Be it in only a few cases (n=3), Berden *et al.* did show disappearance of the α-PLG in two patients after treatment; however, there was also one patient in remission who remained positive for α-PLG.[2] In the study by Bautz *et al.*, nine PR3-ANCA positive patients with thrombotic events during follow-up were reported, and five of them were positive for α-PLG in the presence of active disease, while the others were in remission and were negative for α-PLG at the time of the thrombotic event.[1] Hao *et al.* described 48 patients with sequential samples of whom 7 were positive for α-PLG during active disease; at remission during follow-up only one of the patients remained positive. In fact, Hao *et al.* noted that there was a better association between the levels of α-PLG and disease activity compared to ANCA levels and disease activity.[3] Therefore, these results suggest that α-PLG may be transient in nature. However, no firm conclusions can be drawn because of limited available data. The exact biology behind the occurrence and disappearance of these antibodies is not known and needs further study. In our study, samples were not selected with respect to disease state, since this was beyond the scope of our study.

The various studies on α-PLG in AAV had differences in their assays, which could have been responsible for the discrepancies in the results.[1–3] Different concentrations of coating antigen and different conjugated secondary antibodies were used to detect antibody-antigen complexes. In order to prevent false positive results, we optimized each step in the assay and used purified PLG as coating antigen to prevent background and unspecific binding. One assay used sera as samples while others used purified IgG with different definitions for positivity. In the previous studies a 97.5th percentile

(mean + 2 standard deviation) threshold of healthy controls was used as a cut-off point for α-PLG positivity. Hao *et al.* tested the samples one time.[3] Bautz *et al.* analyzed the highest value measured, not mentioning how often a sample was tested.[1] Berden *et al.* tested all samples 6 times and considered a patient α-PLG positive when in >50% of the occasions the assay was positive.[2] In our study samples were tested one time for α-PLG positivity. In addition, there was little information in these studies regarding the PLG antigen used for coating. Only Hao *et al.* specified their antigen further describing that it was human PLG supplied by Abcam (Cambridge, UK). The product information describes that this is a full length natural human PLG protein, so we assume that this probably is glu-PLG.[3] We demonstrated that using lys-PLG as a coating antigen showed better differentiation between negative and positive controls.

An important discussion point regarding α-PLG remains its epitope-specificity. Also in the field of ANCA-specificity this still is an ongoing discussion. It is thought that varying epitope-specificity of ANCA will influence their physiological effects and their potential for pathogenicity. 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.[8–10] Several epitopes relevant for PR3-ANCA AAV and MPO-ANCA AAV are described in the literature. These are linear and conformational epitopes. Especially, the conformational epitopes form a major problem for diagnostic purposes and pathogenic studies.[8–10] Similar issues will form a challenge in future research for α-PLG. Of the previous studies on α-PLG in AAV, only Bautz *et al.* described the target epitope of the detected α-PLG, which was also found on complementary PR3 as discussed previously.[1] Glu-PLG and lys-PLG are biologically correlated; removing the PAp domain - which consists of several amino acids - changes glu-PLG into lys-PLG.[11] In the activation process, glu-PLG and lys-PLG are both open conformations, which are distinct from each other.[12] Open conformation glu-PLG and open conformation lys-PLG can both be converted to plasmin by tPA or uPA. Glu-PLG can first be converted into lys-PLG and then into plasmin or glu-PLG can directly be converted into plasmin. Unfortunately, little is known about the exact mechanism of conformational changes of PLG in its different states.[13] Reactivity to each conformational state may be important in different ways biologically and must be further investigated for their pathogenic characteristics and potential diagnostic purposes.

In the present study we have combined important technical findings/methods from previous studies which have led to the optimized α-PLG assay here presented. Until now, there were many uncertainties and differences in the setup and results of hitherto

used assays. Our optimized assay can be used for future research purposes and will aid in uniform reporting on α-PLG, opening the way to further explore their relevance in AAV.

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REFERENCES

1. Bautz DJ, Preston GA, Lionaki S, et al. Antibodies with dual reactivity to plasminogen and complementary PR3 in PR3-ANCA vasculitis. *J Am Soc Nephrol*, 2008;19: 2421-2429.
2. Berden AE, Nolan SL, Morris HL, et al. Anti-plasminogen antibodies compromise fibrinolysis and associate with renal histology in ANCA-associated vasculitis. *J Am Soc Nephrol*, 2010;21: 2169-2179.
3. Hao J, Wang C, Gou SJ, Zhao MH, Chen M. The association between anti-plasminogen antibodies and disease activity in ANCA-associated vasculitis. *Rheumatology (Oxford)*, 2014;53: 300-306.
4. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*, 2013;65: 1-11.
5. Cohen D, Berger SP, Steup-Beekman GM, Bloemenkamp KW, Bajema IM. Diagnosis and management of the antiphospholipid syndrome. *BMJ*, 2010;340: c2541.
6. Niewold TB, Harrison MJ, Paget SA. Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis. *QJM*, 2007;100: 193-201.
7. Pendergraft III WF, Preston GA, Shah RR, et al. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med*, 2004;10: 72-79.
8. Rasmussen N, Wiik A, Jayne DR. A historical essay on detection of anti-neutrophil cytoplasmic antibodies. *Nephrol Dial Transplant*, 2015;30 Suppl 1: i8-13.
9. Roth AJ, Ooi JD, Hess JJ, et al. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. *J Clin Invest*, 2013;123: 1773-1783.
10. Sommarin Y, Rasmussen N, Wieslander J. Characterization of monoclonal antibodies to proteinase-3 and application in the study of epitopes for classical anti-neutrophil cytoplasm antibodies. *Exp Nephrol*, 1995;3: 249-256.
11. Miles LA, Castellino FJ, Gong Y. Critical role for conversion of glu-plasminogen to Lys-plasminogen for optimal stimulation of plasminogen activation on cell surfaces. *Trends Cardiovasc Med,* 2003;13: 21-30.
12. Han J, Baik N, Kim KH, et al. Monoclonal antibodies detect receptor-induced binding sites in Glu-plasminogen. *Blood*, 2011;118: 1653-1662.
13. Law RH, Abu-Ssaydeh D, Whisstock JC. New insights into the structure and function of the plasminogen/plasmin system. *Curr Opin Struct Biol*, 2013;23: 836-841.

3

ANCA-ASSOCIATED GLOMERULONEPHRITIS: RISK FACTORS FOR RENAL RELAPSE

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ABSTRACT

Introduction: Relapse in ANCA-associated vasculitis (AAV) has been studied previously, but there are few studies on renal relapse in particular. Identifying patients at high risk of renal relapse may aid in optimizing clinical management. We investigated which clinical and histological parameters are risk factors for renal relapse in ANCA-associated glomerulonephritis (AAGN).

Methods: Patients (n = 174) were newly diagnosed and had mild–moderate or severe renal involvement. Data were derived from two trials of the European Vasculitis Society: MEPEX and CYCAZAREM. The Cox regression model was used to identify parameters increasing the instantaneous risk (= rate) of renal relapse (useful for instant clinical decisions). For identifying predictors of renal relapse during follow-up, we used Fine & Gray's regression model. Competing events were end-stage renal failure and death.

Results: The cumulative incidence of renal relapse at 5 years was 9.5% (95% CI: 4.8-14.3%). In the Cox model, sclerotic class AAGN increased the instantaneous risk of renal relapse. In Fine & Gray's model, the absence of interstitial infiltrates at diagnosis was predictive for renal relapse.

Conclusions: In this study we used two different models to identify possible relationships between clinical and histopathological parameters at time of diagnosis of AAV with the risk of experiencing renal relapse. Sclerotic class AAGN increased the instantaneous risk of renal relapse. This association is most likely due to the high proportion of sclerosed glomeruli reducing the compensatory capacity. The absence of interstitial infiltrates increased the risk of renal relapse which is a warning sign that patients with a relatively benign onset of disease may also be prone to renal relapse. Renal relapses occurring in patients with sclerotic class AAGN and renal relapses occurring in patients without interstitial infiltrates were mutually exclusive, which may indicate that they are essentially different.

INTRODUCTION

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are the major subtypes of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Approximately 80% of patients with GPA and 90% with MPA develop kidney involvement during the disease course.[1] ANCA-associated glomerulonephritis (AAGN) progresses to end-stage renal failure (ESRF) in approximately 20–40% of patients.[2–5] The gold standard for establishing AAGN is a renal biopsy, which typically shows a pauci-immune necrotizing crescentic glomerulonephritis,[6,7] which can be grouped into four classes.[8] Relapse in ANCA-associated vasculitis has been studied previously, but there are few studies on renal relapse in particular. It is important to find a balance between the risk of relapse and the risk of treatment-related adverse effects.

Identifying patients at high risk of renal relapse may aid in optimizing clinical management. Previous relevant studies mainly focused on relapse in general with clinical data,[2,5,9–19] identifying proteinase 3 (PR3)-ANCA, GPA, lung or cardiovascular involvement, and better renal function at presentation as associated with relapse in general.[2,10–14,16,17,19] Note that different statistical analyses were used in these reports to determine the influence of various parameters on relapse; some published studies employed Fine & Gray's regression model while others used the standard Cox regression model. Both models are correct but address different research questions.

If more than one endpoint can occur, a competing risk analysis must be performed. In the case of renal relapse, ESRF and death are competing events, because the occurrence of one of them precludes the occurrence of renal relapse. Fine & Gray's regression model is used to estimate the effect of a risk factor on the cumulative incidence of renal relapse (CIR), which denotes the probability of experiencing renal relapse before time *t*. The classical Cox regression model is used to investigate the effect of risk factors on the rate of renal relapse. The parameters in the Cox regression model are hazard ratios and the interpretation is the traditional one. Note that relationships between risk factors (or explanatory parameters) and cause-specific hazards do not lead to simple relationships between explanatory variables and cumulative incidence. It is important to emphasize that both approaches are valid but that they answer different research questions and may render different results. Thus, the effect of a parameter on the CIR might be different from its effect on the rate of renal relapse. Estimation based on Fine & Gray's model is useful for making predictions from the start of the disease, whereas the rate looks at parameters that increase the instantaneous risk of renal relapse and is useful for instant clinical decisions. In the present study, we apply both methods and discuss implications from their results.

We investigated whether diagnostic clinical and histological parameters are associated with renal relapse in patients with AAV with primary renal involvement. The study aim was to identify diagnostic tools that may be helpful in monitoring and managing patients with AAV, in particular in relation to renal relapse.

METHODS

Patients

Patients included in this study were newly diagnosed with AAV with either mild to moderate or severe renal involvement (serum creatinine ≤ or > 500 μmol/L (≤ or > 5.8 mg/dl)). Patients were derived from two international multicenter randomized clinical European Vasculitis Society (EUVAS) trials: MEPEX and CYCAZAREM.[11,20] Inclusion criteria for both trials are described elsewhere.[11,20] The diagnosis was based on a clinical presentation compatible with ANCA-associated vasculitis and substantiated by a positive ANCA serology and/or histology.

The MEPEX and CYCAZAREM trial follow-up continued until 12 and 18 months after diagnosis, respectively. During the trials, patients received protocolized treatment regimens.[11,20] After these follow-up periods, patients were treated according to their local physician's standards. Patients were included and followed-up in the period June 1, 1995, through 30 November, 2006. Patients were included in this study only if histological data obtained from renal biopsy at the time of study entry, clinical data, and long-term follow-up data were available.

Disease definitions were adapted from the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis and a previous European Union Study.[21,22] Both trials were conducted according to the 1964 Declaration of Helsinki and subsequent amendments. The trials were approved by the local ethics committees of the participating centers throughout Europe. All patients gave written informed consent. The ethics for the use of the data and material for subsequent studies, including this study, was approved by the West Midlands Multi-centre Research Ethics Committee, date 22/09/2004 (reference number: MREC/98/7/37). In addition, this study was performed according to the 'Netherlands Code of Conduct for Scientific Practice', an ethical code for performing observational studies with patient material approved by the Federatie van Medisch Wetenschappelijke Verenigingen (translated: Federation of Medical Scientific Organisations) together with the legal and ethical committee of the Koninklijke Nederlandse Akademie van Wetenschappen (translated: Royal Dutch Academy of Science) and the Nederlandse Organisatie voor Wetenschappelijk

Onderzoek (translated: Dutch Organisation for Scientific Research). The data of the patients were analyzed anonymously.

Clinical and histological parameters

Candidate parameters for clinical predictors of renal relapse in this study were serum creatinine levels, age, sex, diagnosis (GPA or MPA), ANCA-antigen specificity (PR3-ANCA or myeloperoxidase (MPO)-ANCA), and receiving plasma exchange during induction therapy. Patients were subdivided into two groups of GPA and MPA based on the clinical criteria. Renal-limited vasculitis was regarded as a form of MPA.

Candidate parameters for histological predictors were determined from paraffin sections of renal biopsies. Stains used for evaluation were silver, periodic acid–Schiff, hematoxylin and eosin, and trichrome. Sections were reviewed by two of a panel of five participating pathologists (IMB, FF, LHN, RW, and/or JAB). Both pathologists, blinded to patient data and the other observer's results, scored the biopsies separately and according to a previously standardized protocol, which was proven to be comprehensive and reproducible when used for histologic analysis.[23] One of the histological parameters included in this previously standardized protocol is interstitial infiltrates. Interstitial infiltrates were scored according to the following categories:

- None: <10% of the unscarred parenchyma infiltrated.
- Mild: 10 to 25% of the unscarred parenchyma infiltrated.
- Quite dense: 26 to 50% of the unscarred parenchyma infiltrated.
- Very dense: >50% of the unscarred parenchyma infiltrated.

In this study, only biopsies with a minimum of seven whole glomeruli were analyzed for glomerular lesions and the histopathological classification system of AAGN.[8] During plenary meetings, the panel of five pathologists decided upon the final scores to achieve consensus for each biopsy.

Clinical outcomes

The clinical outcome parameter was first renal relapse. A renal relapse was defined as a rise in serum creatinine of >30% or a fall in estimated glomerular filtration rate >25% and/or new hematuria or proteinuria (all attributable to active vasculitis), as indicated by the Birmingham Vasculitis Activity Score.[24–26] Patients were followed up until the last visit or death.

Statistical analyses

In this study, more than one endpoint could occur, namely renal relapse, ESRF, or death. The event of interest was renal relapse, while ESRF and death were competing events. Two regression models used in the competing risks framework were estimated here: Fine & Gray's model and Cox model. To study the effect of risk factors on the CIR the former model is employed while the latter is used to study the effects of risk factors on the rate of renal relapse, i.e. the cause-specific hazard. For more details concerning the difference between the two models, see Andersen *et al*. and Koller *et al*.[27,28] The technical aspects of competing risks were described previously by Putter *et al*. [29]

Univariate analyses with both methods were performed on every clinical and histological parameter. These analyses were performed on all patients without ESRF at baseline (n = 149), except for glomerular lesions and the histopathological class of AAGN. In addition, we performed a $\chi 2$-test to see whether the percent of renal relapse differed significantly between the histopathological classes. Also, a Pearson correlation test was performed to investigate whether the presence of interstitial infiltrate was correlated with interstitial fibrosis. All baseline parameters were included as fixed covariates.

Because of the number of parameters (13 in this study) and the relatively low number of events, inclusion of too many parameters carries the risk of "overfitting".[30] Therefore, predefined smaller sets of entry parameters were included in the multivariate analyses, as follows: based on the original publication of the histopathological classification system of AAGN[8]; based on parameters described previously more than once as being associated with relapse; and based on only histological parameters.

We denoted hazard ratios estimated by employing Cox regression model as cause-specific hazard ratio (csHR) and the hazard ratios estimated by using Fine & Gray's regression model as Fine & Gray's HR (F&G HR). All hazard ratios are provided with 95% confidence intervals (CI). A *P* value of less than 0.05 was considered significant. Statistical analyses were performed in SPSS (version 20.0; SPSS Inc, Chicago, IL) and R 2-18 (http://cran.r-project.org). All analyses concerning competing risks were performed with the mstate library.[31,32]

RESULTS

Patients

A total of 174 patients with newly diagnosed AAV and a renal biopsy at diagnosis were included in this study. Table 1 shows the baseline patient characteristics. The median follow-up time was 102 months (range: 38–136 months).

Table 1 | Baseline characteristics of all patients

Characteristic		Value
Number of patients		174
Age (years)		60.3 ± 13.1[a]
Male		94 (54)
Diagnosis		
	GPA	74 (43)
	MPA	100 (57)
ANCA antigen		
	PR3	81 (47)
	MPO	80 (46)
	Negative	7 (4)
	Double positive	3 (2)
	NR	3 (2)
Serum creatinine		
	≤ 100 µmol/L	23 (13)
	101-200 µmol/L	23 (13)
	≥ 201 µmol/L	128 (74)
ESRF at baseline		25 (14)
PLEX therapy		
	Yes	46 (26)
	No	128 (74)
Histopathological class[b]		
	Focal	23 (20)
	Crescentic	58 (51)
	Mixed	18 (16)
	Sclerotic	14 (12)

Data are presented as n (%) unless otherwise noted.
Abbreviations: ANCA, anti-neutrophil cytoplasmic antibody; ESRF, end-stage renal failure; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; NR, not reported/not performed; PLEX, plasma exchange therapy; PR3, proteinase 3.
[a]Mean (SD).
[b]Only patients with at least 7 whole glomeruli in their renal biopsy and no ESRF at baseline.

Renal relapse

Of the 174 patients, 25 could not experience a renal relapse because they had ESRF at baseline. Of the remaining 149 patients, 22 were chronic kidney disease (CKD) stage 1-2 and 127 were CKD stage 3-5. Of these 22 CKD stage 1-2 patients, 5 (22.7%)

developed CKD stage 3-5 within 5 years of follow-up. None of these 5 had experienced a renal relapse. In total, 31 patients experienced a renal relapse during follow-up. The CIR at 5 years was 9.5% (95% CI: 4.8-14.3%). A total of 19 patients developed ESRF during follow-up without experiencing renal relapse, and 29 died without experiencing renal relapse and without developing ESRF during follow-up. All patients who died during the follow-up period had CKD stage ≥ 3 at baseline. Seventy patients had none of these events during follow-up (Figures 1 and 2).

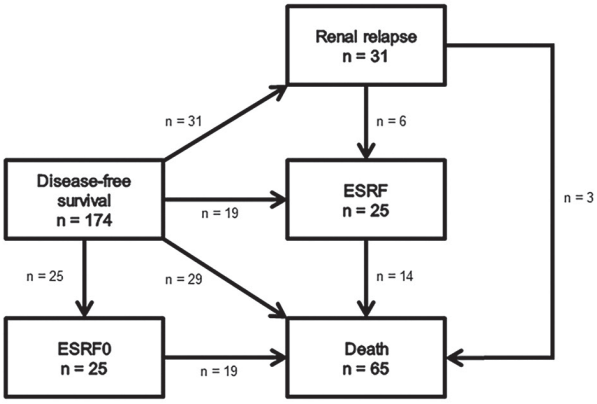

Figure 1 | Events.
Overview of different events experienced by 174 patients during follow-up. Twenty-five patients presented with ESRF at baseline. Nineteen of them died during follow-up. Thirty-one patients experienced renal relapse during follow-up; six of them developed ESRF, of whom four died, and three died without ESRF during follow-up. Nineteen patients developed ESRF without renal relapse (competing event 1), of whom 10 died at a later timepoint. Twenty-nine patients died without experiencing renal relapse or ESRF (competing event 2). Seventy patients experienced no event during follow-up.
Abbreviations: DSF, disease-free survival; ESRF, end-stage renal failure; ESRF0, end-stage renal failure at baseline.

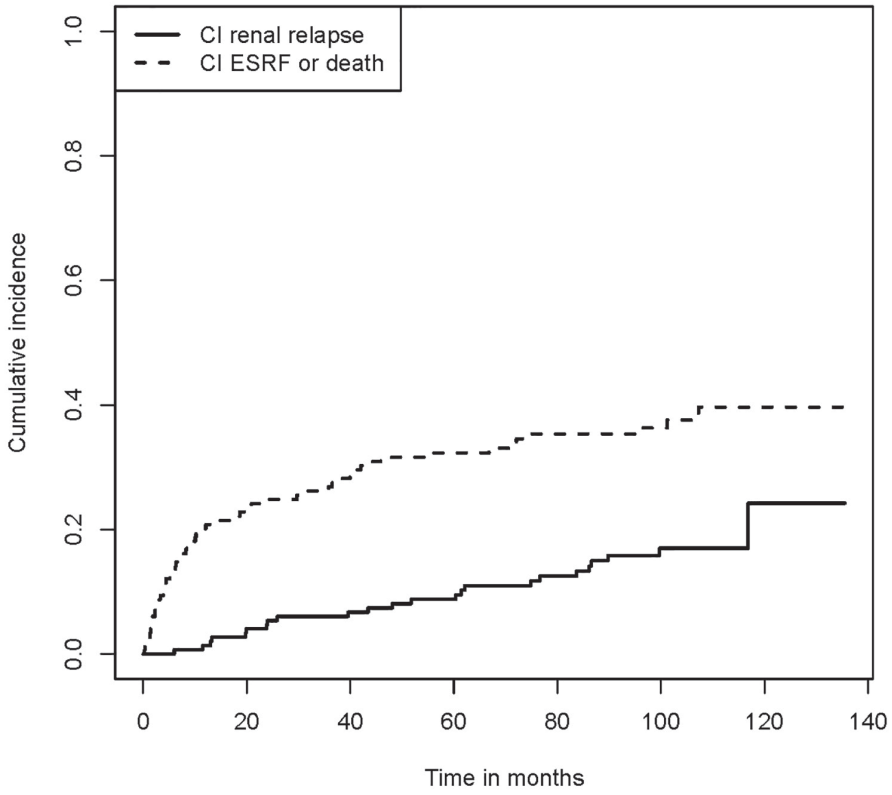

Figure 2 | Cumulative incidence of renal relapse, end-stage renal failure or death.
Cumulative incidence of patients who experienced renal relapse (event of interest) and patients who developed ESRF or died (competing events). This figure illustrates the probability of experiencing a renal relapse and the probability of developing ESRF or dying without experiencing a renal relapse.
Abbreviations: CI, cumulative incidence; ESRF, end-stage renal failure.

Of the 149 patients, 113 had adequate renal tissue samples (at least seven whole glomeruli in the renal biopsy) for classification purposes. Their diagnostic renal biopsies were classified as follows: 23 focal class (20.4%), 58 crescentic class (51.3%), 18 mixed class (15.9%), and 14 sclerotic class (12.4%) (Table 2). Of these 113 patients, 24 experienced a renal relapse during follow-up. The numbers of patients having a renal relapse per class were 5/23 (21.7%) focal class, 9/58 (15.5%) crescentic class, 4/18 (22.2%) mixed class, and 6/14 (42.9%) sclerotic class (χ2-test: P=0.167). The distribution of patient ages did not differ across classes. In particular, patients from the sclerotic class were not older than those in other classes. During the trials, therapies given to the patients did not differ among the four classes. Fourteen patients developed ESRF during follow-up without experiencing renal relapse (competing event 1). Twenty

patients died without experiencing renal relapse and without developing ESRF during follow-up (competing event 2) (Figure 3).

Table 2 | Baseline characteristics of patients per histopathological class

Characteristic		Value per class			
		Focal class	Crescentic class	Mixed class	Sclerotic class
Number of patients		23	58	18	14
Age (years)		55.4 ± 13.8[a]	60.4 ± 13.6[a]	59.8 ± 9.2[a]	63.8 ± 12.3[a]
Male		14 (61)	28 (48)	11 (61)	6 (43)
Diagnosis					
	GPA	16 (70)	25 (43)	6 (33)	3 (21)
	MPA	7 (30)	33 (57)	12 (67)	11 (79)
ANCA antigen					
	PR3	17 (74)	28 (48)	8 (44)	3 (21)
	MPO	5 (22)	25 (43)	9 (50)	11 (79)
	Negative	0 (0)	2 (4)	1 (6)	0 (0)
	Double positive	1 (4)	2 (4)	0 (0)	0 (0)
	NR	0 (0)	1 (2)	0 (0)	0 (0)
Serum creatinine					
	≤ 100 µmol/L	14 (61)	1 (2)	1 (6)	0 (0)
	101-200 µmol/L	3 (13)	7 (12)	3 (17)	3 (21)
	≥ 201 µmol/L	6 (26)	50 (86)	14 (78)	11 (79)
PLEX therapy					
	Yes	1 (4)	18 (69)	5 (28)	4 (29)
	No	22 (96)	40 (31)	13 (72)	10 (71)

Data are presented as n (%) unless otherwise noted.
Sample size: 113 patients (patients with at least 7 whole glomeruli in their renal biopsy and no end-stage renal failure at baseline).
Abbreviations: ANCA, anti-neutrophil cytoplasmic antibody; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; NR, not reported/not performed; PLEX, plasma exchange therapy; PR3, proteinase 3.
[a]Mean (SD).

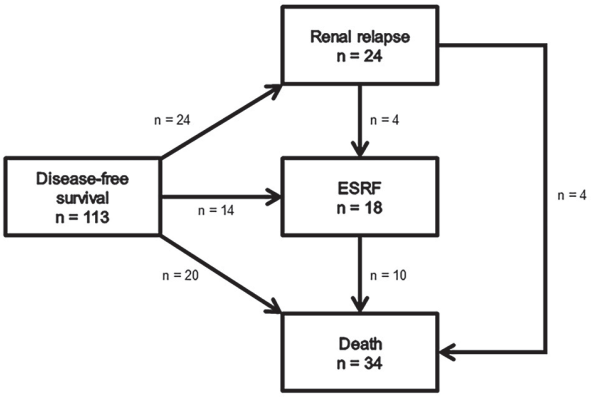

Figure 3 | Events of the 113 patients with ≥7 glomeruli in their renal biopsy without end-stage renal failure at baseline.
Twenty-four patients experienced a renal relapse during follow-up. Of these 24 patients, four developed ESRF, of which three died, and four died without ESRF during follow-up. Fourteen patients developed ESRF without renal relapse (competing event 1), of whom 7 died at a later timepoint. Twenty patients died without renal relapse and without ESRF (competing event 2). Fifty-five patients experienced no event during follow-up.
Abbreviations: DSF, disease-free survival; ESRF, end-stage renal failure.

Competing risks analyses

To investigate which parameters are associated with the CIR, Fine & Gray's regression model was used. The traditional Cox regression model was used to estimate the effect of the parameters on the rate of renal relapse.[27]

Fine & Gray's regression model

The univariate analyses showed that age, interstitial infiltrates, and intra-epithelial infiltrates were associated with CIR (S Table 1 in the supplementary material). Older age was associated with a lower risk for experiencing a renal relapse. Higher scores of interstitial infiltrates and intra-epithelial infiltrates, which are both signs of acute disease activity, were associated with a lower risk for renal relapse. The histopathological class and CKD stage were not significantly associated with the risk of renal relapse.

Among all histological parameters, only interstitial infiltrates had a significant association on the risk of renal relapse (the CIR) in the multivariate analysis (Table 3). Although there was a correlation between interstitial infiltrates and interstitial fibrosis, this correlation was relatively weak (r=0.236, P=0.004). Therefore, interstitial fibrosis was not predictive for renal relapse. Patients with mild infiltrates had a four times lower

risk for renal relapse than patients without interstitial infiltrates (F&G HR: 0.09; 95% CI: 0.02–0.39; *P* = 0.001). This association persisted when correcting for the patient cohort indicator.

Table 3 | Multivariate analysis with Fine & Gray's model based on histological parameters

		Renal relapse	
Parameter		**P Value**	**F&G HR (95% CI)**
Interstitial infiltrates			
	None	-	1
	Mild	0.001	0.09 (0.02-0.39)
	Quite dense	0.1	0.16 (0.02-1.56)
	Very dense	0.5	0.29 (0.01-7.83)
Interstitial fibrosis			
	None	-	1
	Focal	0.9	1.06 (0.24-4.67)
	Diffuse	0.7	1.59 (0.19-13.44)
Tubular atrophy			
	None	-	1
	Small foci	0.7	1.44 (0.23-8.85)
	Extensive	0.9	0.87 (0.05-14.34)
Intra-epithelial infiltrates[a]		0.2	0.34 (0.05-2.08)
Histopathological class			
	Focal	0.2	0.21 (0.02-2.11)
	Crescentic	0.3	0.40 (0.08-2.00)
	Mixed	0.9	1.06 (0.18-6.11)
	Sclerotic	-	1

Sample size: 112 patients

Abbreviations: 95% CI, 95% confidence interval; NS, not significant; F&G HR, Fine and Gray's hazard ratio.

[a]Reference group: No intra-epithelial infiltrates.

Among all clinical parameters, only age, corrected for other parameters, was associated with patients experiencing a renal relapse (S Table 2 in the supplementary material).

Cox regression model for renal relapse

Among all baseline parameters, only the histopathological class was a significant risk factor for renal relapse in the univariate analyses (S Table 3 in the supplementary material). CKD stage was not associated with renal relapse. After correction for age, baseline serum creatinine, and plasma exchange therapy, the histopathological class remained the only statistical significant risk factor for experiencing a renal relapse (Table 4). Focal and crescentic class biopsies were associated with a lower cause-specific hazard ratio compared to sclerotic class biopsies. Focal class had a 10.1 times lower rate than the sclerotic class (csHR: 0.10; 95% CI: 0.02–0.60; $P = 0.01$), and crescentic class had a 4.7 times lower rate than the sclerotic class (csHR: 0.21; 95% CI: 0.07–0.62; $P = 0.004$). Patient cohort (MEPEX or CYCAZAREM) did not affect these associations.

Table 4 | Multivariate analyses with both models based on the original publication of the histopathological classification system of ANCA-associated glomerulonephritis (Berden et al, 2010[8])

Parameter		Renal relapse			
		Cox regression model		Fine & Gray's model	
		P Value	csHR (95% CI)	P Value	F&G HR (95% CI)
Serum creatinine					
	≤ 100 µmol/L	-	1	-	1
	101-200 µmol/L	0.6	0.65 (0.11-3.70)	0.6	0.58 (0.07-4.66)
	≥ 201 µmol/L	0.4	0.45 (0.08-2.43)	0.4	0.42 (0.05-3.80)
Age		0.3	0.98 (0.96-1.01)	0.02	0.97 (0.94-0.996)
Plasma exchange therapy[a]		0.9	1.10 (0.33-3.69)	0.8	0.85 (0.23-3.14)
Histopathological class					
	Focal	0.01	0.10 (0.02-0.60)	0.2	0.24 (0.03-2.19)
	Crescentic	0.004	0.21 (0.07-0.62)	0.07	0.34 (0.10-1.09)
	Mixed	0.08	0.31 (0.08-1.15)	0.6	0.68 (0.18-2.58)
	Sclerotic	-	1	-	1

Sample size: 113 patients

Abbreviations: 95% CI, 95% confidence interval; csHR, cause-specific hazard ratio; NS, not significant; F&G HR, Fine and Gray's hazard ratio.

[a]Reference group: No plasma exchange therapy received.

With the inclusion of histopathological class, baseline serum creatinine, ANCA type, and diagnosis, only the histopathological class was a significant risk factor for renal relapse (Table 5). Again, focal and crescentic classes were associated with a lower rate compared to sclerotic class. In this model, focal class had a 10.8 times lower rate than the sclerotic class (csHR: 0.09; 95% CI: 0.02–0.55; $P = 0.009$), and crescentic class had a 4.8 times lower hazard rate than the sclerotic class (csHR: 0.21; 95% CI: 0.07–0.64; $P = 0.006$). There was no effect of patient cohort on these associations.

Table 5 | Multivariate analyses with both models based on previously described parameters associated with relapse

Parameter		Renal relapse			
		Cox regression model		Fine & Gray's model	
		P Value	csHR (95% CI)	P Value	F&G HR (95% CI)
Serum creatinine					
	≤ 100 µmol/L	-	1	-	1
	101-200 µmol/L	0.6	0.62 (0.12-3.33)	0.5	0.44 (0.05-3.62)
	≥ 201 µmol/L	0.4	0.46 (0.08-2.65)	0.2	0.23 (0.02-2.61)
Diagnosis[a]		0.2	0.55 (0.20-1.51)	0.9	0.96 (0.24-3.87)
PR3-ANCA[b]		0.6	0.61 (0.09-4.22)	0.3	0.45 (0.10-2.03)
MPO-ANCA[b]		0.9	0.84 (0.14-5.07)	0.4	0.62 (0.21-1.79)
Histopathological class					
	Focal	0.009	0.09 (0.02-0.55)	0.3	0.32 (0.04-2.28)
	Crescentic	0.006	0.21 (0.07-0.64)	0.2	0.46 (0.13-1.63)
	Mixed	0.06	0.26 (0.07-1.07)	0.7	0.73 (0.18-2.98)
	Sclerotic	-	1	-	1

Sample size: 112 patients
Abbreviations: 95% CI, 95% confidence interval; ANCA, anti-neutrophil cytoplasmic antibody; csHR, cause-specific hazard ratio; MPO, myeloperoxidase; NS, not significant; PR3, proteinase 3; F&G HR, Fine and Gray's hazard ratio.
[a]Reference group: Granulomatosis with polyangiitis.
[b]Reference group: Negative.

DISCUSSION

This study shows that the histopathological class of AAGN in the renal biopsy at diagnosis is a risk factor for renal relapse. More specifically, sclerotic class was associated with a higher rate of renal relapse during long-term follow-up. It is important to emphasize that the effect of sclerotic class on the risk of renal relapse, i.e. the cumulative incidence, estimated by the Fine & Gray's model is different from its effects on the rate, i.e. the cause-specific hazard, estimated by the Cox regression proportional hazard model. This is because the way in which risk factors (or explanatory variables) are associated with the cause-specific hazards may not coincide with the way these covariates are associated with the cumulative incidence. The sclerotic class in AAGN is defined by ≥50% globally sclerosed glomeruli, meaning that the majority of glomeruli are non-functioning and that the compensatory ability of the kidneys is relied on heavily. Therefore, in these patients, a renal relapse may become more readily apparent because the compensatory capacity of a sclerotic class kidney is reduced. Moreover, with fewer functioning glomeruli, these glomeruli may become more vulnerable to a second hit, i.e., a relapse. In patients with AAGN that is not in the sclerotic class, minor relapses may remain subclinical because of the relatively higher number of preserved glomeruli and their compensatory ability. Patients' treatments were not based on the

histopathological classification. Therefore, the sclerotic class may provide a setting in which renal relapse may be more likely to be detected than in the setting of another histopathological class.

To investigate the effect of risk factors on the risk of renal relapse, i.e. the cumulative incidence, we applied Fine & Gray's regression model. Results show that absence of interstitial inflammatory infiltrates is associated with the risk of renal relapse. Patients with these infiltrates had a lower risk for future renal relapse than patients without these inflammatory infiltrates. The association of interstitial infiltrates with renal relapse persisted when corrected for other histological parameters. Previous EUVAS studies focused on predictive clinical and serological parameters for relapse in general. Walsh *et al.* investigated clinical and serological parameters predictive for relapse in general in a European cohort consisting of 535 patients. In that study, PR3-ANCA, lower serum creatinine levels at presentation, cardiovascular involvement, and GPA were independently associated with an increased risk for relapse, whether in the kidney or any other organ.[19] Our study is based on the histopathological data of those patients from the previous study by Walsh *et al.* who had a renal biopsy with sufficient tissue for proper evaluation; thus, we could investigate which histological parameters are predictive for renal relapse. Our finding that the absence of interstitial infiltrates is predictive for renal relapse is in line with the finding by Walsh *et al.* that better renal function increases the risk for a relapse in general because absence of interstitial infiltrates also correlates with better renal function at the time of biopsy.[33,34] Experiencing a renal relapse has a negative influence on renal outcome.[35] Therefore, clinicians should realize that renal relapses must be identified and treated, and keep in mind that those patients with a relatively benign clinical course at onset in particular will be prone to developing a renal relapse.

This study shows a higher cumulative risk for ESRF or death compared to renal relapse as shown in Figure 2. These results may have been influenced by the inclusion of patients from the MEPEX trial which included patients with serum creatinine >500 µmol/L or immediate dialysis dependency. Nineteen patients (11%) had ESRF during follow-up without experiencing a (clinical) renal relapse. It is possible that these patients had subclinical renal relapses, but they were not detected clinically. Based on this knowledge, we emphasize the need for chronic kidney disease management and renal protective strategies.

Our study has a number of limitations. Because of the sample size and relatively low number of events, we were limited in the size of the predefined multivariate analyses.

To avoid bias, all multivariate analyses were predefined before the start of this study. To use the best possible predefined analyses, we constructed them based on the literature regarding the histopathological classification system of AAGN. Unfortunately, repeat biopsies during the time of renal relapse were not performed because it is generally considered that the risk of taking a biopsy would not weight against the benefit of a histologically proven renal relapse, keeping in mind that these can be diagnosed with a high level of certainty on the basis of the clinical findings.

In conclusion, we used two regression models to identify possible relationships between clinical and histopathological parameters at time of diagnosis of AAV with the risk of renal relapse, i.e. the cumulative incidence, and the effect on the rate of renal relapse, i.e. the cause-specific hazard. The effect of sclerotic class on the risk of renal relapse estimated by the Fine & Gray's regression model was different from its effects on the rate estimated by the Cox regression model. Most likely, the lack of compensatory function in the largely sclerosed kidneys gives rise to the identification of a relatively high number of renal relapses. A strong predictive parameter for renal relapse was the absence of interstitial infiltrates as determined with the Fine & Gray model. Combining these results with those of previous studies, it seems that the patient with AAV characterized by a relatively benign clinical setting at onset is prone to a renal relapse. In this study, renal relapses occurring in patients with sclerotic class AAGN and renal relapses occurring in patients without interstitial infiltrates were mutually exclusive, which may indicate that they are essentially other kinds of relapses. Further studies are called for to look further into the characteristics of renal relapses in AAV, in particular to find out whether the histopathological data at disease onset could serve as a guideline for the management of renal relapses in AAV.

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DISCLOSURES

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The authors have declared that no competing interests exist.

REFERENCES

1. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med*, 1997;337: 1512-1523.
2. Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis*, 2003;41: 776-784.
3. Little MA, Nightingale P, Verburgh CA, et al. Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. *Ann Rheum Dis*, 2010;69: 1036-1043.
4. Slot MC, Cohen Tervaert JW, Franssen CFM, Stegeman CA. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int*, 2003;63: 670-677.
5. Westman KWA, Bygren PG, Olsson H, Ranstam J, Wieslander J. Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J Am Soc Nephrol*, 1998;9: 842-852.
6. Falk RJ, Jennette JC. ANCA small-vessel vasculitis. *J Am Soc Nephrol*, 1997;8: 314-322.
7. Bajema IM, Hagen EC, van der Woude FJ, Bruijn JA. Wegener's granulomatosis: a meta-analysis of 349 literary case reports. *J Lab Clin Med*, 1997;129: 17-22.
8. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol*, 2010;21: 1628-1636.
9. Gordon M, Luqmani RA, Adu D, et al. Relapses in patients with a systemic vasculitis. *Q J Med*, 1993;86: 779-789.
10. Hogan SL, Falk RJ, Chin H, et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. *Ann Intern Med*, 2005;143: 621-631.
11. Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med*, 2003;349: 36-44.
12. Koldingsnes W, Nossent JC. Baseline features and initial treatment as predictors of remission and relapse in Wegener's granulomatosis. *J Rheumatol*, 2003;30: 80-88.
13. Kyndt X, Reumaux D, Bridoux F, et al. Serial measurements of antineutrophil cytoplasmic autoantibodies in patients with systemic vasculitis. *Am J Med*, 1999;106: 527-533.
14. Lionaki S, Blyth ER, Hogan SL, et al. Classification of antineutrophil cytoplasmic autoantibody vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. *Arthritis Rheum*, 2012;64: 3452-3462.
15. Nachman PH, Hogan SL, Jennette JC, Falk RJ. Treatment response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol*, 1996;7: 33-39.
16. Pagnoux C, Hogan SL, Chin H, et al. Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis: comparison of two independent cohorts. *Arthritis Rheum*, 2008;58: 2908-2918.
17. Stegeman CA, Cohen Tervaert JW, Sluiter WJ, Manson WL, De Jong PE, Kallenberg CGM. Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med*, 1994;120: 12-17.
18. Wada T, Hara A, Arimura Y, Sada KE, Makino H. Risk factors associated with relapse in Japanese patients with microscopic polyangiitis. *J Rheumatol*, 2012;39: 545-551.

19. Walsh M, Flossmann O, Berden A, et al. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum*, 2012;64: 542-548.
20. Jayne DRW, Gaskin G, Rasmussen N, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol*, 2007;18: 2180-2188.
21. Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum*, 1994;37: 187-192.
22. Hagen EC, Daha MR, Hermans J, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int*, 1998;53: 743-753.
23. Bajema IM, Hagen EC, Hansen BE, et al. The renal histopathology in systemic vasculitis: an international survey study of inter- and intra-observer agreement. *Nephrol Dial Transplant*, 1996;11: 1989-1995.
24. Luqmani RA, Bacon PA, Moots RJ, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM*, 1994;87: 671-678.
25. Luqmani RA, Exley AR, Kitas GD, Bacon PA. Disease assessment and management of the vasculitides. *Baillieres Clin Rheumatol*, 1997;11: 423-446.
26. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis*, 2009;68: 1827-1832.
27. Andersen PK, Geskus RB, de Witte T, Putter H. Competing risks in epidemiology: possibilities and pitfalls. *Int J Epidemiol*, 2012;41: 861-870.
28. Koller MT, Raatz H, Steyerberg EW, Wolbers M. Competing risks and the clinical community: irrelevance or ignorance? *Stat Med*, 2012;31: 1089-1097.
29. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. *Stat Med*, 2007;26: 2389-2430.
30. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol*, 1996;49: 1373-1379.
31. de Wreede LC, Fiocco M, Putter H. Mstate: an R package for the analysis of competing risks and multi-state models. *J Stat Softw*, 2011;38: 1-30.
32. de Wreede LC, Fiocco M, Putter H. The mstate package for estimation and prediction in non- and semi-parametric multi-state and competing risks models. *Comput Methods Programs Biomed*, 2010;99: 261-274.
33. Bajema IM, Hagen EC, Hermans J, et al. Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int*, 1999;56: 1751-1758.
34. Hauer HA, Bajema IM, Van Houwelingen HC, et al. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int*, 2002;62: 1732-1742.
35. de Joode AA, Sanders JS, Stegeman CA. Renal survival in proteinase 3 and myeloperoxidase ANCA-associated systemic vasculitis. *Clin J Am Soc Nephrol*, 2013;8: 1709-1717.

SUPPLEMENTARY MATERIAL

ANCA-associated glomerulonephritis: risk factors for renal relapse

S Table 1 | Univariate analyses with Fine & Gray's model

Parameter	Renal relapse	
	P Value	F&G HR (95% CI)
Clinical parameters		
Gender[a]	0.9	1.04 (0.47-2.30)
Serum creatinine		
≤ 100 µmol/L	-	1
101-200 µmol/L	0.6	0.74 (0.24-2.31)
≥ 201 µmol/L	0.2	0.51 (0.20-1.32)
Age	0.003	0.96 (0.94-0.99)
Diagnosis (GPA, MPA)[b]	0.4	0.73 (0.33-1.62)
PR3-ANCA[c]	0.4	1.43 (0.63-3.24)
MPO-ANCA[c]	0.4	0.73 (0.32-1.64)
Plasma exchange therapy[d]	0.5	0.70 (0.24-2.02)
CKD stage	0.2	0.59 (0.26-1.34)
Interstitial lesions		
Interstitial infiltrates		
None	-	1
Mild infiltrate	0.006	0.25 (0.09-0.67)
Quite dense infiltrate	0.01	0.26 (0.09-0.72)
Very dense infiltrate	0.5	0.45 (0.05-3.79)
Interstitial fibrosis		
None	-	1
Focal	0.5	0.70 (0.27-1.80)
Diffuse	0.7	0.81 (0.25-2.64)
Tubular lesions		
Tubular atrophy		
None	-	1
Small foci	0.1	0.47 (0.19-1.16)
Extensive	0.5	0.60 (0.20-2.15)
Intra-epithelial infiltrates[e]	0.04	0.43 (0.20-0.94)
Glomerular lesions		
Histopathological class		
Focal	0.7	0.99 (0.24-2.48)
Crescentic	0.8	1.20 (0.29-5.07)
Mixed	0.3	1.89 (0.51-6.93)
Sclerotic	-	1
Fibrinoid necrosis	0.5	0.99 (0.97-1.01)

Abbreviations: 95% CI, 95% confidence interval; ANCA, anti-neutrophil cytoplasmic antibody; CKD, chronic kidney disease; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; NS, not significant PR3, proteinase 3; F&G HR, Fine and Gray's hazard ratio.

[a]Reference group: Female.

[b]Reference group: Granulomatosis with polyangiitis.

[c]Reference group: Negative.

[d]Reference group: No plasma exchange therapy received.

[e]Reference group: No intra-epithelial infiltrates.

S Table 2 | Multivariate analysis with Fine & Gray's model based on clinical parameters and histopathological class

Parameter	Renal relapse	
	P Value	F&G HR (95% CI)
Gender[a]	0.9	1.04 (0.40-2.68)
Serum creatinine		
≤ 100 µmol/L	-	1
101-200 µmol/L	0.6	0.55 (0.06-4.65)
≥ 201 µmol/L	0.4	0.35 (0.03-4.73)
Age	0.02	0.97 (0.94-0.99)
Diagnosis (GPA, MPA)[b]	0.8	0.88 (0.24-3.16)
PR3-ANCA[c]	0.2	0.38 (0.08-1.82)
MPO-ANCA[c]	0.3	0.54 (0.18-1.61)
Plasma exchange therapy[d]	0.7	0.80 (0.21-3.01)
Histopathological class		
Focal	0.2	0.26 (0.03-2.19)
Crescentic	0.1	0.38 (0.11-1.31)
Mixed	0.6	0.72 (0.20-2.68)
Sclerotic	-	1

Sample size: 112 patients
Abbreviations: 95% CI, 95% confidence interval; ANCA, anti-neutrophil cytoplasmic antibody; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; NS, not significant; PR3, proteinase 3; F&G HR, Fine and Gray's hazard ratio.
[a]Reference group: Female.
[b]Reference group: Granulomatosis with polyangiitis.
[c]Reference group: Negative.
[d]Reference group: No plasma exchange therapy received.

S Table 3 | Univariate analyses with Cox regression model

Parameter	Renal relapse	
	P Value	csHR (95% CI)
Clinical parameters		
Gender[a]	0.3	1.53(0.75-3.13)
Serum creatinine		
≤ 100 µmol/L	-	1
101-200 µmol/L	0.9	1.00 (0.32-3.12)
≥ 201 µmol/L	0.6	1.30 (0.52-3.28)
Age	0.4	0.99 (0.97-1.02)
Diagnosis (GPA, MPA)[b]	0.3	0.68 (0.33-1.40)
PR3-ANCA[c]	0.6	1.26 (0.60-2.64)
MPO-ANCA[c]	0.8	0.90 (0.43-1.89)
Plasma exchange therapy[d]	0.6	1.28 (0.52-3.16)
CKD stage	0.5	0.77 (0.33-1.78)
Interstitial lesions		
Interstitial infiltrates		
None	-	1
Mild infiltrate	0.08	0.43 (0.16-1.11)
Quite dense infiltrate	0.2	0.50 (0.18-1.36)
Very dense infiltrate	0.5	1.96 (0.23-16.83)
Interstitial fibrosis		
None	-	1
Focal	0.7	1.21 (0.48-3.06)
Diffuse	0.5	1.46 (0.49-4.35)
Tubular lesions		
Tubular atrophy		
None	-	1
Small foci	0.5	0.75 (0.33-1.72)
Extensive	0.9	1.04 (0.34-3.19)
Intra-epithelial infiltrates[e]	0.1	0.58 (0.29-1.19)
Glomerular lesions		
Histopathological class		
Focal	0.008	0.20 (0.06-0.66)
Crescentic	0.006	0.24 (0.08-0.66)
Mixed	0.1	0.36 (0.10-1.28)
Sclerotic	-	1
Fibrinoid necrosis	0.3	0.99 (0.97-1.01)

Abbreviations: 95% CI, 95% confidence interval; ANCA, anti-neutrophil cytoplasmic antibody; CKD, chronic kidney disease; csHR, cause-specific hazard ratio; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3.
[a]Reference group: Female.
[b]Reference group: Granulomatosis with polyangiitis.
[c]Reference group: Negative.
[d]Reference group: No plasma exchange therapy received.
[e]Reference group: No intra-epithelial infiltrates.

4

HISTOPATHOLOGICAL CLASSIFICATION OF ANCA-ASSOCIATED GLOMERULONEPHRITIS: INTEROBSERVER VARIABILITY AND CLINICAL OUTCOME

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ABSTRACT

Introduction: Renal involvement is very common in antineutrophil cytoplasmic autoantibody (ANCA) associated vasculitis (AAV). Therefore, a histopathological classification of ANCA-associated glomerulonephritis was developed, which consisted of the classes: focal, crescentic, mixed, and sclerotic. This classification was associated with renal outcome. Subsequent validation studies showed contradiction regarding the crescentic and mixed classes and the association with renal outcome. Here, we present a worldwide validation study and also analysed interobserver variability.

Methods: We included 145 patients from 10 centers worldwide with at least five glomeruli in their diagnostic renal biopsy. Seven pathologists scored renal biopsies of patients to determine the histopathological classification and to evaluate tubulointerstitial parameters. In addition, clinical data was collected. The primary outcome of the study was renal function at 5 years ($eGFR_5$). Interobserver variability was a secondary outcome.

Results: Renal function at baseline and during follow-up were most favorable in the focal class and worst in the sclerotic class, consistent with primary findings. However, there was no difference between crescentic and mixed class regarding renal function at baseline and during follow-up. A multivariate analysis showed that the best model for predicting $eGFR_5$ included $eGFR_0$, having sclerotic class, age, $proteinuria_0$, and interstitial fibrosis and tubular atrophy. There was a moderate agreement between the pathologists in classifying the diagnostic renal biopsies; kappa (κ) 0.56.

Conclusions: Our study showed a difference between focal and sclerotic class regarding renal outcome, but no difference between crescentic and mixed class. These findings show that the histopathologic classification of AAGN is a valuable tool in the management of patients with AAV, but needs further improvement to distinguish biopsies with crescentic and mixed features better.

INTRODUCTION

The most common primary systemic small-vessel vasculitis in adults is antineutrophil cytoplasmic autoantibody (ANCA) associated vasculitis (AAV).[1] AAV includes granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome). There is also a limited disease variant called renal limited vasculitis (RLV), which only affects the kidneys. In GPA around 20 percent of the patients have glomerulonephritis at presentation.[2] ANCA-associated glomerulonephritis (AAGN) occurs in approximately 80 to 90 percent of patients with GPA and MPA during the disease course.[1;2] Renal involvement in EGPA is less frequent and less severe.[3] Renal involvement in AAV has an important impact on morbidity and mortality.[4-8] AAGN can rapidly progress to renal failure, especially if treated inappropriately. Currently, 20 to 40% of patients with AAV develop end stage renal disease (ESRD).[9-11]

The gold standard for establishing a diagnosis of AAGN is a renal biopsy. Light microscopy shows necrotizing and crescentic glomerulonephritis accompanied by a pauci-immune pattern, i.e. a negative or subdued granular pattern for immunoglobulins and complement.[12;13] Electron microscopy shows degranulation of neutrophils and subendothelial edema due to endothelial injury with few or no immune deposits.[14] The amount of acute and chronic lesions in the renal biopsy may vary considerably from patient to patient. Also, patients' outcome varies considerably.[15;16] Several clinicopathologic studies showed substantial prognostic value of specific pathologic lesions –or the absence thereof– in renal biopsies for renal outcome in AAV.

Frequently described associations in the literature are: 1) percentage of normal glomeruli and favorable renal outcome, 2) percentage of sclerotic glomeruli and adverse renal outcome.[16-19] Moreover, the presence of cellular crescents, which indicates active disease, was associated with the probability of renal recovery with immunosuppressive therapy.[16;18] These findings led to the introduction of a histopathological classification of AAGN in 2010.[20] The classification is based on four categories named focal, crescentic, sclerotic and mixed class. Depending on the predominant glomerular phenotype, each renal biopsy with AAGN can be classified into one of these classes. The classification system was shown to be associated with ESRD and renal function at 1- and 5-year follow-up in the original study consisting of 100 patients.[20]

Since 2010, several validations studies in adult and pediatric cohorts worldwide have been published.[21-43] All these studies show that the focal class has the best renal

outcome, while the sclerotic class has the worst outcome.[25] Contradiction exists regarding the crescentic and mixed classes; in some studies, renal function was better in the crescentic class, whereas in others renal outcome was significantly better in the mixed class, and in addition some did not show a difference.[25] Because of this contradiction, a large international validation study consisting of a worldwide cohort was called for. We here present a worldwide validation study, driven by the original investigators, including a collaboration between 10 centers worldwide. In this study, we also analysed interobserver variability to investigate to what extent this could play a role in the hitherto described discrepancies.

METHODS

Study cohort

Patients from 10 centers worldwide were included. Inclusion criteria were: histopathologically proven AAGN, available diagnostic renal biopsy with at least five glomeruli and a follow-up of at least 3 years (including patients that developed ESRD or died within the first 3 years). Exclusion criteria were: age under 18 years, overlap syndrome (e.g., AAGN combined with anti-glomerular basement membrane disease), and participation in a previous validation study. This study was conducted in accordance with the 1964 Declaration of Helsinki and subsequent amendments.

Diagnostic renal biopsies

Biopsy slides were collected at Leiden University Medical Center. All biopsies with at least five glomeruli were considered sufficient for this study, based on recent findings that biopsies containing three to nine glomeruli were also valid for the prognostic capability of the classification.[22] The biopsies were scanned with the Ultra-Fast Scanner (UFS) at a magnification of 40x. The scanned biopsy were uploaded on a highly secured website, where they were accessible only for a group of seven pathologists (FF, KJ, YO, SW, LHN, JAB and IMB). These pathologists, blinded to the clinical data, scored the biopsies independently. The scoring form (S Document 1 in the supplementary material) was a modified version of the original scoring form for AAGN that was published in 1996.[44] In short, the scoring consisted of the histopathological class, inflammatory infiltrates, interstitial fibrosis and tubular atrophy (IFTA), and tubulitis. Each case was scored by two pathologists. In case of disagreement between these two pathologists, a third pathologist (IMB or JAB) made the final decision on the case. For analytic purposes, tubulointerstitial scores from two pathologists were averaged and categorized as: inflammatory infiltrate in <25% or ≥25% of unscarred parenchyma; IFTA in <25% or ≥25% of cortical area; and tubulitis foci with <5 or ≥5 cells/tubular cross section.

Clinical data

For each patient we retrieved the following data from the clinical records at participating centers: patient demographics, diagnosis (GPA, MPA, EGPA, or RLV), serum and urine laboratory values, and details on induction and maintenance therapy. Renal function was expressed as eGFR, calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, adjusted for race/ethnicity (Caucasian/Asian or other).[45-47] eGFR was calculated at the time of biopsy ($eGFR_0$) and at 1- and 5-year follow-up ($eGFR_1$ and $eGFR_5$, respectively). The eGFR calculation was omitted, when patients reached ESRD; in that case, eGFR was considered 0 for analytic purposes.

Outcomes

The primary outcome of the study was renal function at 5 years ($eGFR_5$). Secondary outcomes were: renal function at 1 year ($eGFR_1$), renal relapse, ESRD, death and interobserver variability. A renal relapse was defined as a rise in serum creatinine of >30% or a fall in estimated glomerular filtration rate >25% and/or new hematuria or proteinuria (all attributable to active vasculitis), as indicated by the Birmingham Vasculitis Activity Score.[48-50] ESRD was defined as a need for renal replacement therapy (dialysis for at least three months or transplantation) or as an eGFR value below 15 mL/min that persisted for at least three months. Renal survival was expressed as the time between diagnosis and ESRD.

Statistical analyses

Continuous variables were expressed as mean±SD. Categorical variables were expressed as numbers (%). Numerical data of groups were compared with the student's t-test or one-way analysis of variance. Categorical data of groups were compared with Fisher's exact test or the chi-square test. Renal survival was analyzed with the Kaplan Meier survival analysis and log-rank test. Pearson's correlation coefficients were calculated to identify variables correlating with $eGFR_5$. Stepwise multiple linear regression analysis was performed to find the best model for predicting $eGFR_5$. Interobserver agreement was investigated by calculating the κ for the histopathological classification, and the ICC for tubulointerstitial parameters. Values of κ or ICC were interpreted as following: >0.75, excellent agreement; 0.40-0.75, fair to good agreement; and <0.40, poor agreement.[51;52] All analyses were performed with SPSS version 23 (IBM Corp., Armonk, NY, USA). *P* values <0.05 were considered significant.

RESULTS

Patient characteristics

Histopathological and clinical data of 157 patients were collected. Twelve cases were excluded, because of missing clinical data or an insufficient number of glomeruli in the biopsy (i.e. less than five glomeruli). The baseline characteristics of the 145 included patients, all diagnosed between 1991 and 2011, are shown in Table 1.

Table 1 | Characteristics of the total cohort

	Total (*n*=145)
Age at biopsy, year, mean±SD	61.2±12.7
Male (%)	83 (57.2)
Diagnosis (%)[a]	
GPA	63 (45.0)
MPA	71 (50.7)
EGPA	2 (1.4)
RLV	4 (2.9)
Diagnostic delay, months, mean±SD	3.1±9.0
ANCA specificity (%)[b]	
PR3	50 (37.0)
MPO	73 (54.1)
Negative	6 (4.4)
Double positive	6 (4.4)
Center	
Cochin Hospital, Paris	6 (4.1)
General University Hospital in Prague	38 (26.2)
Leiden University Medical Center, Leiden	36 (24.8)
Medical University of Innsbruck	7 (4.8)
Medical University of Vienna	9 (6.2)
Necker Hospital, Paris	4 (2.8)
Rigshospitalet Copenhagen	7 (4.8)
Teinekeijnkai Hospital Sapporo	7 (4.8)
JCHO Sendai Hospital, Sendai	24 (16.6)
Weill Cornell Medical College New York	7 (4.8)

ANCA, antineutrophil cytoplasmic autoantibody; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase-3; RLV, renal-limited vasculitis; SD, standard deviation.
[a] The AAGN diagnosis was not further specified in 5 patients.
[b] ELISA test results were available in 135 patients.

Histopathological classes and clinical parameters

Of the diagnostic biopsies, 52 (35.9%) were focal class, 37 (25.5%) crescentic class, 39 (26.9%) mixed class, and 17 (11.7%) were sclerotic class (Table 2). GPA predominated in the focal class, while MPA predominated in the mixed class. There was no difference between the crescentic and sclerotic class regarding the diagnosis. Although there was no significant difference in the prevalence of myeloperoxidase (MPO-)ANCA and proteinase-3 (PR3-)ANCA specificity among the classes, the crescentic and mixed class showed a predominance of MPO-ANCA, while the focal class showed a slight predominance of PR3-ANCA. MPO- and PR3-ANCA were found in equal numbers in the sclerotic class.

Histopathological classes and renal function

Focal class had the highest eGFR at baseline (Table 2). After one and five year follow-up, focal class still had the highest eGFR. Sclerotic class had the lowest eGFR at baseline and during follow-up. Renal functions at baseline and during follow-up were not significantly different between the crescentic and mixed classes. Proteinuria at the time of the biopsy was lowest in the focal class, and comparable between the crescentic, mixed, and sclerotic class.

Table 2 | Patient characteristics according to histopathological class

	Focal (*n*=52)	Crescentic (*n*=37)	Mixed (*n*=39)	Sclerotic (*n*=17)	P - value
Age at biopsy, year, mean±SD	59.7±12.6	61.6±11.4	60.6±14.4	65.9±11.7	0.37
Male (%)	33 (63.5)	24 (64.9)	18 (46.2)	8 (47.1)	0.22
Diagnosis (%)[a]					0.005
GPA	33 (63.5)	14 (40.0)	8 (21.6)	8 (50.0)	
MPA	17 (32.7)	19 (54.3)	27 (73.0)	8 (50.0)	
EGPA	1 (1.9)	0 (0.0)	1 (2.7)	0 (0.0)	
RLV	1 (1.9)	2 (5.7)	1 (2.7)	0 (0.0)	
ANCA specificity (%)[b]					0.13
PR3	23 (47.9)	13 (37.1)	7 (19.4)	7 (43.8)	
MPO	19 (39.6)	21 (60.0)	25 (69.4)	8 (50.0)	
Negative	3 (6.3)	0 (0.0)	3 (8.3)	0 (0.0)	
Double positive	3 (6.3)	1 (2.9)	1 (2.8)	1 (6.3)	
Diagnostic delay, months, mean±SD	4.7±12.9	1.4±2.7	2.4±3.2	3.6±11.5	0.39
$eGFR_0$, ml/min/1.73 m^2, mean±SD	49.9±29.3	18.0±15.7	26.7±18.6	19.4±11.8	<0.001
Proteinuria class at biopsy (%)[c]					0.005
Normal	4 (8.7)	1 (3.1)	2 (5.3)	0 (0.0)	
Moderately increased	18 (39.1)	4 (12.5)	3 (7.9)	3 (18.8)	
Severely increased	24 (52.2)	27 (84.4)	33 (86.8)	13 (81.3)	
$eGFR_1$, ml/min/1.73 m^2, mean±SD	61.4±23.9	37.3±20.6	37.7±21.1	20.3±16.0	<0.001
$eGFR_5$, ml/min/1.73 m^2, mean±SD	59.7±21.1	34.9±20.4	37.3±23.7	19.3±20.0	<0.001
Renal relapse during follow-up (%)	20 (40.0)	14 (40.0)	11 (28.9)	3 (18.8)	0.33
ESRD during follow-up (%)	1 (1.9)	9 (24.3)	6 (15.4)	8 (47.1)	<0.001
Death during follow-up (%)	15 (28.8)	15 (40.5)	9 (23.1)	6 (35.3)	0.40

ANCA, antineutrophil cytoplasmic autoantibody; $eGFR_{0/1/5}$, estimated glomerular filtration rate at baseline, at 1 year and at 5 years respectively; EGPA, eosinophilic granulomatosis with polyangiitis; ESRD, end-stage renal disease; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase-3; RLV, renal-limited vasculitis; SD, standard deviation.

[a] The AAGN diagnosis was not further specified in 5 patients.

[b] ELISA test results were available in 135 patients.

[c] In accordance with the Kidney Disease: Improving Global Outcomes (KDIGO) clinical guidelines, normal level of proteinuria was defined as protein excretion of <0.15 g/day, or as a negative protein dipstick test; moderately increased proteinuria was defined as a protein excretion rate of 0.15–0.50 g/day, or as trace on protein dipstick test; severely increased proteinuria was defined as total protein excretion >0.50 g/day, or as + or more on protein dipstick. The proteinuria class could be determined in 132 patients.

Predictors of renal function in time

Variables significantly associated with $eGFR_5$ were: age, clinical diagnosis (MPA or GPA), ANCA serology (MPO-ANCA or PR3-ANCA), $eGFR_0$, the amount of proteinuria at time of biopsy, the histopathological classification, the extent of interstitial infiltrate, the amount of interstitial fibrosis and tubular atrophy (IFTA), and the amount of tubulitis. When performing a stepwise multivariate regression analysis, the best predicting model for $eGFR_5$ included $eGFR_0$, having sclerotic class, age, $proteinuria_0$, and IFTA (Table 3).

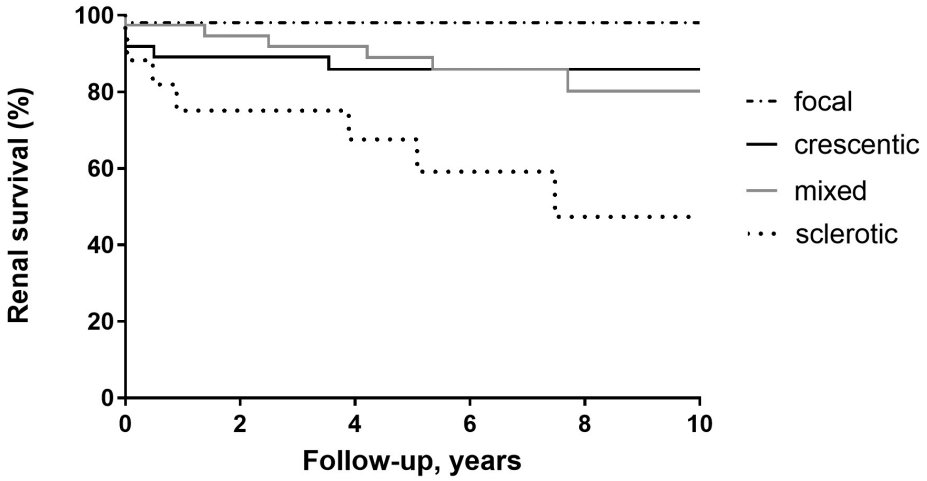
Table 3 | Multivariate prediction models of $eGFR_5$

Variable	Model 1 (R^2 = 0.43)		Model 2 (R^2 = 0.48)		Model 3 (R^2 = 0.50)		Model 4 (R^2 = 0.55)		Model 5 (R^2 = 0.56)	
	β	P-value	β	P-value	β	P-value	β	P-value	β	P-value
$eGFR_0$	0.65	<0.001	0.61	<0.001	0.56	<0.001	0.49	<0.001	0.41	<0.001
Histopathological classification[a] Sclerotic			-0.23	0.001	-0.22	0.002	-0.22	0.002	-0.17	0.02
Age					-0.15	0.04	-0.22	0.004	-0.24	0.002
$Proteinuria_0$							-0.27	0.001	-0.27	<0.001
IFTA category									-0.21	0.006

$eGFR_{0/5}$, estimated glomerular filtration rate at baseline and at 5 years respectively; IFTA, interstitial fibrosis and tubular atrophy.
[a] Focal class as reference.

Renal relapse and end-stage renal disease

During a mean follow-up duration of 8.0±5.4 years, 48 (33.1%) patients experienced at least one renal relapse. There was no difference between crescentic and mixed class regarding the number of patients who experienced a renal relapse (P=0.34; S Table 1 in the supplementary material). Twenty-four (16.6%) patients developed ESRD. At 10 years follow-up renal survival was significantly different between the four histopathological classes, but not between crescentic and mixed class (Figure 1). A total of 45 (31.0%) patients died. When combining death and/or ESRD within 10 years as one outcome, no difference was seen between crescentic and mixed class (S Figure 1 in the supplementary material).



N at risk						
Focal	52	49	43	27	17	17
Crescentic	37	29	27	14	10	7
Mixed	39	34	32	23	14	13
Sclerotic	17	11	9	7	3	2

Figure 1 | Renal survival according to histopathological class.
At 10-year follow-up, renal survival was different between the four classes (P<0.001), but not between crescentic and mixed class (P=0.98).

Treatment

The majority of patients received corticosteroids and cyclophosphamide as induction therapy (106 [74.1%] patients; S Table 2 in the supplementary material). Patients receiving corticosteroids and cyclophosphamide had a similar 10-year renal survival rate compared to patients receiving other treatment regimens as induction therapy (P=0.17). Maintenance therapy consisted for most patients of corticosteroids together with azathioprine or mycophenolate mofetil (83 [61.5%] patients). Ten-year renal survival rates did not differ between patients with no or minimal maintenance therapy (only corticosteroids) compared to patients with maintenance therapy consisting of multiple immunosuppressive drugs (P=0.37). Plasma exchange was more frequently used in patients with crescentic class compared to the other classes (24.3% versus 10.4%, P=0.04; S Table 2 in the supplementary material). Within the crescentic class, renal survival at 10 years did not differ between patients that received and patients that did not receive plasma exchange therapy (P=0.34).

Interobserver agreement

Agreement on the histopathological class between the two pathologists was observed in 99 (68.3%) cases; kappa (κ) 0.56 (Figure 2). This agreement rate corresponds to moderate agreement. In seven cases there was complete disagreement between three pathologists. We discovered three possible reasons for disagreement when re-evaluating the cases that lacked agreement (*n*=46); technical (e.g., differences between histological stains), interpretative (e.g., different interpretations of the definitions) and errors (e.g., miscalculations and incomplete scorings). The intraclass correlation coefficient (ICC) between two pathologists was 0.57 for interstitial infiltrate, 0.46 for IFTA, and 0.36 for tubulitis.

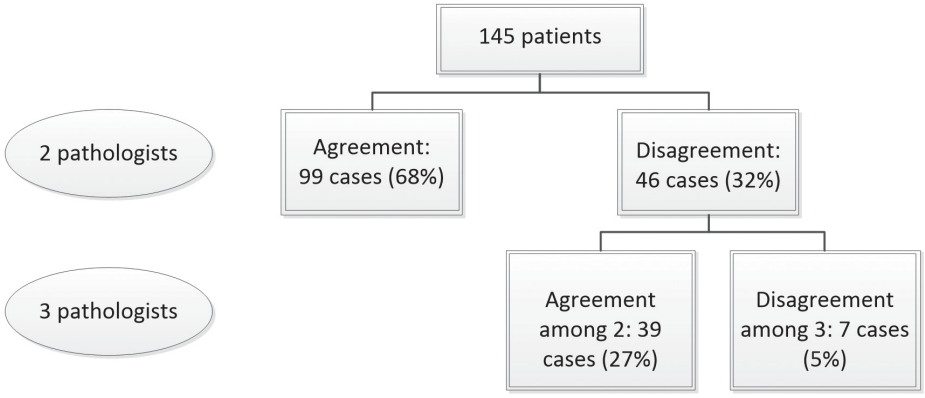


Figure 2 | Interobserver agreement on histopathological class.

DISCUSSION

Our worldwide multicenter study on the histopathological classification of AAGN showed a favorable outcome in the focal class and a poor outcome in the sclerotic class, which is in line with our original study and previous validation studies. There was no difference between renal outcome in the crescentic and mixed class, which is in contrast to findings of the original study, but it is in line with results from a recent meta-analysis.[25]

Overall, our data show that the histopathological classification is associated with renal function and the development of ESRD during follow-up. This association with $eGFR_5$ persists after correcting for other baseline values. However, the crescentic and mixed class were indiscriminate regarding renal function and developing ESRD. Although these findings suggest that a revision of the original classification is called for, we are reluctant to lump the crescentic and mixed class, in particular because

other studies showed that cellular crescents are an important factor for predicting potential reversibility of renal impairment during follow-up.[16;18] This kind of information can be used for therapeutic purposes and tailoring treatment for individual patients. An additional factor not yet incorporated in the current classification, but which have shown some predictive value for long-term renal outcome, are fibrous crescents.[15] Currently it is investigated whether these can be of additive value for predicting renal outcome in the current classification.

There are four possible explanations for the conflicting results regarding crescentic and mixed class: differences in patient populations, differences in treatment, moderate interobserver agreement and insufficiency of the histopathologic classification. Epidemiologic studies showed that there is a difference in distribution of GPA/MPA/EGPA and ANCA-specificity around the globe.[53;54] Since there is an association between the histopathological class and diagnosis, and both variables are associated with renal outcome as shown above, differences in patient population could partly be an explanation for the conflicting results. Unfortunately, our cohort was not large enough to analyze this hypothesis. Regarding treatment, our study showed that patients with crescentic class received plasma exchange more frequently than patients in the other classes. However, a subanalysis showed that the use of plasma exchange did not affect renal survival within the crescentic class. This is an interesting finding, because crescentic class is considered an active and potentially reversible disease state, which could be reversed with the right treatment.[16;18;20] Our study is insufficient for firm conclusions on whether the therapy must be considered in addition to the histopathological classification for predicting outcome. A third explanation is interobserver agreement which was moderate in this study. In addition, the scoring was performed by seven experienced nephropathologists, and therefore we cannot translate this level of agreement to the clinical practice in a one-on-one fashion. This agreement level could influence the observed contradiction in the previous studies.

A fourth explanation is insufficiency of the histopathologic classification. The current histopathologic classification of AAGN is only based on glomerular lesions, not taking tubulointerstitial parameters into account, because these did not have an additional predictive value in the original study and made the classification only more complicated.[20] The importance of tubulointerstitial parameters for renal outcome in AAGN, even when used in addition to the histopathologic classification of AAGN, has been shown in other studies.[15;16;18;29;36;40;42;55-58] In our study, the extents of interstitial infiltrate, IFTA, and tubulitis were significantly associated with $eGFR_5$ in the univariate analysis. In the multivariate analysis, only IFTA remained significantly associated

with $eGFR_5$. These data suggest that including tubulointerstitial parameters in the classification system will lead to refinements for the prognostication of patients at time of diagnosis, although the poor to moderate interobserver agreement regarding tubulointerstitial variables must also be considered.

The international patient cohort from three different continents was a strength of this study. Only one previous validation study included patients from more than two countries.[40] In addition, our study included a large group of different pathologists for scoring the renal biopsies, which made it possible to investigate interobserver agreement. Our study also has some limitations. Due to the international character there was a wide variety of therapeutic regimens and the study had a retrospective design. Therefore, there was some missing data; but less than 7% of data were missing on renal function, diagnosis, serology, and therapy.

In conclusion, our study showed a significant difference between focal and sclerotic class regarding renal outcome, but no difference between crescentic and mixed class. This last observation was also described by a number of previous studies. These findings show that the histopathologic classification of AAGN is a valuable tool in the management of patients with AAV, but in the near future, adjustments are needed to improve its prognostic value, especially for the crescentic and mixed class. Currently, we are performing a study to evaluate a more detailed scoring system for both glomerular and interstitial variables. Results from that study will determine how to adjust the histopathological classification for AAGN for more sophisticated prognostic value.

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DISCLOSURES

None to declare.

REFERENCES

1. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med,* 1997;337: 1512-1523.
2. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med,* 1992;116: 488-498.
3. Masi AT, Hunder GG, Lie JT, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum,* 1990;33: 1094-1100.
4. Bligny D, Mahr A, Toumelin PL, Mouthon L, Guillevin L. Predicting mortality in systemic Wegener's granulomatosis: a survival analysis based on 93 patients. *Arthritis Rheum,* 2004;51: 83-91.
5. Bourgarit A, Le TP, Pagnoux C, et al. Deaths occurring during the first year after treatment onset for polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: a retrospective analysis of causes and factors predictive of mortality based on 595 patients. *Medicine (Baltimore),* 2005;84: 323-330.
6. Luqmani RA, Bacon PA, Beaman M, et al. Classical versus non-renal Wegener's granulomatosis. *Q J Med,* 1994;87: 161-167.
7. Mukhtyar C, Flossmann O, Hellmich B, et al. Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force. *Ann Rheum Dis*, 2008;67: 1004-1010.
8. Reinhold-Keller E, Beuge N, Latza U, et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. *Arthritis Rheum,* 2000;43: 1021-1032.
9. Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis,* 2003;41: 776-784.
10. de Joode AA, Sanders JS, Stegeman CA. Renal survival in proteinase 3 and myeloperoxidase ANCA-associated systemic vasculitis. *Clin J Am Soc Nephrol,* 2013;8: 1709-1717.
11. Little MA, Nazar L, Farrington K. Outcome in glomerulonephritis due to systemic small vessel vasculitis: effect of functional status and non-vasculitic co-morbidity. *Nephrol Dial Transplant,* 2004;19: 356-364
12. Jennette JC, Wilkman AS, Falk RJ. Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol,* 1989;135: 921-930.
13. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*, 2013;65: 1-11.
14. Joh K, Muso E, Shigematsu H, et al. Renal pathology of ANCA-related vasculitis: proposal for standardization of pathological diagnosis in Japan. *Clin Exp Nephrol,* 2008;12: 277-291.
15. de Lind van Wijngaarden RAF, Hauer HA, Wolterbeek R, et al. Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: a prospective analysis of 100 patients with severe renal involvement. *J Am Soc Nephrol*, 2006;17: 2264-2274.
16. Hauer HA, Bajema IM, Van Houwelingen HC, et al. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int*, 2002;62: 1732-1742.

17. Aasarod K, Bostad L, Hammerstrom J, Jorstad S, Iversen BM. Renal histopathology and clinical course in 94 patients with Wegener's granulomatosis. *Nephrol Dial Transplant*, 2001;16: 953-960.
18. Bajema IM, Hagen EC, Hermans J, et al. Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int,* 1999;56: 1751-1758.
19. Haroun MK, Stone JH, Nair R, Racusen L, Hellmann DB, Eustace JA. Correlation of percentage of normal glomeruli with renal outcome in Wegener's granulomatosis. *Am J Nephrol,* 2002;22: 497-503.
20. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol,* 2010;21: 1628-1636.
21. Andreiana I, Stancu S, Avram A, Taran L, Mircescu G. ANCA positive crescentic glomerulonephritis outcome in a Central East European cohort: a retrospective study. *BMC Nephrol,* 2015;16: 90.
22. Bjorneklett R, Sriskandarajah S, Bostad L. Prognostic Value of Histologic Classification of ANCA-Associated Glomerulonephritis. *Clin J Am Soc Nephrol,* 2016;11: 2159-2167.
23. Chang DY, Wu LH, Liu G, Chen M, Kallenberg CG, Zhao MH. Re-evaluation of the histopathologic classification of ANCA-associated glomerulonephritis: a study of 121 patients in a single center. *Nephrol Dial Transplant,* 2012;27: 2343-2349.
24. Chen Y, Bao H, Liu Z, et al. Risk Factors for Renal Survival in Chinese Patients with Myeloperoxidase-ANCA-Associated GN. *Clin J Am Soc Nephrol,* 2017;12: 417-425.
25. Chen YX, Xu J, Pan XX, et al. Histopathological Classification and Renal Outcome in Patients with Antineutrophil Cytoplasmic Antibodies-associated Renal Vasculitis: A Study of 186 Patients and Metaanalysis. *J Rheumatol,* 2017;44: 304-313.
26. Cordova-Sanchez BM, Mejia-Vilet JM, Morales-Buenrostro LE, Loyola-Rodriguez G, Uribe-Uribe NO, Correa-Rotter R. Clinical presentation and outcome prediction of clinical, serological, and histopathological classification schemes in ANCA-associated vasculitis with renal involvement. *Clin Rheumatol,* 2016;35:1805-1816.
27. Diaz-Crespo F, Villacorta J, Acevedo M, et al. The predictive value of kidney biopsy in renal vasculitis: a multicenter cohort study. *Hum Pathol,* 2016;52: 119-127.
28. Ellis CL, Manno RL, Havill JP, Racusen LC, Geetha D. Validation of the new classification of pauci-immune glomerulonephritis in a United States cohort and its correlation with renal outcome. *BMC Nephrol,* 2013;14: 210.
29. Ford SL, Polkinghorne KR, Longano A, et al. Histopathologic and clinical predictors of kidney outcomes in ANCA-associated vasculitis. *Am J Kidney Dis,* 2014;63: 227-235.
30. Hilhorst M, Wilde B, van Breda Vriesman P, van Paassen P, Cohen Tervaert JW. Estimating renal survival using the ANCA-associated GN classification. *J Am Soc Nephrol,* 2013;24: 1371-1375.
31. Iwakiri T, Fujimoto S, Kitagawa K, et al. Validation of a newly proposed histopathological classification in Japanese patients with anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. *BMC Nephrol,* 2013;14: 125.
32. Khalighi MA, Wang S, Henriksen KJ, et al. Pauci-immune glomerulonephritis in children: a clinicopathologic study of 21 patients. *Pediatr Nephrol,* 2015;30: 953-959.
33. Kristensen T, Gregersen JW, Krag SR, Ivarsen P. The relation between histopathological classification and renal outcome, ANCA subtype and treatment regimens in ANCA-associated vasculitis. *Clin Exp Rheumatol,* 2016;34: S105-S110.

34. Li X, Liang S, Zheng C, et al. Clinicopathological characteristics and outcomes of pediatric patients with systemic small blood vessel vasculitis. *Pediatr Nephrol,* 2014;29: 2365-2371.
35. Moroni G, Binda V, Leoni A, et al. Predictors of renal survival in ANCA-associated vasculitis. Validation of a histopatological classification schema and review of the literature. *Clin Exp Rheumatol,* 2015;33:S-63.
36. Muso E, Endo T, Itabashi M, et al. Evaluation of the newly proposed simplified histological classification in Japanese cohorts of myeloperoxidase-anti-neutrophil cytoplasmic antibody-associated glomerulonephritis in comparison with other Asian and European cohorts. *Clin Exp Nephrol,* 2013;17: 659-662.
37. Naidu GS, Sharma A, Nada R, et al. Histopathological classification of pauci-immune glomerulonephritis and its impact on outcome. *Rheumatol Int,* 2014;34: 1721-1727.
38. Nohr E, Girard L, James M, Benediktsson H. Validation of a histopathologic classification scheme for antineutrophil cytoplasmic antibody-associated glomerulonephritis. *Hum Pathol,* 2014;45: 1423-1429.
39. Noone DG, Twilt M, Hayes WN, et al. The new histopathologic classification of ANCA-associated GN and its association with renal outcomes in childhood. *Clin J Am Soc Nephrol,* 2014;9: 1684-1691.
40. Quintana LF, Perez NS, De Sousa E, et al. ANCA serotype and histopathological classification for the prediction of renal outcome in ANCA-associated glomerulonephritis. *Nephrol Dial Transplant,* 2014;29: 1764-1769.
41. Sacri AS, Chambaraud T, Ranchin B, et al. Clinical characteristics and outcomes of childhood-onset ANCA-associated vasculitis: a French nationwide study. *Nephrol Dial Transplant,* 2015;30 Suppl 1: i104-i112.
42. Tanna A, Guarino L, Tam FW, et al. Long-term outcome of anti-neutrophil cytoplasm antibody-associated glomerulonephritis: evaluation of the international histological classification and other prognostic factors. *Nephrol Dial Transplant,* 2015;30: 1185-1192.
43. Togashi M, Komatsuda A, Nara M, et al. Validation of the 2010 histopathological classification of ANCA-associated glomerulonephritis in a Japanese single-center cohort. *Mod Rheumatol,* 2014;24: 300-303.
44. Bajema IM, Hagen EC, Hansen BE, et al. The renal histopathology in systemic vasculitis: an international survey study of inter- and intra-observer agreement. *Nephrol Dial Transplant,* 1996;11: 1989-1995.
45. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med,* 2009;150: 604-612.
46. Matsushita K, Mahmoodi BK, Woodward M, et al. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA,* 2012;307: 1941-1951.
47. Teo BW, Xu H, Wang D, et al. GFR estimating equations in a multiethnic Asian population. *Am J Kidney Dis,* 2011;58: 56-63.
48. Luqmani RA, Bacon PA, Moots RJ, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM,* 1994;87: 671-678.
49. Luqmani RA, Exley AR, Kitas GD, Bacon PA. Disease assessment and management of the vasculitides. *Baillieres Clin Rheumatol,* 1997;11: 423-446.
50. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis,* 2009;68: 1827-1832.

51. Fleiss JL, Cohen J. The Equivalence of Weighted Kappa and the Intraclass Correlation Coefficient as Measures of Reliability [Abstract]. *Educational and Psychological Measurement*,1973;33: 613-619.
52. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics,* 1977;33: 159-174.
53. Watts RA, Lane SE, Scott DG, et al. Epidemiology of vasculitis in Europe. *Ann Rheum Dis,* 2001;60: 1156-1157.
54. Watts RA, Scott DG, Jayne DR, et al. Renal vasculitis in Japan and the UK--are there differences in epidemiology and clinical phenotype? *Nephrol Dial Transplant*, 2008;23: 3928-3931.
55. Berden AE, Jones RB, Erasmus DD, et al. Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. *J Am Soc Nephrol,* 2012;23: 313-321.
56. Hogan SL, Nachman PH, Wilkman AS, Jennette JC, Falk RJ, Glomerular Disease Collaborative Network. Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol*, 1996;7: 23-32.
57. Levi C, Meas-Yedid V, Daniliuc C, et al. Computerized Instersitial Fibrosis Is the Most Powerful Histological Predictor of Renal Outcome in ANCA-Associated Vasculitis [Abstract]. *J Am Soc Nephrol,* 2012: 710A-711A.
58. Muso E, Endo T, Yumura W, Joh K. Need of Interstitial Fibrosis Parameter on the Newly Proposed Simplified Glomerular Histological Classification to Predict the Longterm Outcome in Japanese Cohort of MPO-ANCA Associated RPGN [Abstract]. *J Am Soc Nephrol,* 2012: 532A.

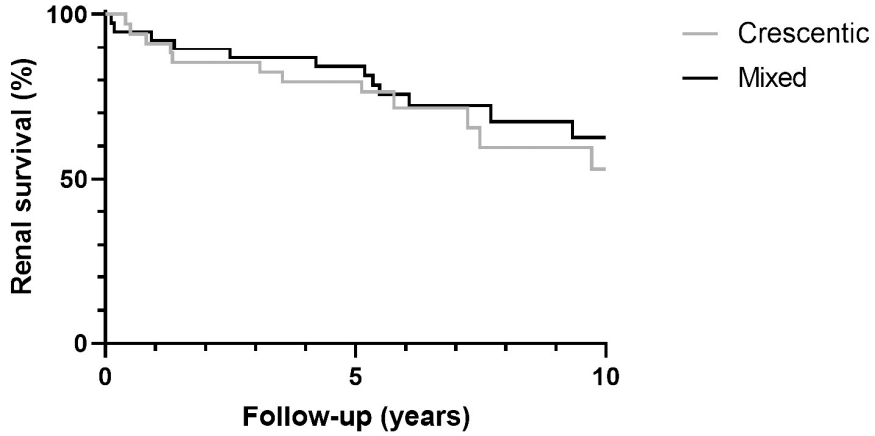
SUPPLEMENTARY MATERIAL

Histopathological classification of ANCA-associated glomerulonephritis: interobserver variability and clinical outcome

S Table 1 | Subanalyses for outcomes in the crescentic and mixed classes

	Crescentic (*n*=37)	Mixed (*n*=39)	P-value
Follow-up, yrs	7.3 ± 5.2	8.3 ± 5.3	0.41
Renal relapses	14 (40.0)	11 (28.9)	0.34
ESRD within 10 yrs	9 (24.3)	6 (15.4)	0.40
Time to ESRD, yrs	6.2 ± 6.7	3.5 ± 2.8	0.31
Death	15 (40.5)	9 (23.1)	0.14
Time to death, yrs	6.6 ± 5.5	4.6 ± 4.5	0.39
Death within 10 yrs	11 (29.7)	7 (17.9)	0.29

Data are presented as mean ± SD or as number (percentage).
ESRD, end-stage renal disease



S Figure 1 | Combined outcome of ESRD/death in the crescentic and mixed classes over time.
P-value (log-rank) = 0.57

S Table 2 | Treatment according to histopathological class

Induction therapy	Total (*n*=143)[a]	Focal class (*n*=51)	Crescentic class (*n*=37)	Mixed class (*n*=39)	Sclerotic class (*n*=16)
Plasma exchange	20 (14.0)	5 (9.8)	9 (24.3)	4 (10.3)	2 (12.5)
Corticosteroids only	19 (13.3)	4 (7.8)	4 (10.8)	6 (15.4)	5 (31.3)
Corticosteroids and cyclophosphamide	106 (74.1)	43 (84.3)	30 (81.1)	23 (59.0)	10 (62.5)
Corticosteroids and azathioprine or MMF	8 (5.6)	2 (3.9)	1 (2.7)	4 (10.3)	1 (6.3)
Corticosteroids and mizoribine	5 (3.5)	1 (2.0)	0 (0.0)	4 (10.3)	0 (0.0)
Corticosteroids and rituximab[b]	5 (3.5)	1 (2.0)	2 (5.4)	2 (5.2)	0 (0.0)

Maintenance therapy	Total (*n*=136)[c]	Focal class (*n*=49)	Crescentic class (*n*=35)	Mixed class (*n*=36)	Sclerotic class (*n*=16)
Initially none	5 (3.7)	2 (4.1)	1 (2.9)	2 (5.6)	0 (0.0)
Corticosteroids only	27 (19.9)	8 (16.3)	4 (11.4)	7 (19.4)	8 (50.0)
Corticosteroids and cyclophosphamide	8 (5.9)	5 (10.2)	1 (2.9)	2 (5.6)	0 (0.0)
Corticosteroids and azathioprine or MMF	83 (61.0)	29 (59.2)	25 (71.4)	21 (58.3)	8 (50.0)
Azathioprine or MMF	5 (3.7)	3 (6.1)	2 (5.7)	0 (0.0)	0 (0.0)
Corticosteroids and mizoribine	8 (5.9)	2 (4.1)	2 (5.7)	4 (11.1)	0 (0.0)

MMF, mycophenolate mofetil.

[a] Data on induction therapy was missing in 2 patients.

[b] One of these patients also received 2 doses of intravenous cyclophosphamide.

[c] Data on maintenance therapy was available in 142 patients. Six patients did not receive maintenance therapy due to death or dialysis dependency.

S Document 1 | Scoring questionnaire

Overall

1 Total number of glomeruli:

2 AAGN class

a) Focal

b) Crescentic

c) Mixed

d) Sclerotic

Inflammatory infiltrate present in:

3 Infiltrates

a) <10% of unscarred parenchyma

b) 10 to 25% of unscarred parenchyma

c) 26 to 50% of unscarred parenchyma

d) >50% of unscarred parenchyma

4 Dominant cell type of infiltrate

a) Neutrophils

b) Mononuclear cells

c) Eosinophils

5 Interstitial fibrosis and tubular atrophy

a) No interstitial fibrosis and tubular atrophy

b) Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)

c) Moderate interstitial fibrosis and tubular atrophy (26-50% of cortical area)

d) Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)

6 Intra-epithelial infiltrate

a) No mononuclear cells in tubules

b) Foci with 1 to 4 cells/tubular cross section or 10 tubular cells

c) Foci with 5 to 10 cells/tubular cross section

d) Foci with >10 cells/tubular cross section

Vessels

7 Is vasculitis present in the small vessels (arterioles and/or arteries)?

a) Yes

b) No

8 Are large vessels present in the biopsy?

a) Yes (please answer question 9)

b) No (proceed to question 10)

9 Is vasculitis present in the large vessels?

a) Yes

b) No

Granulomas

10 Are granulomas present?

a) Yes

b) No

Conclusion

11 Do you have any comments?

5

THE DUTCH TRANSPLANTATION IN VASCULITIS (DUTRAVAS) STUDY: OUTCOME OF RENAL TRANSPLANTATION IN ANCA-ASSOCIATED GLOMERULONEPHRITIS

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ABSTRACT

Introduction: Data on the outcome of renal transplantation in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (AAGN) patients are still limited. In particular, how disease recurrence in the renal allograft defines graft outcome is largely unknown. Therefore, we conducted a multi-center observational clinical and histopathological study to establish recurrence rate of AAGN in the allograft, and the impact of recurrence on allograft survival.

Methods: Using the nationwide Dutch Pathology Registry (PALGA), we retrospectively collected clinical and histopathological data of consecutive AAGN patients who had developed end-stage renal failure and received a kidney allograft in one of six Dutch university hospitals between 1984 and 2011. Transplant biopsies were scored using the Banff '09 classification. Renal disease recurrence was scored using the histopathological classification of AAGN.

Results: The post-transplantation recurrence rate of AAGN was 2.8% per patient year, accumulating to recurrence in a total of 11 out of 110 AAGN patients within the first 5 years after transplantation. Four of these 11 patients lost their graft, with one-year and 5-year graft survival rates of 94.5% and 82.8%, respectively. By multivariate analysis, AAGN recurrence was independently associated with subsequent graft loss.

Conclusions: In this study in 110 Dutch patients, the recurrence rate of AAGN within 5 years after kidney transplantation appeared slightly higher than in previous reports. Moreover, recurrence of AAGN contributed independently to kidney allograft loss, emphasizing the importance of clinical vigilance, since early treatment might be critical to rescuing the allograft.

INTRODUCTION

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are the major subtypes of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Approximately 80% of patients with GPA and 90% of patients with MPA develop kidney involvement during the disease course.[1] ANCA-associated glomerulonephritis (AAGN) progresses to end-stage renal failure (ESRF) in approximately 20-40% of patients.[2-5] Data regarding the outcome of renal transplantation in AAGN patients are limited with only a few multi-center cohort studies. The studies vary widely with respect to graft survival and disease relapse rates; they reported 1-year graft survival rates of 86-100% and 5-year graft survival rates of 69-100%. In these studies, relapse rates ranged from 1.0-2.0% per patient year of follow-up.[6-10] National and international registry studies reported 1-, 3-, 5, and 10-year graft survival rates of 95%,[11] 70%,[12] 82-96%,[11, 13] and 80%,[14] respectively. These registry studies focused solely on graft survival and provided limited in-depth disease-specific data. Almost none of the published studies took into account histopathological findings. Only Little *et al.* commented briefly on vascular changes within the renal graft, but the complete renal transplant histopathology was not formally reviewed.[9]

In the current study, we investigated 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. We formally reviewed the complete renal transplant histopathology and assessed the impact of disease recurrence within the allograft on graft survival, since it is largely unknown how disease recurrence in the renal allograft affects graft outcome.

METHODS

Patients

The study cohort included 113 patients who were retrospectively recruited from six academic hospitals in the Netherlands using a nationwide search for AAGN patients who received a renal transplant. We used the Dutch Pathology Registry (PALGA) (www.palga.nl), a nationwide network and registry of histo- and cytopathology in the Netherlands, encompassing all pathology laboratories.[15] Only patients with one of the following diagnoses were included: GPA (formerly Wegener's granulomatosis), MPA, proteinase 3 (PR3-)AAV, myeloperoxidase (MPO-)AAV, AAV not further specified, renal limited vasculitis (RLV), systemic vasculitis, or pauci-immune rapidly progressive glomerulonephritis. Experienced, academically based nephrologists and nephropathologists verified the diagnosis of each patient by reviewing the medical and histology reports. The diagnosis was based on a clinical presentation compatible

with AAGN and substantiated by a positive ANCA serology and/or histology (light microscopy and immunofluorescence and/or electron microscopy). Because the PALGA search was limited to patients with a renal transplant sample, we performed an additional, center-specific search for transplanted AAGN patients. Nephrologists at the participating centers searched their hospital's clinical database for additional patients. In summary, 113 patients with 34 native kidney biopsies and 136 renal transplant samples were included in this study. Diagnostic definitions were adapted from the 1994 Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis.[16] All transplantations occurred between 1984 and 2011. We verified our search strategy using the Renine database (https://www.renine.nl/page?id=registry), which registers all patients in the Netherlands who receive chronic renal replacement therapy. This verification revealed no additional AAGN patients who were transplanted between 1984 and 2011. All patients were Caucasian and had ESRF secondary to AAGN. Only the first renal transplantation of each patient was analyzed.

We also performed a search for native renal biopsies of all included patients. This resulted in 34 native renal biopsies from 31 patients. This number was relatively low, because many of the native renal biopsies were taken in regional non-academic hospitals, where the tissue specimens were either not accessible or not preserved. In case of non-accessibility or non-availability, the histology reports were additionally reviewed centrally by an experienced, academically based nephropathologist (I.M.B.).

Patients transplanted after September 2000 received daclizumab as induction therapy (100 mg/day on the day of transplantation and 10 days after transplantation). The general maintenance regimen of the transplanted patients in this cohort was a triple therapy consisting of prednisolone, mycophenolate mofetil (MMF) and a calcineurin inhibitor (CNI). Fifty-one patients remained on this triple therapy regimen. In 22 patients, MMF or CNI was switched to azathioprine. Sixteen patients continued taking prednisolone and CNI, seven continued taking MMF and CNI, one continued taking prednisolone only, and four continued taking CNI only. In one patient, fingolimod was added to the standard regimen. In five patients, CNI was switched to a mammalian target of rapamycin (mTOR) inhibitor (three patients received sirolimus, and two received everolimus); in one patient, MMF was switched to everolimus, and a protein kinase C inhibitor was added. One patient continued on MMF, CNI and everolimus, and one patient continued on CNI and sirolimus.

Data collection

Patient data included clinical data, histology reports and —where available— native renal biopsies and/or renal transplant samples. Clinical data were collected using a questionnaire for review of the medical reports, which was completed by experienced nephrologists working in the participating academic hospitals. Native renal biopsies, renal transplant samples and histology reports were collected by experienced nephropathologists working at the participating academic centers. Data until January 2013 were collected. This study was conducted in accordance with the Declarations of Helsinki and Istanbul.

Clinical parameters

The following clinical data of the recipient between the time of diagnosis and the time of renal transplantation were collected: gender, date of birth, diagnosis (GPA/MPA), ANCA positivity during active disease (yes/no, PR3-ANCA/MPO-ANCA, cytoplasmic (c)ANCA/perinuclear (p)ANCA), time interval between diagnosis and transplantation, and (time on) dialysis. Clinical data at the time of transplantation included age, date of transplantation, donor type (deceased/living), and ANCA positivity. Clinical data collected after transplantation involved serum creatinine levels, date of last visit, disease relapse/renal disease recurrence, graft loss, death, ANCA positivity two and five years after transplantation, and transplantation-related immunosuppressive therapy. In case of disease relapse, the therapy regimen used to achieve disease remission was noted.

Patients were subdivided into two groups of GPA and MPA in accordance with established diagnostic definitions. RLV was regarded as a form of MPA. ANCA was categorized as PR3-ANCA in case of a positive PR3-ANCA ELISA or a positive cANCA pattern by indirect immunofluorescence (IIF) microscopy, and as MPO-ANCA in case of a positive MPO-ANCA ELISA or a pANCA pattern on IIF microscopy.

Parameters considered for analyses were: gender, age at transplantation, GPA/MPA, ANCA-type, time between diagnosis and transplantation, time on dialysis, donor type, ANCA status at transplantation, renal disease recurrence, and an acute rejection episode.

Histology

Native renal biopsies and renal disease recurrence in the graft with an available biopsy were scored according to the histopathological classification of AAGN.[17] All renal transplant samples were scored according to the Banff ’09 classification.[18-20]

The class was based on the first renal graft biopsy confirming the renal disease recurrence. All samples were scored centrally by an experienced, academically based nephropathologist (I.M.B.) who was blinded with respect to the clinical data.

Study outcomes

The primary clinical outcomes were renal disease recurrence and graft loss. The secondary outcome was disease relapse. Renal disease recurrence refers to the manifestation of the disease in the graft, independent of other organ involvement. Disease relapse refers to renal disease recurrence and/or extra-renal disease manifestations. Renal disease recurrence was defined as an increase in serum creatinine and new-onset hematuria or proteinuria (all attributable to active vasculitis) and/or histological confirmation. The appearance of cellular crescents and/or fibrinoid necrosis in the renal graft biopsy was considered evidence of renal disease recurrence. Extra-renal disease relapse was defined as new, worsened or recurred manifestations (all attributable to active vasculitis). Disease relapse was based on the expert opinion of the academically based nephrologists and nephropathologist (I.M.B.).

Statistical Analysis

Graft survival censored for death with a functioning graft was analyzed using the Kaplan-Meier survival method. For univariate analyses, log rank test and Cox regression analyses were used, with graft loss, renal disease recurrence and disease relapse as outcomes. In the univariate analyses, significant associations were found only with graft loss as outcome; therefore, multivariate Cox regression analyses were only performed with graft loss as dependent variable. All baseline parameters were considered to be fixed covariates. To assess the effect of renal disease recurrence and acute rejection on graft loss, both were considered to be time-dependent covariates. Due to the relatively low number of events, inclusion of all variables in the multivariate Cox regression analysis was not statistically feasible.[21] Therefore, we analyzed one multivariate Cox regression model based on the outcomes of the univariate analyses (all variables with $P<0.05$). For Cox regression analyses, hazard ratio (HR) and 95% confidence interval (CI) were calculated. A P-value <0.05 was considered statistically significant. All statistical calculations were performed using SPSS (v20.0; IBM Corp, Armonk, NY) and GraphPad Prism software (v6; GraphPad Software Inc, La Jolla, CA).

RESULTS

Patients and events

A total of 113 AAGN patients who received a renal transplant from 1984 through 2011 were included (Table 1). In three patients (3%) the graft did not gain proper function after transplantation and these were excluded from analyses. Of the remaining 110 patients, 88 (80%) retained the graft and did not die during the follow-up period. Fifty-seven of them (65%) completed the five years of follow-up. The remaining 31 patients (35%) had their last visit within five years of transplantation (median: 27.9 months, interquartile range (IQR): 21.0-41.8); two of them were lost to follow-up (reason unknown).

During follow-up, 79 patients (72%) experienced no event and six patients (5%) died with a functioning graft within five years of transplantation without experiencing a disease relapse (Figure 1). These six patients died due to infection (*n*=3), cancer (*n*=2), or a cardiovascular event (*n*=1).

Thirteen patients (12%) experienced 16 disease relapses (three patients experienced two relapses) within five years of transplantation (Figure 1). Of these 16 relapses, two involved extra-renal organs only, five involved the renal graft only, and nine involved both the renal graft and extra-renal organs (Table 2). The first disease relapse occurred within a median of 22.1 months (IQR: 10.3-46.3) following transplantation. The risk of experiencing a first disease relapse within 5 years of renal transplantation was 3.3% per patient year. The first renal disease recurrence occurred within a median of 18.0 months (IQR: 10.3-45.9). The risk of experiencing a first renal disease recurrence within five years was 2.8% per patient year. The relapses were equally distributed between 1986 and 2012.

Table 1 | Baseline characteristics of renal transplant recipients with end-stage renal failure due to ANCA-associated vasculitis

Characteristic	Value
Number of patients	113
Age at transplantation (years)	52.2 (14.7)[a]
Male	77 (68.1)
Diagnosis	
GPA	77 (68.1)
MPA	36 (31.9)
ANCA type	
PR3	37 (32.7)
MPO	52 (46.0)
ACPA[b]	6 (5.3)
Negative	5 (4.4)
Double positive	1 (0.9)
ANCA-positive not further specified	4 (3.5)
NR	8 (7.1)
Time between diagnosis and transplantation (months)	50.0 (26.6 – 95.2)[c]
Dialysis before transplantation[d]	
Yes	107 (94.7)
No (preemptive transplantation)	4 (3.5)
NR	2 (1.8)
Time on dialysis before transplantation[d] (months)	24.0 (16.7 – 40.3)[c]
Donor type	
Deceased	67 (59.3)
Living	46 (40.7)
ANCA status at time of transplantation	
Positive	27 (23.9)
Negative	25 (22.1)
NR	61 (54.0)
Era of transplantation	
Before 1990	8 (7)
1990 - 2000	37 (33)
After 2000	68 (60)

Data are presented as *n* (%) unless otherwise noted.
[a]Mean (SD).
[b]Former nomenclature for ANCA, and referred primarily to PR3-ANCA. These patients were tested before 1990, and no further specified test results were reported at a later time point.
[c]Median (25^{th} and 75^{th} percentile).
[d]Irreversible dialysis dependency.
Abbreviations: GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; ANCA, anti-neutrophil cytoplasmic antibody; PR3, proteinase 3; MPO, myeloperoxidase; ACPA, anti-cytoplasmic antibody; SD, standard deviation, NR, not reported/not performed.

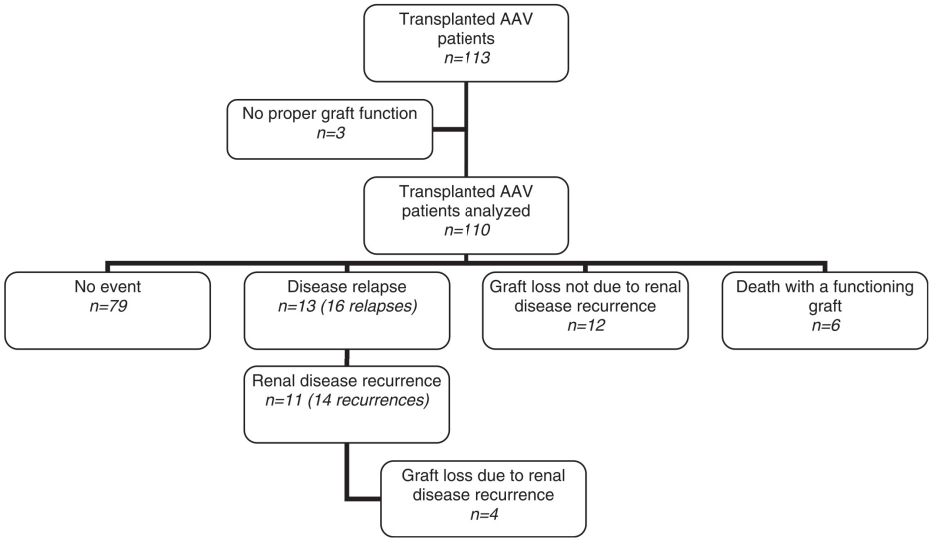


Figure 1 | Events.
Flowchart of the different events, which the 113 patients experienced within five years of renal transplantation.
Abbreviation: AAV, ANCA-associated vasculitis.

The occurrence of (and time to first) relapse was not associated with any of the baseline characteristics listed in table 1, including type of immune-suppressive regimen. Four patients with renal disease recurrence in the graft, lost their grafts due to the disease recurrence within five years of transplantation. One of these patients experienced the first renal disease recurrence ten months after transplantation, which entered into remission following plasmapheresis, prednisolone and cyclophosphamide treatment. This patient's second renal disease recurrence occurred 13 months after the first event, and despite treatment with an increased dose of prednisolone with continuation of MMF and CNI, the patient lost the graft. In the other patients, the disease recurrences leading to graft loss were treated with prednisolone and cyclophosphamide (Table 2). These four grafts were lost before remission was achieved. All other patients were treated to remission without graft loss. At the time of relapse, 13 patients were ANCA-positive, two patients were ANCA-negative, and in one, the ANCA titer was not reported.

Table 2 | Characteristics of patients with disease relapse within five years of transplantation

Patient	Gender	Diagnosis	ANCA type	Age at Tx[a]	ANCA at Tx	Donor type	Year of Tx	Medication[b]	Disease relapse	Time after Tx[c]	Treatment relapse	ANCA status at relapse	Elevated ANCA titer before relapse	Graft loss ≤5 years of Tx[d]	Δ time[e]	Histopathological class
1	F	MPA	MPO	63	Positive	DD	1998	P, MMF, CNI	R	0.3	P, CYC	Positive	NR	Yes	1.0	Focal
2	M	GPA	PR3	51	Negative	DD	2000	P, MMF, CNI, AZA	RER	15.8	P, CYC	Negative	No	Yes	8.0	Mixed
3	F	GPA	PR3	58	Negative	LD	2001	P, MMF, CNI	RER	45.9	P, CYC	Positive	NR	Yes	3.5	Mixed
4	M	GPA	PR3	45	Positive	DD	2004	P, MMF, CNI, AZA	RER	10.3	Pph, P, CYC	Positive	Yes	No	N/A	Crescentic
4 (2nd relapse)									RER	23.0	P	Positive	Yes	Yes	8.0	N/A
5	M	GPA	PR3	51	NR	DD	2001	P, MMF, CNI	RER	18.0	MeP, P, CYC	Positive	NR	No	N/A	Focal
6	M	GPA	PR3	35	Positive	LD	2010	P, MMF, CNI, sirolimus	RER	10.4	P, RTX	Positive	Yes	No	N/A	Focal
7	M	GPA	ACPA	52	NR	DD	1984	P, CNI	RER	27.2	Cs	Positive	NR	No	N/A	Focal
7 (2nd relapse)									RER	46.1	P, CYC	Positive	NR	No	N/A	N/A
8	M	MPA	MPO	50	NR	LD	2010	P, MMF, CNI, AZA	R	8.1	P, CYC	Positive	NR	No	N/A	Focal
8 (2nd relapse)									R	26.6	P, RTX	Positive	Yes	No	N/A	N/A
9	M	MPA	MPO	40	Positive	DD	1990	P, CNI	R	59.2	P, AZA	Positive	No	No	N/A	Mixed
10	M	GPA	MPO	42	Positive	LD	1996	P, CNI	R	22.1	P, CYC	Positive	Yes	No	N/A	Mixed
11	M	GPA	MPO	62	NR	DD	1995	P, MMF, CNI	RER	55.7	NR	NR	NR	No	N/A	N/A
12	M	GPA	NR	50	NR	DD	1991	P, CNI	ER	36.0	NR	Positive	NR	No	N/A	N/A
13	F	GPA	PR3	18	Negative	DD	2008	P, MMF, CNI	ER	46.7	P	Negative	No	No	N/A	N/A

[a]Age in years.
[b]Transplantation-related immunosupressive medication.
[c]Time in months.
[d]Graft loss within five years of transplantation due to disease relapse with renal graft involvement (renal disease recurrence).
[e]Time difference in months between renal disease recurrence and graft loss.
Abbreviations: ANCA, anti-neutrophil cytoplasmic antibody; Tx, transplantation; F, female; M, male; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; ACPA, anti-cytoplasmic antibody; DD, deceased donor; LD, living donor; P, prednisolone; MMF, mycophenolate mofetil; CNI calcineurin inhibitor; AZA, azathioprine; R, disease relapse with solely renal graft involvement; RER, disease relapse with renal graft and extra-renal involvement; ER, disease relapse with solely extra-renal involvement; CYC, cyclophosphamide; Pph, plasmapheresis; MeP, methylprednisolone; Cs, cyclosporine; RTX, rituximab; NR, not reported/not performed; N/A, no biopsy of the renal disease recurrence available or not applicable.

Twelve patients lost their grafts due to other causes than renal disease recurrence of AAGN: interstitial fibrosis and tubular atrophy (*n*=4), acute rejection (*n*=3), (uro)sepsis (*n*=2), post-transplant lymphoproliferative disorder (*n*=1), infarction (*n*=1) and acute cyclosporine toxicity (*n*=1). The 1-year graft survival rate was 94.5% (95% CI, 90.2%-98.8%), and the 5-year graft survival rate was 82.8% (95% CI, 75.0%-90.6%) (Figure 2). The era in which the transplantation was performed had no significant effect on graft survival (Figure 3).

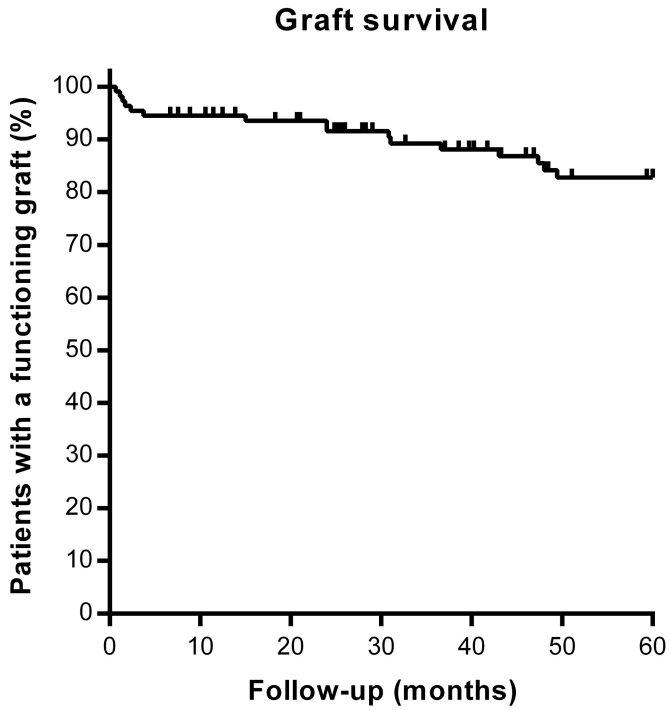
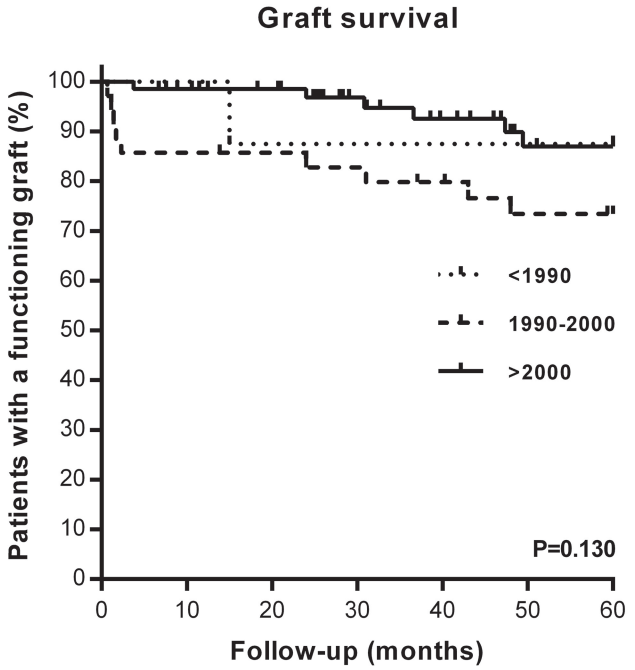


Figure 2 | Death-censored renal graft survival.
Kaplan-Meier curve of renal graft survival among 110 renal transplant recipients with ANCA-associated vasculitis. During follow-up 16 patients lost their graft within five years of transplantation. The one-year graft survival rate was 94.5% (95% CI, 90.2%-98.8%), and the 5-year graft survival rate was 82.8% (95% CI, 75.0%-90.6%). The data was censored for death with a functioning graft.



Patients at risk							
<1990	8	8	7	7	7	7	7
1990-2000	35	30	29	28	26	23	22
>2000	67	63	59	45	39	29	28

Figure 3 | Death-censored renal graft survival according to transplantation era.
Kaplan-Meier curves of renal graft survival according to the era's in which the 110 renal transplant recipients with ANCA-associated vasculitis were transplanted. The patients were divided into three era's: before 1990, 1990-2000 and after 2000. These era's were chosen in accordance with the study by Little *et al.*(9) There was no difference in graft survival between the groups (log-rank test: P=0.13).

Histopathology

Of the 136 renal transplant samples taken from these 110 patients within five years of transplantation, 108 fulfilled the criteria (≥7 glomeruli and ≥1 artery) for scoring using the Banff '09 classification (Table 3).[18-20] Of the 108 suitable samples, 24 were protocol biopsies (median: 4 months, IQR: 3 – 14), all the other biopsies were taken for cause. During follow-up, 23 patients experienced 28 biopsy-confirmed acute rejection episodes, three of which showed signs of humoral rejection. Six episodes were subclinical acute rejections detected by protocol biopsies.

Table 3 | Overview of the histological findings in 108 renal transplant samples of 110 patients

Histological finding[a]	Number of samples[b]	Number of patients[c]	Time after Tx[d]
Normal	13	11	0.7 (0.3-3.0)
Acute rejection	28[e]	23	5.2 (0.5-16.8)
Borderline changes	9[e]	8	1.3 (0.8-5.5)
IFTA	25	21	12.0 (1.3 – 28.3)
CNI toxicity	8	8	2.1 (0.9-10.5)
ATN	4	4	0.3 (0.2-0.3)
Renal disease recurrence	10[e]	10	19.0 (9.7-31.8)
BK-nephropathy	1	1	22.3
TMA	1	1	0.4
Pyelonephritis	1	1	0.4
Infarction	2	2	0.1 and 1.7
Extensive vasculopathy	1	1	0.9
Slight hyalinosis	1	1	1.1

[a]Adapted from the Banff '09 classification.
[b]The total number of samples/episodes differs from 108, since categories may coincide in the same sample and some numbers represent number of episodes rather than number of samples (see [e]).
[c]The total number of patients in the table differs from the total number of the cohort (*n*=110), since histological lesions may coincide in the same patient and not all patients were biopsied after transplantation.
[d]Time in months: median (IQR).
[e]These numbers represent episodes: in some cases more than one biopsy has been performed on the same episode.
Abbreviations: Tx, transplantation; IFTA, interstitial fibrosis and tubular atrophy; CNI, calcineurin inhibitor; ATN, acute tubular necrosis; TMA, thrombotic microangiopathy; IQR, interquartile range.

Renal biopsies were available for 10 of the 14 renal disease recurrences. All ten biopsy-confirmed recurrences were first-time renal disease recurrences. The histopathological classes of these ten recurrences were focal (5), crescentic (1), and mixed class (4) (Table 2). The biopsies contained a median of 16 glomeruli (IQR: 12-22). In the three patients who had lost their graft within five years of transplantation as a result of their first renal disease recurrence, one recurrence was focal, and two were mixed class. In one of the patients with a mixed class recurrence, the recurrence changed to crescentic class before graft loss (S Table 1 in the supplementary material).

Outcome statistical analyses

Four baseline parameters were associated with graft loss in a univariate Cox regression analysis (S Table 2 in the supplementary material). Male gender of the recipient (HR 0.33, 95% CI 0.12-0.88, P=0.03) and a living donor (HR 0.22, 95% CI 0.05-0.95, P=0.04) were associated with better graft survival. Experiencing renal disease recurrence (HR 12.43, 95% CI 3.61-42.89, P<0.001) and experiencing an acute rejection (HR 3.18, 95% CI 1.08-9.36, P=0.04) were significantly associated with worse graft survival. No baseline parameter was associated with either renal disease recurrence or disease relapse (S Table 3 in the supplementary material).

In the multivariate analysis with graft loss as outcome and including all baseline parameters that were statistically significant in the univariate analysis, only the associations with gender of the recipient (HR, 0.27; 95% CI, 0.09-0.81; *P*=0.02) and experiencing renal disease recurrence persisted (HR, 18.48; 95% CI, 4.96-68.89; *P*<0.001) (Table 4).

Table 4 | Outcome of the multivariate analysis regarding graft loss

	Parameter	P-value	HR (95% CI)
Multivariate model	Male gender recipient	0.02	0.27 (0.09-0.81)
	Living donor	0.08	0.26 (0.06-1.18)
	Renal disease recurrence	<0.001	18.48 (4.96-68.89)
	Acute rejection episode	0.25	1.94 (0.62-6.03)

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

DISCUSSION

We investigated the outcome of renal transplantation within the first five years of transplantation in a Dutch cohort of 113 AAGN patients transplanted between 1984 and 2011. In this cohort, the 1-year and 5-year graft survival rates were 94.5% and 82.8%, respectively, which is similar to graft survival rates reported by registry data of the general transplantation population in Europe and North America.[22-24] Compared to the Dutch general renal transplantation population transplanted in the period 1984-2011, the graft survival in our AAV cohort seemed to be better: 1-year graft survival 94.5% vs. 86.0%, 5-year graft survival 82.8% vs. 70.0% (Dutch Transplantation Foundation (NTS) database; registers all transplantations in the Netherlands, accessed 12-06-2015). This discrepancy is most likely due to the larger proportion of transplantations in the earlier years in the general population compared to our cohort. In addition, compared to the European and North American registry data, transplantations from an earlier period have been included in the Dutch registry data. The risk of experiencing a first disease relapse or renal disease recurrence within 5 years of transplantation was 3.3% and 2.8% per patient year, respectively. The principal finding in this cohort was that renal disease recurrence was an important cause of graft loss within the first 5 years of transplantation.

Our graft survival rates are similar to those reported in previous studies on AAGN, although some studies reported a slightly better 1- or 5-year graft survival rate.[7-9,13] Most of these studies included transplantations performed in a later time period compared to our study; from the late 90's on. In our cohort the era in which the transplantation was performed had no significant effect on graft survival. These slight differences can also be a consequence of the relative small cohorts in the different studies. The largest cohort with 919 recipients transplanted between 1997-2007 showed similar graft survival rates as our study.[11] There were no noteworthy differences in treatment protocols between the studies that could explain the differences.

Our relapse and recurrence rates are slightly higher compared to those reported in other studies. Most studies on the outcome of renal transplantation in AAV described rates of 1.0-2.0% per patient year.[6-9] Three studies reported higher rates of 7.6%, 6.0% and 10.0% per patient year.[25-27] Several of these studies described patients with renal disease recurrence with consequent graft loss, sometimes in >50% of the patients with recurrence.[7, 9-11, 13] Our study is the first study which specifically assesses the impact of renal disease recurrence on graft survival, showing that renal disease recurrence is associated with subsequent graft loss.

The patients who lost their graft after renal disease recurrence were not treated more or less aggressively than the patients who did not lose their graft. In view of recent evidence from the RAVE trial (NCT00104299) showing that rituximab treatment has an advantage over cyclophosphamide treatment in case of relapse with severe disease manifestations,[28, 29] we suggest that also in the transplantation setting there may be a benefit of treating recurrent disease with rituximab. In fact, two patients in our cohort who were treated with rituximab for a renal disease recurrence did not lose their graft. Another study also described remission induction with rituximab in patients with renal disease recurrence in the graft.[30] However, we were unable to compare the rituximab-treated patients with standard cyclophosphamide-treated patients due to the small patient numbers. Our results show that the recurrence of AAGN is an important cause of graft loss, and therefore, continuously monitoring these transplanted patients for relapse is highly important.

Currently no distinction is made in the clinical setting based on the clinical defined diseases or ANCA-specifity. They are treated the same way and have the same procedure regarding renal transplantation in AAGN. Our study showed that there is no difference in graft loss, renal disease recurrence or disease relapse when comparing disease subtypes or ANCA-specificity. This is also described in other studies on renal transplantation in AAGN.[7, 9, 13] Further studies are needed to determine whether distinction in ANCA specificity or disease subtype is needed for optimal clinical management.

Our study and other studies revealed that patients can relapse without a positive ANCA titer before the relapse. This finding is consistent with other renal transplantation studies.[26, 31-34] A meta-analysis found that a rise in—or persistence of—ANCA has modest predictive value for future disease relapse in AAV patients without ESRF.[35] Therefore, it may be concluded that the isolated use of serial ANCA measurements is not reliable for management decision-making in transplanted AAGN patients.

This is the first study that formally reviewed the complete renal transplant histopathology and classified renal disease recurrence in the graft according to the histopathological classification of AAGN. With regards to the histopathological presentation of the renal disease recurrence in the grafts, we found that the disease can recur as a different class in the graft and that histopathological class can change over time in the renal graft after disease recurrence. These findings, although based on small numbers (S Table 1 and S Table 4 in the supplementary material), may suggest that the histopathological classes represent different phases of the disease. This is

in line with recent evidence from a study by Hruskova *et al*. describing a class change over native renal biopsies in 86% of the patients in protocolized repeat biopsies after one year.[36]

In the last 30 years, important developments were made in the field of post-transplantation immunosuppressive therapy. In 1983, cyclosporine was introduced after the 'azathioprine era'. This resulted in improved graft survival rates.[37] After increased use of cyclosporine over time, in 1992 new trends developed including waning of cyclosporine and the rise of induction therapy. The use of induction therapy gradually increased resulting in 59% of all recipients receiving induction therapy in 2001. During this period, corticosteroid therapy was an important component of maintenance therapy. The waning of cyclosporine was accompanied with a rise of tacrolimus use. In 1992, azathioprine was predominantly used as antimetabolite, but by 2001 most centers used MMF. In 1996, rapamycine was introduced as an alternative to spare other immunosuppressive drugs, in particular the nephrotoxic CNI. These developments resulted in the currently most used regimen of induction therapy followed by a triple therapy consisting of prednisolone, MMF and tacrolimus.[38] This probably has decreased the relapse rates of AAV after renal transplantation when comparing the different studies over time, but none of them could demonstrate this, probably due to a lack of power.[6-9, 25-27]

AAV is diagnosed on the clinical manifestation compatible with AAV and substantiated by a positive ANCA serology and/or histology. In the 90s, solid phase assays (ELISAs) for PR3-ANCA and MPO-ANCA detection were developed and standardized.[39] In addition, a disease specific activity index was introduced, which was revised two times since then; Birmingham Vasculitis Activity Score.[40-42] The gold standard for establishing AAGN is a renal biopsy. These diagnostic tools are also used in the setting of transplantation for detecting disease relapse. When detecting disease relapse in general the patient receives induction therapy based on a cyclophosphamide regimen as is the case with newly diagnosed AAV. As discussed above, rituximab is now also more frequently used in case of renal disease recurrence after transplantation.

The retrospective design is a limitation of our study. Although complete data were available for most patients, some cases had missing data or material was not available. This made it impossible to determine the duration of remission prior to transplantation in relation to post-transplant outcome. Moreover, numbers were too low to detect an impact of maintenance immunosuppression or therapy of recurrent disease on graft loss. Nevertheless, given our data we do not expect that a specific regimen was

associated with a negative outcome, as the majority of patients who experienced disease relapse, renal disease recurrence and/or graft loss received conventional therapy.

We conclude that in a substantial proportion (36%) of AAGN patients with disease recurrence in the renal graft, the recurrence led to graft loss within five years of transplantation. In a multivariate analysis, renal disease recurrence was independently associated with subsequent graft loss. So, although the risk of renal disease recurrence is rather low, once a recurrence of the disease occurs in the graft, the risk of graft loss is considerable. This study confirms that renal transplantation is a viable treatment option for AAGN patients with ESRF, but it also serves as a warning that clinicians must remain cognizant of the risk of graft loss when the disease has recurred in the renal graft.

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DISCLOSURES

None to declare.

REFERENCES

1. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med,* 1997;337: 1512-1523.
2. Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis,* 2003;41: 776-784.
3. Little MA, Nazar L, Farrington K. Outcome in glomerulonephritis due to systemic small vessel vasculitis: effect of functional status and non-vasculitic co-morbidity. *Nephrol Dial Transplant,* 2004;19: 356-364.
4. Slot MC, Tervaert JW, Franssen CF, Stegeman CA. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int,* 2003;63: 670-677.
5. Westman KW, Bygren PG, Olsson H, Ranstam J, Wieslander J. Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J Am Soc Nephrol,* 1998;9: 842-852.
6. Allen A, Pusey C, Gaskin G. Outcome of renal replacement therapy in antineutrophil cytoplasmic antibody-associated systemic vasculitis. *J Am Soc Nephrol,* 1998;9: 1258-1263.
7. Geetha D, Eirin A, True K, et al. Renal transplantation in antineutrophil cytoplasmic antibody-associated vasculitis: a multicenter experience. *Transplantation,* 2011;91: 1370-1375.
8. Gera M, Griffin MD, Specks U, Leung N, Stegall MD, Fervenza FC. Recurrence of ANCA-associated vasculitis following renal transplantation in the modern era of immunosupression. *Kidney Int,* 2007;71: 1296-1301.
9. Little MA, Hassan B, Jacques S, et al. Renal transplantation in systemic vasculitis: when is it safe? *Nephrol Dial Transplant,* 2009;24: 3219-3225.
10. Marco H, Mirapeix E, Arcos E, et al. Long-term outcome of antineutrophil cytoplasmic antibody-associated small vessel vasculitis after renal transplantation. *Clin Transplant,* 2013;27: 338-347.
11. Shen J, Gill J, Shangguan M, Sampaio MS, Bunnapradist S. Outcomes of renal transplantation in recipients with Wegener's granulomatosis. *Clin Transplan,* 2011;25: 380-387.
12. Briggs JD, Jones E. Renal transplantation for uncommon diseases. Scientific Advisory Board of the ERA-EDTA Registry. European Renal Association-European Dialysis and Transplant Association. *Nephrol Dial Transplant,* 1999;14: 570-575.
13. Tang W, Bose B, McDonald SP, et al. The outcomes of patients with ESRD and ANCA-associated vasculitis in Australia and New Zealand. *Clin J Am Soc Nephrol,* 2013;8: 773-780.
14. Schmitt W, Opelz G, Van Der Woude F. Renal transplantation (RTx) is safe and successful in Wegener's granulomatosis (WG): data from the Collaborative Transplant Study [abstract]. *J Am Soc Nephrol,* 2002;13: 564A-565A.
15. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol,* 2007;29: 19-24.
16. Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum,* 1994;37: 187-192.
17. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol,* 2010;21: 1628-1636.

18. Sis B, Mengel M, Haas M, et al. Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant,* 2010;10: 464-471.
19. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int,* 1999;55: 713-723.
20. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant,* 2008;8: 753-760.
21. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol,* 1996;49: 1373-1379.
22. Stel VS, van de Luijtgaarden MW, Wanner C, Jager KJ, on behalf of the European Renal Registry I. The 2008 ERA-EDTA Registry Annual Report-a precis. *NDT Plus,* 2011;4: 1-13.
23. Cecka JM. The OPTN/UNOS Renal Transplant Registry. *Clin Transpl,* 2005: 1-16.
24. Gondos A, Dohler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation,* 2013;95: 267-274.
25. Schmitt WH, Haubitz M, Mistry N, Brunkhorst R, Erbsloh-Moller B, Gross WL. Renal transplantation in Wegener's granulomatosis. *Lancet,* 1993;342: 860.
26. Moroni G, Torri A, Gallelli B, et al. The long-term prognosis of renal transplant in patients with systemic vasculitis. *Am J Transplant,* 2007;7: 2133-2139.
27. Haubitz M, Kliem V, Koch KM, et al. Renal transplantation for patients with autoimmune diseases: single-center experience with 42 patients. *Transplantation,* 1997;63: 1251-1257.
28. Specks U, Merkel PA, Seo P, et al. Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med,* 2013;369: 417-427.
29. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med,* 2010;363: 221-232.
30. Murakami C, Manoharan P, Carter-Monroe N, Geetha D. Rituximab for remission induction in recurrent ANCA-associated glomerulonephritis postkidney transplant. *Transpl Int,* 2013;26: 1225-1231.
31. Nyberg G, Akesson P, Norden G, Wieslander J. Systemic vasculitis in a kidney transplant population. *Transplantation,* 1997;63: 1273-1277.
32. Elmedhem A, Adu D, Savage CO. Relapse rate and outcome of ANCA-associated small vessel vasculitis after transplantation. *Nephrol Dial Transplant,* 2003;18: 1001-1004.
33. Rostaing L, Modesto A, Oksman F, Cisterne JM, Le Mao G, Durand D. Outcome of patients with antineutrophil cytoplasmic autoantibody-associated vasculitis following cadaveric kidney transplantation. *Am J Kidney Dis,* 1997;29: 96-102.
34. Deegens JK, Artz MA, Hoitsma AJ, Wetzels JF. Outcome of renal transplantation in patients with pauci-immune small vessel vasculitis or anti-GBM disease. *Clin Nephrol,* 2003;59: 1-9.
35. Tomasson G, Grayson PC, Mahr AD, Lavalley M, Merkel PA. Value of ANCA measurements during remission to predict a relapse of ANCA-associated vasculitis--a meta-analysis. *Rheumatology (Oxford),* 2012;51: 100-109.
36. Hruskova Z, Honsova E, Berden AE, et al. Repeat protocol renal biopsy in ANCA-associated renal vasculitis. *Nephrol Dial Transplant,* 2014;29: 1728-1732.

37. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med,* 2000;342: 605-612.
38. Helderman JH, Bennett WM, Cibrik DM, Kaufman DB, Klein A, Takemoto SK. Immunosuppression: practice and trends. *Am J Transplant,* 2003;3 Suppl 4: 41-52.
39. Hagen EC, Andrassy K, Csernok E, et al. Development and standardization of solid phase assays for the detection of anti-neutrophil cytoplasmic antibodies (ANCA). A report on the second phase of an international cooperative study on the standardization of ANCA assays. *J Immunol Methods,* 1996;196: 1-15.
40. Luqmani RA, Bacon PA, Moots RJ, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM,* 1994;87: 671-678.
41. Luqmani RA, Exley AR, Kitas GD, Bacon PA. Disease assessment and management of the vasculitides. *Baillieres Clin Rheumatol,* 1997;11: 423-446.
42. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis,* 2009;68: 1827-1832.

SUPPLEMENTARY MATERIAL

The Dutch transplantation in vasculitis (DUTRAVAS) study: outcome of renal transplantation in ANCA-associated glomerulonephritis

S Table 1 | Overview of the patients with follow-up biopsies of their biopsy-confirmed renal disease recurrence

Patient[a]	Class recurrence - first biopsy[b]	Class recurrence - follow-up biopsy 1[b]	Class recurrence - follow-up biopsy 2[b]	Second renal disease recurrence ≤5 years of Tx	Graft loss ≤5 years of Tx[c]
1	Focal (0.3)	Focal (0.6)	Focal (1.1)	No	Yes
2	Mixed (15.8)	Crescentic (17.1)	-	No	Yes
7	Focal (27.2)	Mixed (32.8)	-	Yes	No
8	Focal (8.1)	Focal (10.0)	-	Yes	No

[a]The patient numbers correspond with Table 2.
[b]Class (months after transplantation).
[c]Graft loss within five years of transplantation due to disease relapse with renal graft involvement (renal disease recurrence).
Abbreviations: Tx, transplantation.

S Table 2 | Outcomes of the univariate analyses regarding graft loss

Parameter	Graft loss	
	P-value	HR (95% CI)
Gender recipient[a]	0.03	0.33 (0.12-0.88)
Age at transplantation (years)	0.36	0.99 (0.96-1.02)
Diagnosis[b]	0.20	1.92 (0.72-5.17)
PR3-ANCA[c]	0.60	0.75 (0.25-2.24)
MPO-ANCA[c]	0.81	1.14 (0.40-3.29)
Time between diagnosis and transplantation (months)	0.85	1.00 (0.99-1.01)
Time on dialysis (months)	0.27	1.01 (0.99-1.04)
Donor type[d]	0.04	0.22 (0.05-0.95)
ANCA status at transplantation[e]	0.31	0.49 (0.12-1.96)
Renal disease recurrence[f]	<0.001	12.43 (3.61-42.89)
Acute rejection episode[g]	0.04	3.18 (1.08-9.36)

[a]Ref: female.
[b]Ref: granulomatosis with polyangiitis.
[c]Ref: negative.
[d]Ref: deceased donor.
[e]Ref: ANCA-negative.
[f]Ref: patients experiencing no renal disease recurrence (time-dependent covariate).
[g]Ref: patients experiencing no acute rejection episode (time-dependent covariate).
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; PR3, proteinase 3; ANCA, anti-neutrophil cytoplasmic antibody; MPO, myeloperoxidase.

S Table 3 | Outcomes of the univariate analyses regarding renal disease recurrence and disease relapse

Parameter	Renal disease recurrence		Disease relapse	
	P-value	HR (95% CI)	P-value	HR (95% CI)
Gender recipient[a]	0.42	1.89 (0.41-8.74)	0.59	1.43 (0.39-5.19)
Age at transplantation (years)	0.64	0.99 (0.95-1.03)	0.27	0.98 (0.95-1.01)
Diagnosis[b]	0.52	1.50 (0.44-5.12)	0.81	1.15 (0.36-3.74)
PR3-ANCA[c]	0.64	1.35 (0.39-4.67)	0.43	1.62 (0.49-5.31)
MPO-ANCA[c]	0.81	0.86 (0.25-2.97)	0.58	0.72 (0.22-2.36)
Time between diagnosis and transplantation (months)	0.64	1.00 (0.99-1.02)	0.74	1.00 (0.99-1.02)
Time on dialysis (months)	0.20	1.02 (0.99-1.05)	0.10	1.02 (1.00-1.05)
Donor type[d]	0.78	0.84 (0.25-2.88)	0.51	0.67 (0.21-2.18)
ANCA status at transplantation[e]	0.27	2.54 (0.49-13.12)	0.47	1.69 (0.40-7.08)

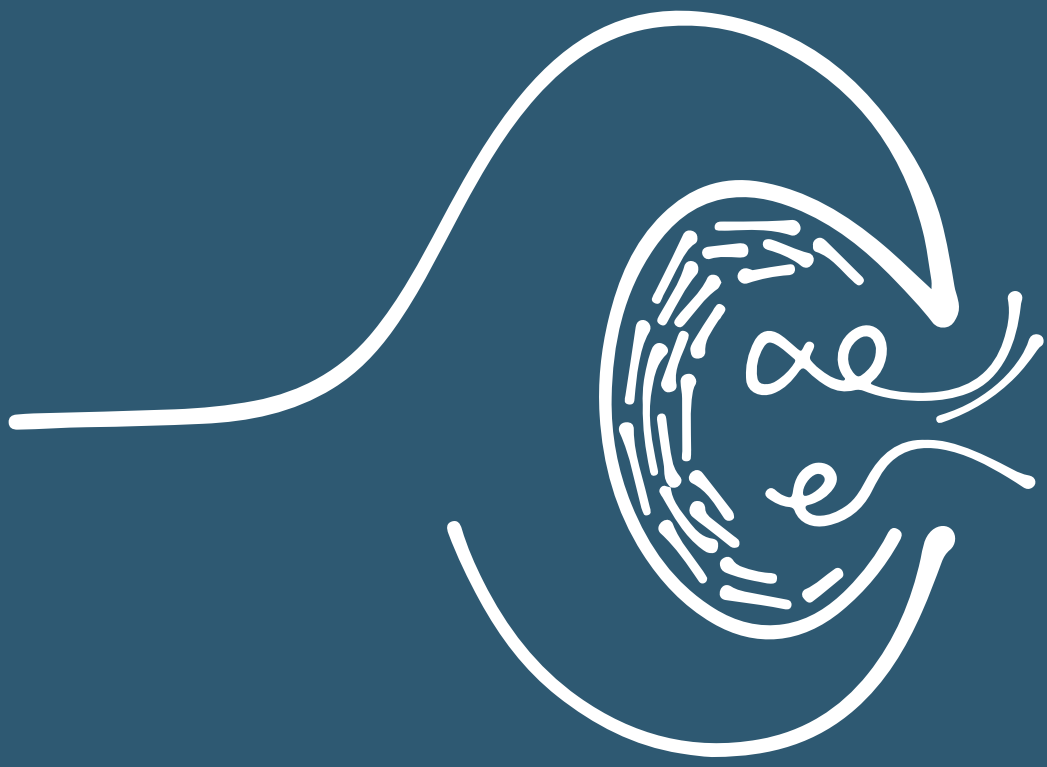
[a]Ref: female.
[b]Ref: granulomatosis with polyangiitis.
[c]Ref: negative.
[d]Ref: deceased donor.
[e]Ref: ANCA-negative.
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; PR3, proteinase 3; ANCA, anti-neutrophil cytoplasmic antibody; MPO, myeloperoxidase.

S Table 4 | Overview of the patients with a native renal biopsy and a biopsy-confirmed renal disease recurrence after transplantation

Patient[a]	Class diagnostic native biopsy	Class follow-up native biopsy, if available	Class renal disease recurrence[b]
1	Focal	Mixed	Focal (10.4)
2	Crescentic	-	Mixed (45.9)
3	Mixed*	-	Mixed (59.2)
4	Sclerotic	-	Mixed (142.4)

[a]Patient 1, 2 and 3 correspond with patient 6, 3 and 9, respectively, in Table 2.
[b]Class (months after transplantation).
*One week after cyclophosphamide treatment was started.

6



DIAGNOSIS AND MANAGEMENT OF ANCA-ASSOCIATED VASCULITIS

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SUMMARY POINTS

Consider antineutrophil cytoplasmic antibody (ANCA) associated vasculitis when inflammatory disease cannot be ascribed to any other disease and inflammation progresses despite antibiotics.

Avoid diagnostic delay to prevent end organ damage, particularly renal disease.

Test for ANCA in patients with chronic destructive upper airway disease, pulmonary nodules, renal and pulmonary inflammatory disease, rapidly progressive glomerulonephritis, skin vasculitis with systemic illness, mononeuritis multiplex, subglottic stenosis of the trachea, and retro-orbital mass.

Patients should be managed by a specialist in vasculitides.

Remission is usually induced with high dose glucocorticoids and cyclophosphamide and followed by remission maintenance treatment.

Adverse responses to treatment are common, as are relapses, so long term follow-up is needed.

Vasculitides associated with antineutrophil cytoplasmic antibodies (ANCA) are systemic autoimmune diseases of unknown cause that affect small to medium sized blood vessels. They include granulomatosis with polyangiitis (formerly Wegener's granulomatosis), microscopic polyangiitis, and eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome). This review mainly focuses on granulomatosis with polyangiitis and microscopic polyangiitis. Although they are relatively rare, they must be diagnosed and treated early because untreated disease may rapidly develop into multiple organ failure and death. With modern treatment, these diseases are no longer fatal but chronic. Early diagnosis and treatment may prevent progression to end organ damage and lengthen healthier life. A recent large survey of patients with ANCA associated vasculitis found a lag of three to 12 months between disease onset and diagnosis, suggesting that diagnostic delay is a problem.[1] We review the diagnosis and management of ANCA associated vasculitides for the generalist reader, drawing on the findings of observational studies, randomised controlled trials, and meta-analyses.

SOURCES AND SELECTION CRITERIA

We searched PubMed (original search performed in August 2011, updated in December 2011) for relevant articles on epidemiology, diagnosis, and management of antineutrophil cytoplasmic antibody (ANCA) associated vasculitis. Where possible, we sought data from prospective randomised clinical trials and meta-analyses. We also screened personal archives for relevant papers and consulted experts in otolaryngology (NR), nephrology (DJ), and rheumatology (RL). All relevant keyword variations were used. All searches contained the keywords "ANCA" or "vasculitis", or both. We limited results to articles written in English.

WHO GETS IT?

The overall annual incidence of ANCA associated vasculitis in Europe and Northern America is approximately 20 per million (with point prevalence of 130/million for granulomatosis with polyangiitis and 47.9/million for microscopic polyangiitis in the United Kingdom in 2008).[2,3] Disease onset usually occurs at 65-74 years, although it can occur at any age.[3] Prevalence is generally higher in men, but women more often develop disease at a younger age.[1] The overall prevalence of ANCA associated vasculitis is highest in Caucasians.[1,4] The incidence of granulomatosis with polyangiitis is higher in northern Europe, whereas that of microscopic polyangiitis is higher in southern Europe and Japan.[2,5]

HOW DO PATIENTS PRESENT?

Patients typically present with prodromal "flu-like" symptoms of several weeks' or months' duration,[6,7] such as fever, polymyalgia, polyarthralgia, headache, malaise, anorexia, and unintended weight loss. These non-specific symptoms overlap with symptoms of non-vasculitic processes such as post-viral syndrome, infections, or malignancy. Consider vasculitis as a differential diagnosis in patients with general symptoms and signs of inflammatory disease. Some patients may initially present with focal vasculitic disease such as rash, cutaneous vasculitis, bloody-purulent rhinitis, scleritis, or arthritis. In such patients, careful examination of other organ systems may show other disease manifestations.

Figure 1 shows the many ways in which vasculitis can manifest. Patients may report different symptoms over time. Symptoms of the different ANCA associated vasculitides overlap, but some symptoms are more common in certain diseases. For example, ear, nose, and throat problems—such as hearing loss, otalgia, (bloody nasal) rhinorrhoea, otorrhoea, sinusitis, nasal crusting, and recurrent otitis media—occur in about 90% of patients with granulomatosis with polyangiitis and in 35% of those with microscopic polyangiitis.[6,7] Large observational studies have shown that the airways and lung parenchyma are commonly affected, as are the kidneys, although this may not be apparent until renal failure occurs.[6,8,9] Urinalysis may therefore identify renal involvement early on in the disease. About 50% of patients have cutaneous manifestations of disease such as urticarial rash or tender skin nodules. The eyes and nervous system are also commonly affected.[6,8,9] A careful physical examination is needed to determine the full extent of disease.

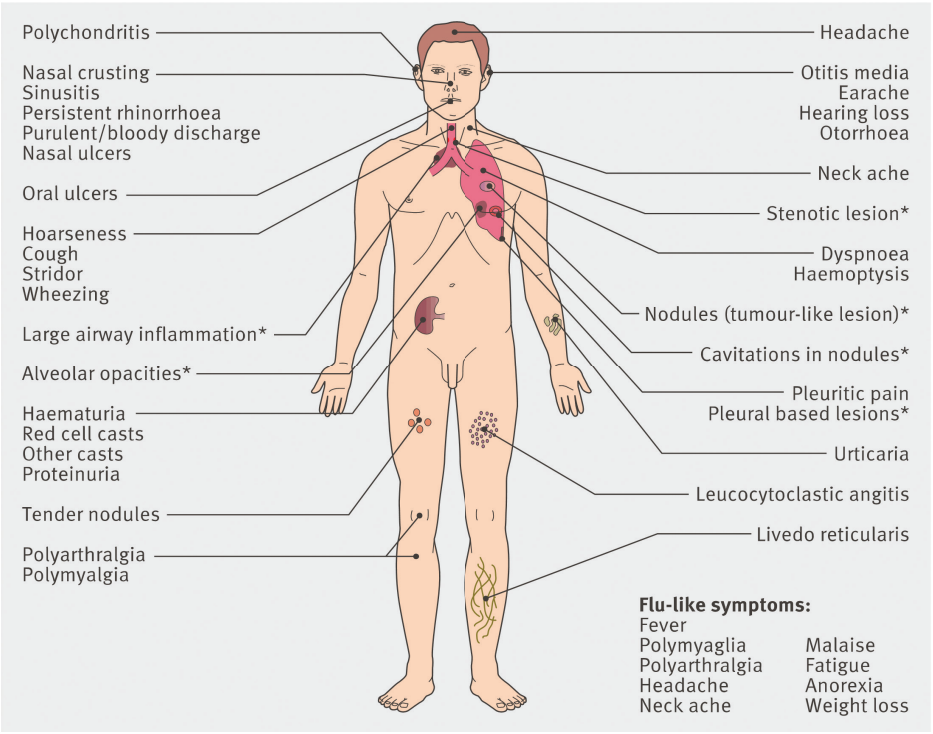


Figure 1 | Clinical manifestations of antineutrophil cytoplasmic antibody associated vasculitis. Alveolar haemorrhage is an important cause of mortality. Renal involvement manifests with early detectable haematuria, red cell and other casts, and proteinuria. It is an important cause of morbidity and mortality. The most common skin lesion is leucocytoclastic angiitis, which mostly causes purpura on the lower extremities, sometimes accompanied by focal necrosis and ulcerations. Skin lesions can appear on parts of the body not shown here. Eye disease presents as a painful or painless red eye. Mononeuritis multiplex is seen in 20% of patients.
*These lesions can be seen on chest radiography and computed tomography.

HOW CAN IT BE DIAGNOSED?

Investigations that can be undertaken in primary care

Blood tests requested in primary care may show leucocytosis, thrombocytosis, raised erythrocyte sedimentation rate and C reactive protein values, normochromic-normocytic anaemia, and a raised serum creatinine.[6] Patients with symptoms and signs of vasculitis and abnormalities on these blood tests require urinalysis, including urinary sedimentation, to look for haematuria and proteinuria. An increased serum creatinine indicates that renal damage has already occurred. Chest radiography in patients with pulmonary symptoms such as dyspnoea, cough, or haemoptysis may show infiltrates, nodules, or cavitations in the lung parenchyma.

An ANCA assay can be requested in primary care. The test is indicated in a patient with unexplained illness that has lasted more than a few weeks (box 1) and is associated with a raised erythrocyte sedimentation rate or C reactive protein, particularly if more than one organ system is affected. Four large international randomised controlled trials found that the test is positive in 90-95% of patients with active generalised granulomatosis with polyangiitis or microscopic polyangiitis before treatment.[10-13] Two types of assay are generally used: indirect immunofluorescence (IIF) and the enzyme linked immunosorbent assay (ELISA). Table 1 outlines the properties of these tests. An international multicentre observational study found that IIF is more sensitive but that ELISA is more specific.[14] The current international standard approach is to use IIF as a screening test and ELISA to confirm positive results.[15] ANCA assays should be performed only in experienced laboratories. Testing is not standardised, so sensitivity and specificity vary between laboratories and reference values are unavailable. Although the ANCA test is positive in most patients with untreated disease, a negative result does not exclude the diagnosis of ANCA associated vasculitis because 5-10% of patients do not develop ANCA. Neither does a negative ANCA test exclude the presence of other non-ANCA associated small and medium vessel vasculitic syndromes. Such patients may require more systematic investigation to ascertain the extent of their disease. Although the ANCA test is widely used as a routine screening tool for vasculitis in secondary care, it provides poor sensitivity and specificity in this setting.[16,17] When there is a clearer indication and likelihood of vasculitis the yield from testing is higher. If there is a high index of suspicion on the basis of clinical findings but the ANCA test is negative, an ANCA assay can be repeated a few weeks after the original test, but if patients have disease manifestations in multiple sites immediate referral is indicated.

Box 1 | Targeted testing for antineutrophil cytoplasmic antibodies

Test when patients have one or more of the following sets of symptoms:

- General: Persistent flu-like condition with headache, myalgias, arthralgias, and weight loss
- Ear, nose, and throat: Hearing loss that slowly develops over days to weeks without a preceding cold, but with "chronic flu"; slowly developing nasal stenosis with midfacial pain and increasing bloody purulent secretion with crust formation that does not respond to antibiotics (granulomatosis with polyangiitis)
- Eyes: Unexplained conjunctivitis combined with general symptoms, uveitis, unilateral proptosis, and paresis of the ocular motor nerves (granulomatosis with polyangiitis)
- Lungs: Slowly developing cough and shortness of breath possibly with bloody-purulent sputum, bilateral infiltrates on radiography that do not respond to antibiotics, non-tuberculous cavitating lesions (granulomatosis with polyangiitis), alveolar haemorrhage (microscopic polyangiitis)
- Skin: Bursts of small cutaneous vasculitis elements, pyoderma gangraenosum, and oedema
- Kidneys: Haematuria, proteinuria, hypertension, decreasing renal function (granulomatosis with polyangiitis and microscopic polyangiitis)

Table 1 | Properties of ANCA tests and their clinical importance

	PR3-ANCA	MPO-ANCA
Test methods	Finely granular staining of the cytoplasm (c-ANCA) is seen on IIF; PR3 is the antigen in direct, capture, anchor, and luminex ELISAs	Perinuclear staining of the neutrophils (p-ANCA) is seen on IIF; MPO is used as the antigen in direct, capture, and luminex ELISAs
Diagnostic potential	Almost all patients in northern Europe with untreated acute granulomatosis with polyangiitis will be positive	Most patients in northern Europe with untreated acute microscopic polyangiitis and some with granulomatosis with polyangiitis will be positive
Relation to disease activity	Immunomodulatory treatment reduces positivity for PR3-ANCA, but positivity increases when treatment is tapered off; reappearance of PR3-ANCA in non-treated patients may reflect disease activity	Immunomodulatory treatment also reduces positivity for MPO-ANCA and it increases when treatment is tapered off, but fluctuations often occur that are not related to disease activity

ANCA=antineutrophil cytoplasmic autoantibodies; ELISA=enzyme linked immunosorbent assay; IIF=indirect immunofluorescence; MPO=myeloperoxidase; PR3=proteinase 3.

Refer any patient with a positive ANCA test result to a specialist in vasculitis, usually a rheumatologist or a nephrologist, or possibly a chest physician or ear, nose, and throat surgeon, depending on clinical presentation (box 2). Referral to specialists without experience with vasculitis may delay diagnosis. Many conditions can be associated

with a positive ANCA test result, including inflammatory bowel disease, chronic infections (such as tuberculosis), and autoimmune conditions such as systemic lupus erythematosus and rheumatoid arthritis, and ANCA can be induced by several drugs. This highlights the need for judicious testing. The clinical setting in which the test is performed is crucial for interpreting the results. We recommend ANCA testing routinely in the following circumstances: acute or chronic destructive upper airway disease; evidence of renal inflammatory disease as indicated by an active urine sediment or laboratory parameters indicative of rapidly progressive glomerulonephritis; evidence of pulmonary inflammatory disease as indicated by a variety of clinical symptoms or radiographic abnormalities; skin vasculitis associated with systemic illness; and mononeuritis multiplex. ANCA testing is also indicated in subglottic stenosis of the trachea manifesting as slowly progressive dyspnoea and retro-orbital mass manifesting as protrusion of the eye bulb and diplopia, although these conditions may be difficult to recognise without specialist tests.

Box 2 | Specialist otolaryngological management of patients with granulomatosis with polyangiitis

These patients most often have problems in the nose and sinuses. Initially midfacial pain, nasal stenosis, bloody-purulent rhinorrhoea, crust formations that may grow into large "casts" of the entire nasal cavities, and hearing loss are important problems, but these symptoms often disappear on treatment. However, sometimes symptoms do not resolve or slowly reappear as treatment is tapered. Most patients with nasal symptoms have a chronic nasal infection with *S aureus*. Daily nasal lavage with saline is helpful but not curative. Long term antibiotics can be effective, but the infection often reappears when they are tapered. As chronic infection may be difficult to differentiate from smouldering disease, patients may need to be seen by ear, nose, and throat surgeons with experience in vasculitis.

Some patients eventually develop destructive midface lesions, such as the saddle nose deformity, and in these cases plastic surgery can be performed when the disease is inactive and patients are on low dose immunomodulatory treatment.

Investigations performed in specialist care

Computed tomography scanning will provide additional information on the location and nature of lesions identified on chest radiography. A diagnosis of ANCA associated vasculitis is confirmed by specific abnormalities found on tissue biopsies obtained from sites of active disease, such as vasculitis, giant cells, "geographical necrosis," and granulomas. In granulomatosis with polyangiitis, biopsies from the respiratory tract—mainly nose and sinuses—often do not show more than one of the histopathological

hallmarks.[18] In such cases, the doctor has to treat the patient on the basis of typical clinical findings or a positive ANCA test (or both). Multiple biopsies from active lesions, if possible taken from different organs at different times, increase the chance of establishing a histological diagnosis. When the kidneys are affected, renal biopsies have a higher diagnostic yield, generally showing variable amounts of focal necrotising glomerulonephritis. In international randomised unblinded controlled trials that investigated more than 95 renal biopsies, a histopathological diagnosis of ANCA associated glomerulonephritis could be established in 80-98% of biopsies.[11,13,19]

HOW CAN A SPECIALIST IN VASCULITIS BE FOUND?

Not all hospitals will have an expert on vasculitis, but many regional centres have local or national experts or groups of experts. It is not always easy to contact such experts; local patient organisations, under the umbrella of Vasculitis UK or the Vaculitis Foundation are a useful resource because they have regular contact with regional experts in their area. In the UK, experts are often linked to societies or organisations because they run educational or research meetings and are usually engaged in active research programmes. International organisations such as the Vasculitis Foundation in the United States and the European Vasculitis Society (EUVAS) can help with inquiries to locate an expert.

WHAT IS THE NATURAL COURSE OF DISEASE IF LEFT UNTREATED?

A key natural history study of 56 patients in 1958 found that average patient survival was about five months; 82% of patients did not survive the first year after diagnosis and more than 90% of patients died within two years. The main cause of death was “uraemia” as a result of rapidly progressive renal failure, and the second most common cause was respiratory failure.[20]

HOW IS ANCA ASSOCIATED VASCULITIS CURRENTLY TREATED?

Standard treatment consists of inducing remission with high dose glucocorticoids and high dose oral or intravenous pulse cyclophosphamide for three to six months, and maintaining remission with azathioprine or methotrexate while glucocorticoids are slowly reduced and withdrawn.[21]

Trials with intravenous pulse cyclophosphamide as induction therapy have used a minimum six month course. Courses of intravenous pulse cyclophosphamide of less than six months may still be appropriate when rapid disease control is obtained.

Treatment requires specialist supervision; it aims to control disease activity to prevent further damage to organs and to prevent the recurrence of vasculitis. Managing treatment toxicity is an important part of patient care, and the general practitioner may be confronted with this problem (box 3).

According to a large randomised unblinded trial, 75% of patients treated with daily oral cyclophosphamide and prednisolone achieve remission by three months. By that time, prednisolone is usually reduced to 10-15 mg/day,[12] and cyclophosphamide is withdrawn because of the risk of cumulative toxicity and replaced with an alternative immunosuppressant. Patients who do not respond initially to treatment continue with induction treatment for longer and may be considered for second line treatment.[22]

The optimum duration of maintenance treatment is not known and practice differs widely between centres. Alternative maintenance immunosuppressive agents, such as mycophenolate mofetil, might be indicated in individual patients.

Although methotrexate has been used as induction therapy in place of the potentially more toxic cyclophosphamide for limited or non-severe disease, an unblinded randomised controlled trial of 100 patients found that it was associated with higher relapse rates, so its use remains controversial.[10]

In the longer term, regular visits to the specialist are needed (every three months at least) to check on disease activity and treatment side effects, and to manage the consequences of irreversible tissue damage, such as renal failure.

Box 3 | Side effects of commonly used immunosuppressive agents

Cyclophosphamide*: Leucopenia or neutropenia, infections (usually respiratory and urinary tract), infertility, cancer (especially bladder cancer and leukaemia), haemorrhagic cystitis, alopecia, and amenorrhea.

Glucocorticoids: Osteoporosis, candida infection (oral and vaginal), other infections, weight gain, hyperglycaemia or diabetes, hypertension, Cushingoid appearance, skin atrophy, and cataract.

Azathioprine: Nausea, leucopenia or neutropenia, infection, hypersensitivity, cancer, alopecia, cholestasis, and thrombocytopenia.

Methotrexate: Nausea, oral ulcers, liver dysfunction, infection, hypertension, leucopenia.

Mycophenolate mofetil: Infection, leucopenia, gastrointestinal tract manifestations, anaemia, thrombocytopenia.

Rituximab: Infections (encephalitis is particularly dangerous), cancer, anaemia, neutropenia, thrombocytopenia, hypogammaglobulinaemia.

*Cyclophosphamide can be given in two ways: daily oral cyclophosphamide and pulse cyclophosphamide. In the CYCLOPS trial the group that received pulse cyclophosphamide had significantly less leucopenia than the daily oral group. During pulse administration, the patient can be given prehydration and 2-mercaptoethanesulfonate sodium to protect the bladder against the toxicity of cyclophosphamide.[11]

With modern treatment ANCA associated vasculitis has changed from being an imminently life-threatening condition to a chronic condition prone to relapse throughout life. A large observational study of 107 patients found that about 50% of treated patients experience one or more relapses by five years.[23]

Long term follow-up studies have clearly shown that reducing exposure to cyclophosphamide is associated with a higher risk of late relapse, so a balance is needed between reduced exposure to cyclophosphamide and the increased risk of relapse.[24]

Prospectively collected data from 524 patients showed that important risks of treatment with cyclophosphamide include infection, infertility, and incident cancer.[25] Long term treatment with corticosteroids also has many side effects. These adverse effects drive the search for more efficacious and safer treatment modalities.

NEWER THERAPEUTIC AGENTS UNDER STUDY

Two recent prospective randomised controlled trials found that the B cell depleting agent rituximab effectively induced remission in patients with ANCA associated vasculitis, and that its safety profile was comparable with that of standard treatment.[26,27] Further investigation of the efficacy and safety of rituximab is needed, although it was recently approved by the Food and Drug Administration in the US for use in combination with glucocorticoids to treat patients with granulomatosis with polyangiitis and microscopic polyangiitis.

Plasma exchange has been investigated as an adjunct to standard treatment for patients with severe renal disease. In 2007, a large international randomised controlled trial of 137 patients favoured plasma exchange over methylprednisolone as adjunctive treatment with regard to recovery of renal function,[13] but a recent meta-analysis concluded that more data are needed to establish the long term benefit of this treatment.[28]

Plasma exchange is generally safe. A large study of 7538 exchanges in 887 patients showed side effects and technical incidents in 16.8% of all exchanges. This included a transfusion reaction in 6.9%, insufficient flow rate in 5%, hypotension in 2.9%, electrocardiographic alterations in 1.8%, hypocalcaemia in 1.4%, collapse in 0.9%, and pulmonary distress in 0.5%. Plasma exchange was discontinued in only 4% of cases.[29]

WHAT IS THE LONG TERM OUTLOOK FOR PATIENTS WITH ANCA ASSOCIATED VASCULITIS?

With modern treatment the disease has changed from being universally fatal to being a chronic relapsing and remitting disease. Several organs are often affected; renal involvement is common, and glomerulonephritis results in end stage renal failure and a need for renal replacement in 20-40% of patients according to observational studies (within a median follow-up of 3.1 to ≥5 years).[30-33]

The risk of death for patients treated with current treatments is still 2.6 times higher than that of age matched background controls.[34] The increased risk of death is greatest in the first year after diagnosis, when infections and active vasculitis account for most early deaths. Older patients with severe renal impairment have a particularly high risk of dying in the first few months after presentation; this reflects the severity of their disease as well as their increased susceptibility to the toxicity of current treatments.[34] Mortality in patients who survive the first year after diagnosis is still 1.3 times higher than that of age matched population controls. Death after the first year is mainly caused by infections, cardiovascular disease, and cancer.[34]

Patients are at lifelong increased risk of infections and often need treatment with antibiotics. In a population based case-control study, many patients reported fatigue, which affected employment and overall quality of life, as a major problem.[35] The socioeconomic impact of the disease, however, has proved difficult to assess.[36]

WHAT SHOULD GENERALISTS BE AWARE OF WITH REGARD TO TREATMENT?

Patients may turn to their general practitioner for support and information. The first few weeks of treatment can be difficult because patients usually still have symptoms associated with vasculitis. Frequent hospital visits and blood tests are needed to monitor disease activity and response to treatment.

Before each treatment with high dose cyclophosphamide, platelet and white cell counts, particularly the neutrophil count, must be above the lower limit of normal and liver function must be stable. Creatinine concentrations are needed to make dose adjustments. For daily oral cyclophosphamide, azathioprine, or mycophenolate mofetil, and for weekly methotrexate, routine monitoring of blood counts, liver function, and renal function are important to avoid drug toxicity. It is usually more convenient for patients to attend the primary care practice for these routine blood tests, but this requires good communication between primary care and secondary care. Guidelines

on how treatment should be changed in response to unexpected results should be agreed on before treatment.

Patients are susceptible to infections particularly during induction treatment (most usually respiratory or urinary tract infections), and early intervention with antibiotics is necessary for confirmed infections. In case of pulse cyclophosphamide, which is routinely followed by antiemetics and oral antifungal treatment, the most vulnerable time for patients is seven to 10 days after each pulse. The drug suppresses the bone marrow, causing neutropenia and increased risk of infection. Patients are advised not to visit or be visited by anyone with an upper or lower respiratory tract infection during this time. Patients on pulse cyclophosphamide may become profoundly tired in the two to three days after administration but will gradually improve. However, be aware of potential drug interactions. For example, patients with granulomatosis with polyangiitis may be treated with high doses of methotrexate and could develop severe neutropenia if treated for a urinary tract infection with standard dose co-trimoxazole; prophylactic use of low dose co-trimoxazole (960 mg three times a week) is therefore suggested as standard concomitant treatment with methotrexate.

Remember to consider consequences of damage caused by the disease, such as chronic kidney disease, and the effects of treatment, such as osteoporosis and impaired glycaemic control caused by chronic exposure to glucocorticoids. Patients are also at increased risk of cancer and premature and accelerated atherosclerosis, which predisposes them to early cardiovascular disease, including strokes.[37-41] It is important to control and treat all well known cardiovascular risk factors. Three observational studies found an increased incidence of venous thromboembolic events, which did not seem to be attributable to classic prothrombic risk factors.[42-44] Proton pomp inhibitors are prescribed to prevent mucosal damage of the stomach as a result of high doses of glucocorticoids. Patients taking immunosuppressive drugs usually require prophylaxis with co-trimoxazole because of an increased risk of acquiring *Pneumocystis jiroveci* pneumonia (box 4).

Box 4 | Important points for generalists

Regularly follow up the patient and be aware of some specific problems and prophylactic strategies*

Specific problems and prophylactic strategies

- Osteoporosis: Use of bisphosphonates
- Cardiovascular disease: Use of statins and smoking cessation
- Hyperglycaemia or diabetes: Regular glycaemic control
- Hypertension: Regular blood pressure control
- Gastric mucosal damage: Use of a proton pump inhibitor
- Oral ulcers: Use of folic acid or folinic acid
- *Pneumocystis jiroveci* pneumonia: Prophylactic co-trimoxazole
- Fatigue: Informal support or formal counselling

*The specialist will supervise this, but generalists need to be aware. If one of these problems occurs, the patient must be seen immediately by the doctor in charge at the vasculitis clinic. Rapid referral to specialist care is also needed if patients become ill with no obvious cause, especially if they report unusual symptoms.

Because relapse can occur after many years in remission, patients often remain on indefinite follow-up. Disease flares are common and patients must be encouraged to seek urgent medical attention if they experience a flare—that is, recurrence, deterioration, or new onset of symptoms and signs attributable to active vasculitis (box 5). If patients have a flare or experience serious complications of treatment they may require hospital admission. If early symptoms are missed or ignored, a serious episode of vasculitis with renal or respiratory failure may ensue. Patients are usually educated to look out for early symptoms of relapse so that this kind of avoidable disaster can be prevented.

Box 5 | Characteristics of a flare

Flare: Recurrence, deterioration, or new onset of symptoms and signs attributable to active vasculitis.

Major flare: Recurrence, deterioration, or new onset of at least one item on the Birmingham vasculitis activity score (see supplementary material),[45-47] indicating a threat to vital organ function as a result of active vasculitis. Examples include:

- 30% increase of creatinine or 25% decrease of glomerular filtration rate within three months
- Evidence of severe pulmonary haemorrhage or granulomata
- Threatened vision (including orbital granuloma and retinal vasculitis)
- Sensorineural deafness
- New multifocal neurological lesions or mononeuritis multiplex
- Gastrointestinal haemorrhage or perforation

Minor flare: Recurrence, deterioration, or new onset of at least three other items on the Birmingham vasculitis activity score related to non-vital organs attributable to active vasculitis. Examples include:

- Epistaxis, nasal crusting, lesions on nasal endoscopy
- Conductive deafness
- Deafness
- Rash
- Myalgia, arthralgia, arthritis
- (Epi)scleritis
- Pulmonary symptoms not characteristic of a major relapse

WHAT CAUSES VASCULITIS?

Although the precise causes are unknown, ANCA associated vasculitis probably results from an interplay between genetic and environmental factors. Several associations have been made with genes that encode proteins involved in immunity, which are also often associated with other autoimmune diseases.[48] However, family members or twins of patients with granulomatosis with polyangiitis are rarely reported to have disease manifestations, which goes against a strong genetic predisposition.[1] A recent study using Swedish nationwide registers did show some increased familial occurrence of granulomatosis with polyangiitis, which might denote genetic susceptibility to the disease, but exposure to similar environmental factors might also induce familial clustering.[49]

Among the environmental factors that have been implicated are occupational exposure to silica (such as farming, construction work), antithyroid and antihypertensive drugs (propylthiouracil and hydralazine), and several microbial agents, particularly *Staphylococcus aureus*.[50] Chronic nasal carriage of this agent has been associated with a higher incidence of relapse in granulomatosis with polyangiitis,[51] and a multicentre randomised double blind controlled trial showed that prophylactic treatment with co-trimoxazole reduces the incidence of relapses in granulomatosis with polyangiitis.[52]

All these factors may play a role in the development of ANCA, which are generally considered a pathogenic factor. The most direct clinical evidence for their pathogenicity is the development of pulmonary renal syndrome in a neonate shortly after being born to a mother with myeloperoxidase (MPO)-ANCA positive microscopic polyangiitis, probably because of transplacental transmission of maternal MPO-ANCA.[53,54] No other such cases have been reported, however, and a full term healthy normal child was recently born to a mother with microscopic polyangiitis, despite transplacental transfer of MPO-ANCA.[55] The development of a mouse model in which injection of MPO-ANCA induced glomerulonephritis and vasculitis similar to that seen in human disease provides strong in vivo evidence for the pathogenicity of these antibodies.[56] However, to date, an equally good model has not been developed for proteinase 3 (PR3)-ANCA.

A key role for neutrophils in the acute injury to the blood vessel wall is firmly established in ANCA associated vasculitis. Priming of circulating neutrophils by cytokines, possibly during infection, is thought to underlie local accumulation of neutrophils in the disease. Neutrophil priming causes PR3 and MPO to be expressed on the neutrophil cell membrane, where it becomes accessible to ANCA. Neutrophils activated by ANCA degranulate, produce reactive oxygen species, and release proteolytic enzymes that damage blood vessel walls.[57,58] Apart from ANCA and neutrophils, abnormalities in cellular immunity are probably important,[59] and in recent years an important role for the alternative pathway of complement has also been proposed. Because human ANCA associated glomerulonephritis is pauci-immune, with virtually no deposition of complement in the renal tissue, it had been assumed that complement played no role in this disease. However, recent studies support a role for the alternative pathway of complement in experimental models and in human pauci-immune MPO-ANCA associated vasculitis.[60,61]

Questions for future research

What is the optimum duration of maintenance treatment? This question may be answered by the findings of the REMAIN trial (AVERT project BIOMED-2: BMH-CT93-1078, trial registration number REMAIN 08.022006; www.vasculitis.org) conducted by EUVAS, which is investigating benefits of prolonged maintenance treatment.

With increasing insights into the pathogenesis of the disease and the detection of new biomarkers, might new targeted treatments replace existing standard treatments?

How might we develop patient tailored treatments for patients on the basis of clinical signs and symptoms in combination with genetic information? CCX168, an antagonist of complement factor C5a, is currently a candidate for clinical development.

What are the benefits of plasma exchange? A EUVAS/Vasculitis Clinical Research Consortium (VCRC) trial (PEXIVAS), which is designed to confirm and further explore the benefit of adjuvant plasma exchange, is currently under way.

A question that is important to patients is "How can fatigue associated with vasculitis and its treatment be managed?"

What causes the development of antineutrophil cytoplasmic antibodies (ANCA) and how do we prevent ANCA-associated vasculitis?

More basic research studies are needed to answer these questions.

DISCLOSURES

DJ has received a grant and consulting fee/honorarium from Roche; RL does consultancy of Chemocentryx and Nordic, reports for solicitors on individual cases (expert testimony), is in discussion with Nordic for administrative support to oversee data collection for a Nordic funded study in vasculitis (grants/grants pending), gets paid for lectures including service on speakers bureaus for UCB, receives royalties from EPS research for software that he originally designed to manage use of biologic therapy within NICE guidelines, and receives travel, accommodation and registration fees to attend the annual American College of Rheumatology and European League Against Rheumatism meetings, NR gets paid for lectures including service on speakers bureaus for Phadia and EuroDiagnostica, IB does consultancy of Roche on lupus nephritis; no other relationships or activities that could appear to have influenced the submitted work.

A patient's perspective

Nine years ago I visited my general practitioner with pain in my right knee. He tested twice for rheumatoid arthritis but the results were negative. I was then referred to the rheumatologist at the university clinic. A few months later most of my joints were affected and it seemed that I would be condemned to a wheelchair. The rheumatologist diagnosed rheumatoid arthritis and treated me accordingly. One month later my condition had worsened—I was vomiting two or three times a day, my eyes were reddish, and I had nasal crusting. My rheumatologist had seen a patient with these symptoms once before and she tested me for various factors including antineutrophil cytoplasmic antibody. The next day I was seen by her and a nephrologist. The new diagnosis was Wegener's granulomatosis. This news was a blessing in disguise. I would probably walk again, but I had lost 50% of my renal function. My treatment consisted of heavy immunosuppression including corticosteroids. Over the nine years that followed I had two cataract operations, a prosthetic knee implant, many erythropoietin injections, and a kidney transplant (a gift from my wife); I also experienced Guillain-Barré syndrome, cerebral haemorrhage, and constant bronchitis. Now I am fine. At the age of 69, I now have enough energy to enjoy life.

Henk van Wilpe, *Utrecht*

Additional educational resources

Resources for clinicians

Europe European Vaculitis Society (www.vasculitis.org/) and US Vasculitis Clinical Research Consortium (http://rarediseasesnetwork.epi.usf.edu/vcrc/)—Research collaboratives that focus on vasculitis.

Johns Hopkins Vasculitis Center (www.hopkinsvasculitis.org/)—Provides detailed information on vasculitis.

Fries JF, Hunder GG, Bloch DA, Michel BA, Arend WP, Calabrese LH, et al. The American College of Rheumatology 1990 criteria for the classification of vasculitis. Summary. *Arthritis Rheum* 1990;33:1135-6

Resources for patients

Vasculitis Foundation (www.vasculitisfoundation.org)—US website providing relevant information for doctors and patients.

Vasculitis Foundation Canada (www.vasculitis.ca)—Canadian website providing information and support for patients.

The Dutch Vaculitis Patient Foundation (www.vasculitis.nl)—Dutch website providing useful information for medical professionals and patients.

Vasculitis UK (www.vasculitis-uk.org.uk/news.html)—Provides information on vasculitis and on local support groups.

REFERENCES

1 Abdou NI, Kullman GJ, Hoffman GS, et al. Wegener's granulomatosis: survey of 701 patients in North America. Changes in outcome in the 1990s. *J Rheumatol,* 2002;29: 309-316.

2 Watts RA, Scott DG, Jayne DR, et al. Renal vasculitis in Japan and the UK—are there differences in epidemiology and clinical phenotype? *Nephrol Dial Transplant,* 2008;23: 3928-3931.

3 Watts RA, Lane SE, Bentham G, Scott DG. Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. *Arthritis Rheum,* 2000;43: 414-419.

4 Mahr A, Guillevin L, Poissonnet M, Ayme S. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum,* 2004;51: 92-99.

5 Watts RA, Lane SE, Scott DG, et al. Epidemiology of vasculitis in Europe. *Ann Rheum Dis,* 2001;60: 1156-1157.

6 Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med,* 1992;116: 488-498.

7 Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med,* 1997;337: 1512-1523.

8 Reinhold-Keller E, Beuge N, Latza U, et al. An interdisciplinary approach to the care of patients with Wegener's granulomatosis: long-term outcome in 155 patients. *Arthritis Rheum,* 2000;43: 1021-1032.

9 Stone JH. Limited versus severe Wegener's granulomatosis: baseline data on patients in the Wegener's granulomatosis etanercept trial. *Arthritis Rheum,* 2003;48: 2299-2309.

10 De Groot K, Rasmussen N, Bacon PA, et al. Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum,* 2005;52: 2461-2469.

11 De Groot K, Harper L, Jayne DR, et al. Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial. *Ann Intern Med,* 2009;150: 670-680.

12 Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med,* 2003;349: 36-44.

13 Jayne DR, Gaskin G, Rasmussen N, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol,* 2007;18: 2180-2188.

14 Hagen EC, Daha MR, Hermans J, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int,* 1998;53: 743-753.

15 Savige J, Gillis D, Benson E, et al. International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am J Clin Pathol,* 1999;111: 507-513.

16 Arnold DF, Timms A, Luqmani R, Misbah SA. Does a gating policy for ANCA overlook patients with ANCA associated vasculitis? An audit of 263 patients. *J Clin Pathol,* 2010;63: 678-680.

17 McLaren JS, Stimson RH, McRorie ER, Coia JE, Luqmani RA. The diagnostic value of anti-neutrophil cytoplasmic antibody testing in a routine clinical setting. *QJM*, 2001;94: 615-621.
18 Rasmussen N, Petersen J, Jensen H, Andersen V. Histopathological findings in biopsies from patients with Wegener's granulomatosis. *APMIS Suppl*, 1990;19: 15-16.
19 Hauer HA, Bajema IM, van Houwelingen HC, et al. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int*, 2002;62: 1732-1742.
20 Walton EW. Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *BMJ*, 1958;2: 265-270.
21 Pagnoux C, Mahr A, Hamidou MA, et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med*, 2008;359: 2790-2803.
22 Mukhtyar C, Guillevin L, Cid MC, et al. EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis*, 2009;68: 310-317.
23 Hogan SL, Nachman PH, Wilkman AS, Jennette JC, Falk RJ. Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol*, 1996;7: 23-32.
24 Harper L, Morgan MD, Walsh M, et al. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. *Ann Rheum Dis*, 2012;71: 955-960.
25 Little MA, Nightingale P, Verburgh CA, et al. Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. *Ann Rheum Dis*, 2010;69: 1036-1043.
26 Jones RB, Tervaert JW, Hauser T, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med*, 2010;363: 211-220.
27 Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med*, 2010;363: 221-232.
28 Walsh M, Catapano F, Szpirt W, et al. Plasma exchange for renal vasculitis and idiopathic rapidly progressive glomerulonephritis: a meta-analysis. *Am J Kidney Dis*, 2011;57: 566-574.
29 Bussel A, Jais JP. Side effects and mortality associated with plasma exchange: a three year experience with a regional register. *Life Support Syst*, 1987;5: 353-358.
30 Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis*, 2003;41: 776-784.
31 Koldingsnes W, Nossent H. Predictors of survival and organ damage in Wegener's granulomatosis. *Rheumatol (Oxford)*, 2002;41: 572-581.
32 Little MA, Nazar L, Farrington K. Outcome in glomerulonephritis due to systemic small vessel vasculitis: effect of functional status and non-vasculitic co-morbidity. *Nephrol Dial Transplant*, 2004;19: 356-364.
33 Slot MC, Tervaert JW, Franssen CF, Stegeman CA. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int*, 2003;63: 670-677.
34 Flossmann O, Berden A, De Groot K, et al. Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis*, 2011;70: 488-494.
35 Basu N, Jones GT, Fluck N, et al. Fatigue: a principal contributor to impaired quality of life in ANCA-associated vasculitis. *Rheumatol (Oxford)*, 2010;49: 1383-1390.

36 Cotch MF. The socioeconomic impact of vasculitis. *Curr Opin Rheumatol,* 2000;12: 20-23.
37 De Leeuw K, Sanders JS, Stegeman C, Smit A, Kallenberg CG, Bijl M. Accelerated atherosclerosis in patients with Wegener's granulomatosis. *Ann Rheum Dis,* 2005;64: 753-759.
38 Faurschou M, Mellemkjaer L, Sorensen IJ, Svalgaard TB, Dreyer L, Baslund B. Increased morbidity from ischemic heart disease in patients with Wegener's granulomatosis. *Arthritis Rheum,* 2009;60: 1187-1192.
39 Heijl C, Harper L, Flossmann O, et al. Incidence of malignancy in patients treated for antineutrophil cytoplasm antibody-associated vasculitis: follow-up data from European Vasculitis Study Group clinical trials. *Ann Rheum Dis,* 2011;70: 1415-1421.
40 Morgan MD, Turnbull J, Selamet U, et al. Increased incidence of cardiovascular events in patients with antineutrophil cytoplasmic antibody-associated vasculitides: a matched-pair cohort study. *Arthritis Rheum,* 2009;60: 3493-3500.
41 Suppiah R, Judge A, Batra R, et al. A model to predict cardiovascular events in patients with newly diagnosed Wegener's granulomatosis and microscopic polyangiitis. *Arthritis Care Res (Hoboken),* 2011;63: 588-596.
42 Merkel PA, Lo GH, Holbrook JT, et al. Brief communication: high incidence of venous thrombotic events among patients with Wegener granulomatosis: the Wegener's Clinical Occurrence of Thrombosis (WeCLOT) study. *Ann Intern Med,* 2005;142: 620-626.
43 Stassen PM, Derks RP, Kallenberg CG, Stegeman CA. Venous thromboembolism in ANCA-associated vasculitis—incidence and risk factors. *Rheumatol (Oxford),* 2008;47: 530-534.
44 Weidner S, Hafezi-Rachti S, Rupprecht HD. Thromboembolic events as a complication of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum,* 2006;55: 146-149.
45 Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham vasculitis activity score (version 3). *Ann Rheum Dis,* 2009;68: 1827-1832.
46 Luqmani RA, Bacon PA, Moots RJ, et al. Birmingham vasculitis activity score (BVAS) in systemic necrotizing vasculitis. *QJM,* 1994;87: 671-678.
47 Luqmani RA, Exley AR, Kitas GD, Bacon PA. Disease assessment and management of the vasculitides. *Baillieres Clin Rheumatol,* 1997;11: 423-446.
48 Willcocks LC, Lyons PA, Rees AJ, Smith KG. The contribution of genetic variation and infection to the pathogenesis of ANCA-associated systemic vasculitis. *Arthritis Res Ther,* 2010;12: 202.
49 Knight A, Sandin S, Askling J. Risks and relative risks of Wegener's granulomatosis among close relatives of patients with the disease. *Arthritis Rheum,* 2008;58: 302-307.
50 De Lind van Wijngaarden RA, van Rijn L, Hagen EC, et al. Hypotheses on the etiology of antineutrophil cytoplasmic autoantibody associated vasculitis: the cause is hidden, but the result is known. *Clin J Am Soc Nephrol,* 2008;3: 237-252.
51 Stegeman CA, Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CG. Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med,* 1994;120: 12-17.

52 Stegeman CA, Tervaert JW, de Jong PE, Kallenberg CG. Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N Engl J Med*, 1996;335: 16-20.

53 Bansal PJ, Tobin MC. Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. *Ann Allergy Asthma Immunol*, 2004;93: 398-401.

54 Schlieben DJ, Korbet SM, Kimura RE, Schwartz MM, Lewis EJ. Pulmonary-renal syndrome in a newborn with placental transmission of ANCAs. *Am J Kidney Dis*, 2005;45: 758-761.

55 Silva F, Specks U, Sethi S, Irazabal MV, Fervenza FC. Successful pregnancy and delivery of a healthy newborn despite transplacental transfer of antimyeloperoxidase antibodies from a mother with microscopic polyangiitis. *Am J Kidney Dis*, 2009;54: 542-545.

56 Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest*, 2002;110: 955-963.

57 Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A*, 1990;87: 4115-4119.

58 Porges AJ, Redecha PB, Kimberly WT, Csernok E, Gross WL, Kimberly RP. Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. *J Immunol,* 1994;153: 1271-1280.

59 Berden AE, Kallenberg CG, Savage CO, et al. Cellular immunity in Wegener's granulomatosis: characterizing T lymphocytes. *Arthritis Rheum,* 2009;60: 1578-1587.

60 Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol,* 2007;170: 52-64.

61 Xing GQ, Chen M, Liu G, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. *J Clin Immunol,* 2009;29: 282-291.

SUPPLEMENTARY MATERIAL

Diagnosis and management of ANCA-associated vasculitis

Birmingham Vasculitis Activity Score (version 3)

Patient ID: **Date of birth:** **Total score:**

Assessor: **Date of assessment:**

Tick an item **only** if attributable to active vasculitis. If there are no abnormalities in a section, please tick 'None' for that organ-system.			If **all** abnormalities are due to persistent disease (active vasculitis which is not new/worse in the prior 4 weeks), tick the **PERSISTENT** box at the bottom right corner		
Is this the patient's first assessment?			**Yes** ○	**No** ○	
	None	Active disease		None	Active disease
1. General	○		**6. Cardiovascular**	○	
Myalgia		○	Loss of pulses		○
Arthralgia / arthritis		○	Valvular heart disease		○
Fever ≥38° C		○	Pericarditis		○
Weight loss ≥2 kg		○	**◊ Ischaemic cardiac pain**		○
2. Cutaneous	○		**◊ Cardiomyopathy**		○
Infarct		○	**◊ Congestive cardiac failure**		○
Purpura		○	**7. Abdominal**	○	
Ulcer		○	Peritonitis		○
◊ Gangrene		○	Bloody diarrhoea		○
Other skin vasculitis		○	**◊ Ischaemic abdominal pain**		○
3. Mucous membranes / eyes	○		**8. Renal**	○	
Mouth ulcers		○	Hypertension		○
Genital ulcers		○	Proteinuria >1+		○
Adnexal inflammation		○	**◊Haematuria ≥10 RBCs/hpf**		○
Significant proptosis		○	Creatinine 125-249µ/L(1.41-2.82mg/dl)*		○
Scleritis / Episcleritis		○	Creatinine 250-499 µ/L(2.83-5.64mg/dl)*		○
Conjunctivitis / Blepharitis / Keratitis		○	**◊Creatinine ≥500 µ/L (≥5.66mg/dl)***		○
Blurred vision		○	**◊Rise in serum creatinine >30% or fall in creatinine clearance >25%**		○
Sudden visual loss		○			
Uveitis		○	***Can only be scored on the first assessment**		
◊Retinal changes (vasculitis / thrombosis / exudate / haemorrhage)		○	**9. Nervous system**	○	
			Headache		○
			Meningitis		○
4. ENT	○		Organic confusion		○
Bloody nasal discharge / crusts / ulcers / granulomata		○	Seizures (not hypertensive)		○
			◊Cerebrovascular accident		○
Paranasal sinus involvement		○	**◊Spinal cord lesion**		○
Subglottic stenosis		○	**◊Cranial nerve palsy**		○
Conductive hearing loss		○	Sensory peripheral neuropathy		○
◊Sensorineural hearing loss		○	**◊Mononeuritis multiplex**		○

5. Chest	○		
Wheeze	○	**10. Other**	○
Nodules or cavities	○	a.	○
Pleural effusion / pleurisy	○	b.	○
Infiltrate	○	c.	○
Endobronchial involvement	○	d.	○
◊ **Massive haemoptysis / alveolar haemorrhage**	○	**PERSISTENT DISEASE ONLY:**	
◊ **Respiratory failure**	○	(Tick here if **all** the abnormalities are due to persistent disease)	☐

◊ Major items highlighted
References: 45-47

7

SUMMARY AND GENERAL DISCUSSION

CHANGED CLINICAL ASPECTS

There were some recent changes in the daily practice of antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) patients. Indirect immunofluorescence (IIF) for the detection of ANCA is not standardly used anymore. Recent study showed a large variability between different IIF methods and a high diagnostic performance of proteinase (PR3)-ANCA and myeloperoxidase (MPO)-ANCA by Enzyme-Linked Immuno Sorbent Assay (ELISA). Therefore, the use of both IIF and ELISA testing of each sample is not necessary for maximal diagnostic accuracy.[1] Currently a biopsy is still the golden standard for obtaining a diagnosis. In case a biopsy is not possible or should be delayed, a presumptive diagnosis of AAV can be made in case there is a high probability of AAV based on the clinical presentation, ANCA positivity with ELISA and a low suspicion for another disease. With a presumptive diagnosis, initial therapy can be started, although a biopsy should be obtained as soon as possible to confirm the diagnosis.

The approach for initial therapy is based on the severity of the disease and the organs involved. In case of non-organ-threatening and non-life-threatening disease, i.e. in the absence of active glomerulonephritis, pulmonary hemorrhage etc., a regimen with glucocorticoids and methotrexate can be given. Rituximab or cyclophosphamide can be chosen instead of methotrexate. In case of organ-threatening or life-threatening disease glucocorticoids in combination with cyclophosphamide or rituximab can be started. In case of rapidly deteriorating kidney function, severe kidney dysfunction, pulmonary hemorrhage or severe respiratory impairment, adjunctive plasma exchange therapy is advised. Depending on local protocol, prophylaxis against opportunistic infections during induction therapy is given, i.e. trimethoprim-sulfamethoxazole.[2] For maintenance therapy glucocorticoids in combination with azathioprine or methotrexate are mostly used, although rituximab is also being investigated as maintenance therapy in the MAINRITSAN trial[3] and the RITAZAREM trial (RITAZAREM, ClinicalTrials.gov Identifier: NCT01697267). The MAINRITSAN trial showed that at 28 months more patients had remained in remission with rituximab compared to azathioprine. There was no difference in severe adverse events.[3] The optimal duration for maintenance therapy was investigated in the REMAIN trial. 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.[4] There is still controversy about the duration of glucocorticoids use.

ANTI-PLASMINOGEN AUTOANTIBODIES

It is a big challenge in AAV to detect the presence of anti-plasminogen autoantibodies (α-PLG). We developed an optimized ELISA for the detection of α-PLG, focusing on its usefulness in studies on AAV. We tested different assay set-ups. Purified lysine-plasminogen (lys-PLG) showed better differentiation between positive samples and negative samples compared to glutamic acid-plasminogen (glu-PLG). Therefore, lys-PLG was used as coating antigen. With the optimized α-PLG ELISA we found the presence of α-PLG in 14.3% of MPO-ANCA patients, whereas all our PR3-ANCA patients tested negative in our newly developed assay.

The available studies on α-PLG show a discrepancy in the presence of these autoantibodies in patients with AAV. We mainly detected α-PLG in MPO-ANCA patients, while others detected these antibodies mainly in PR3-ANCA patients or in both patient groups. These discrepancies could be due to differences in the assays used in the studies.[5-7] Examples of differences are different concentrations of coating antigen, different conjugates, different samples (sera vs. purified immunoglobulin G), and different definitions of positivity. We have combined important technical findings and methods from the previous studies which have led to the optimized α-PLG assay presented in chapter 2 of this thesis.

There are two main conformations of PLG, namely glu-PLG and lys-PLG, with glu-PLG being the native form. Lys-PLG is formed after cleavage of a peptide consisting of 77 amino acids at the N-terminal of glu-PLG and is an intermediate step towards the formation of active plasmin.[8] Both conformations differ in physical and functional properties.[9-11] Lys-PLG is more efficiently activated by PLG activators than glu-PLG.[12-14] The different studies on α-PLG published so far did not define which subtype of PLG was used for coating. For the assay developed by us, we used lys-PLG as coating, because it was better at differentiating between positive and negative control samples in our study.

The epitope(s) recognized by α-PLG in AAV are not yet fully defined. Different autoantibodies against different (conformational) epitopes of PLG may exist and this could (partly) explain the described discrepancies. Originally, α-PLG were described in view of anti-complementary PR3 antibodies which were suggested to develop within an idiotypic/anti-idiotypic antibody response.[5,15] Dual reactivity to PLG and complementary PR3 (cPR3) was described in PR3-ANCA patients.[5] This scenario assumed a combined presence of α-PLG with only PR3-ANCA and not MPO-ANCA. The cPR3 sequence has similarities with genetic sequences of microbial and fungal

organisms that code for peptides large enough to be antigenic.[15] If exogenous antigens like peptides of micro-organisms cause the development of autoantibodies against PLG, distinct geographic areas with different micro-organisms could influence the development of these autoantibodies. On the other hand, our study together with those of Berden *et al*. and Hao *et al*. reported the presence of α-PLG in MPO-ANCA patients in the absence of PR3-ANCA.[6,7] This makes the hypothesis regarding cPR3 and α-PLG less likely. In addition, some studies describe α-PLG which did not react to denatured PLG, plasmin or thrombin, while other studies showed cross-reactivity of antibodies between PLG, plasmin and prothrombin.[5,6,16-18] More knowledge about pathogenic epitopes is needed for further optimization of the α-PLG assay and to detect relevant autoantibodies in patients. In the meantime, our currently proposed α-PLG assay can be used for research purposes and further optimization.

The presence of α-PLG is associated with the presence of more active renal lesions in the biopsy, i.e. fibrinoid necrosis and cellular crescents, and worse renal function at 1 year follow-up.[6] The idea is that fibrinoid necrosis is a product of a flaw in the vascular repair system that fails to remove the fibrin clot caused by the vascular injury.[19-23] This flaw is caused by the inhibition of PLG due to α-PLG. In addition, the leaked fibrin seems to stimulate extracapillary proliferation.[24-26] This shows that the presence of these antibodies has an influence on the severity of renal involvement. Therefore, detecting these patients early with a validated α-PLG assay will help improve clinical management of these patients with an improved renal outcome.

PREDICTION OF RENAL RELAPSE

One of the main questions in daily practice when confronted with patients with AAV is how to recognize those who are at risk for disease relapse and whether relapses can be prevented. Identifying patients at high risk of renal relapse may aid in optimizing clinical management. Our study on risk factors for renal disease relapse showed that the histopathological class of ANCA-associated glomerulonephritis (AAGN) and the absence/presence of interstitial inflammatory infiltrates in the renal biopsy at diagnosis are risk factors for renal relapse. More specifically, sclerotic class is associated with a higher rate of renal relapse during long-term follow-up and the absence of interstitial inflammatory infiltrates is associated with the risk of renal relapse.

Previous European Vasculitis Society (EUVAS) studies focused on predictive clinical and serological parameters for relapse in general. Predictive parameters described in the literature are the presence of PR3-ANCA, lower serum creatinine levels at presentation, lung or cardiovascular involvement, and diagnosis of GPA.[27-37] Our

finding that the absence of interstitial infiltrates in renal biopsies predicts renal disease relapse is in line with better renal function increasing the risk for a relapse in general, because absence of interstitial infiltrates also correlates with better renal function at the time of biopsy.[38,39] Interestingly, in a cohort of 535 patients, no clinical parameter at baseline was associated with developing renal relapse.[40] Our study showed histological parameters at baseline that are risk factors for developing renal relapse. A clinical manifestation that is described to be predictive of renal relapse is persistent hematuria during follow-up.[41] Experiencing a renal disease relapse has a negative influence on renal outcome and is associated with end-stage renal failure (ESRF).[40,42] Therefore, clinicians should realize that renal disease relapses must be identified and treated as such.

In the sclerotic class most glomeruli are non-functioning with a decreased compensatory ability of the kidneys. Therefore, renal relapse may become more readily apparent and the functioning glomeruli may become more vulnerable to a second hit, i.e. a relapse. In the focal, crescentic and mixed class, minor relapses may remain subclinical. Patients with interstitial infiltrates (acute disease activity), have a more aggressive clinical disease presentation, making it easier to diagnose the disease early. Treatment will be started earlier and can therefore decrease the risk for renal disease relapse. Therefore, clinicians must keep in mind that those patients with a presumably benign clinical course at onset in particular might be prone to developing a renal disease relapse.

We hypothesize that patients may have smoldering disease in the kidney in which renal relapses may go by unnoticed in case of focal, crescentic and mixed class AAGN. This phenomenon has been described in systemic lupus erythematosus patients after renal transplantation, where subclinical class I, II or III recurrences in the kidney have been encountered unexpectedly in protocol biopsies of 22 patients from a total cohort of 41 patients.[43] Smoldering progression of disease may be devastating to the graft.[43,44] Smoldering disease in general and specifically in the upper and lower airways in AAV has been described.[45-47] In addition, autopsy studies in patients with AAV/GPA showed that persistent airway inflammation is more common than has been appreciated clinically.[48] In our study cohort several patients achieved remission, did not experience a renal disease relapse, but still developed ESRF after achieving remission. In these patients ESRF could be the result of smoldering disease with subclinical renal relapses.

It is also possible that the histopathological classes represent distinct autoimmune syndromes, rather than representing phases according to which the disease progresses.

In our cohort the ANCA-specificity was associated with the histopathologic class. PR3-ANCA was associated with focal class biopsies and MPO-ANCA was associated with sclerotic class. A recent genome-wide association study and meta-analysis in AAV showed that the pathogenesis of AAV has a genetic component. The differences found between GPA and microscopic polyangiitis (MPA) regarding genetic associations were driven by ANCA-specificity and not by clinically defined syndromes. So, PR3–AAV and MPO-AAV seem to have distinct genetic backgrounds and this supports the concept that PR3-AAV and MPO-AAV might be two distinct autoimmune syndromes.[49,50]

HISTOPATHOLOGICAL CLASSIFICATION OF ANCA-ASSOCIATED GLOMERULONEPHRITIS

Several studies described an association between renal histological parameters and renal outcome, for example percentage of normal glomeruli, crescentic glomeruli and sclerotic glomeruli.[38,39,51,52] This led to the introduction of a histopathological classification of AAGN in 2010.[53] This classification classifies each diagnostic renal biopsy into one of four classes; focal, crescentic, sclerotic and mixed class, based on the predominant glomerular phenotype. The first validation of this classification system in 100 patients showed an association with ESRD and renal function at 1- and 5-year follow-up.[53] Several subsequent validation studies confirmed this association regarding focal and sclerotic class, but had contradictory results regarding crescentic and mixed class.[54] Our worldwide validation study also confirmed a favorable outcome in the focal class and a poor outcome in the sclerotic class. Regarding crescentic and mixed class, there was no difference between renal outcome. This is in contrast to the findings of the original study, but is in line with results from 2 recent meta-analyses.[54,55]

The overall histopathological classification showed to be associated with renal function, even after correcting for other baseline parameters, and the development of ESRD during follow-up. However, the crescentic and mixed class were indiscriminate regarding renal function and developing ESRD. There are different possible explanations for these conflicting results regarding crescentic and mixed class. First, there could be differences between the patient populations studied. GPA, MPA and ANCA-specificity are differently distributed around the globe.[56,57] Our validation study showed that the histopathological class and diagnosis are associated with each other, and both variables are associated with renal outcome. Together, differences in patient population could partly be an explanation for the conflicting results. Unfortunately, our cohort was not large enough to analyze this hypothesis. Second, treatment regimens could be different between the patients in the different validation studies. Third, the interobserver agreement between nephropathologists was moderate in

our validation study. It is hard to translate this finding to the clinical practice in a one-on-one fashion, because of the differences in experience, but scoring the same diagnostic biopsy differently can cause the observed differences between the studies. Fourth, the current classification only incorporates normal, crescentic and sclerotic glomeruli. Adding other histological parameters could possibly refine the classification system. Fibrous crescents are not yet incorporated, although they showed to have some predictive value for long-term renal outcome.[39,58] In addition, tubulointerstitial parameters are not considered in the current histopathologic classification of AAGN, while histological studies showed that tubulointerstitial parameters have an association with renal outcome, even when used in addition to the histopathologic classification of AAGN.[38,39,58-67] Our study on renal relapse (chapter 3) and our validation study (chapter 4) confirmed this association.[68] Including tubulointerstitial parameters in the classification system might lead to refinements for the prognostication of patients at time of diagnosis.

The histopathological classification of AAGN is a valuable tool in the management of patients with AAV, but in the near future, adjustments are needed to improve its prognostic value, especially for the crescentic and mixed class. We are not considering to lump the crescentic and mixed class, in particular because other studies showed that cellular crescents are an important factor for predicting potential reversibility of renal impairment during follow-up.[38,39,53] Adding tubulointerstitial parameters could lead to refinements for the prognostication of patients at time of diagnosis, although the poor to moderate interobserver agreement regarding tubulointerstitial variables must also be considered. Currently, we are performing a study to evaluate a more detailed scoring system for both glomerular and interstitial variables. Results from that study will determine how to adjust the histopathological classification for AAGN for more sophisticated prognostic value.

RENAL TRANSPLANTATION

Approximately 20-40% of patients with AAGN progress, with or without clinically evident renal disease relapse, to ESRF.[27,47,69,70] One of the therapeutic options for these patients is a renal transplantation. The Dutch cohort described in this thesis of 113 AAGN patients transplanted between 1984 and 2011 showed one year and five year graft survival rates of 94.5% and 82.8%, respectively. This is similar to previous studies on AAGN and to the general transplantation population in Europe and North America.[71-80] The risk of experiencing a first disease relapse or renal disease recurrence within five years of transplantation was 3.3% and 2.8% per patient year, respectively. This is slightly higher compared to other studies, which described rates of 1.0-2.0%

per patient year.[72,73,75,79,81] Renal disease recurrence was an important cause of graft loss within the first five years after transplantation. Renal transplantation is a viable treatment option for patients with ESRF due to AAGN with rather low renal disease recurrence rates, but once renal disease recurrence has occurred the risk for graft loss is considerable.

The DUTRAVAS study showed that disease recurrence in the renal graft can occur at any time after transplantation. The first disease recurrence in the renal graft occurred nine days after transplantation. This patient was transplanted with ongoing disease activity, which explains the early recurrence. One patient even had a disease recurrence in the renal graft after 142 months. Early (even within days after transplantation, although transplanted during disease remission) and late recurrences of AAGN in the graft have also been described by others.[82-85] This supports that disease recurrence in the renal graft can occur at any time after transplantation. In case of deterioration of renal function, proteinuria and hematuria, even several years after transplantation, disease recurrence in the renal graft should be considered. Continuous and careful monitoring of these patients is important.

An important clinical question with no current formal consensus is time to renal transplantation after reaching clinical remission. A questionnaire sent out by Little *et al.* to transplant units in different countries revealed that 100% of the transplant physicians consulted (n=32) claim that a patient should be in remission at the moment of transplantation.[75] One patient in our cohort was transplanted with ongoing disease activity, had a renal disease recurrence and consequent graft loss within a month. An association between renal transplantation <12 months after reaching clinical remission and mortality was reported.[75] No association was found between the duration of remission before transplantation and disease relapse when taking three months of remission as a cut-off point.[72] However, current practice seems to be delaying the transplantation until the disease is in remission.[73,75,86,87]

Another important question regarding renal transplantation in AAV is whether a patient with a positive ANCA-test can be transplanted. There is still controversy whether a patient must be ANCA negative at the moment of transplantation.[75] Evidence is accumulating that patients with a positive ANCA can be transplanted.[69,72,73,86-92] A paper in 2013 pooled different studies and described an association between ANCA positivity at transplantation and relapse rate, although they mention very scarce information about how they pooled and analyzed these data.[84] The DUTRAVAS study shows that ANCA status at transplantation is not associated with renal disease recurrence and

graft survival. The number of patients in the cohort was too small to discuss PR3-ANCA and MPO-ANCA separately. Therefore, it seems that ANCA positivity at transplantation is not predictive for disease relapse and/or graft loss, which suggests that a patient with a positive ANCA can be transplanted safely. More research on this aspect is needed.

In the DUTRAVAS-study, 36% (4/11) of the patients with disease recurrence in the renal graft lost their graft due to the recurrence within five years after transplantation. Renal disease recurrence in the graft was an important cause of graft loss in this study; four of the 16 graft losses. Graft loss due to AAV recurrence in the graft has also been described in other studies; range 18-60%.[75,85,87,89,91] Briganti *et al*. described a cohort of 1505 patients with biopsy-proven glomerulonephritis due to various causes transplanted between 1988 and 1997 in which at one, five and 10 years after transplantation, recurrent glomerulonephritis was the third most common cause of graft loss. Acute rejection, chronic rejection and death with a functioning graft were more common. In the subgroup analysis, the same order of causes of graft loss was found at 10 years for the pauci-immune crescentic glomerulonephritis patients.[93] Concluding, renal disease recurrence is an important cause of graft loss and the risk for graft loss due to disease recurrence is something clinicians must be aware of.

FUTURE ISSUES

Although a substantial amount of research has been performed and much insight in AAV has been gained, there are still many questions for future research.

One of the clinical challenges in the care of AAV is to predict which patients will have a (renal) relapse of the disease. As literature and our study showed, clinical parameters and histologic lesions in the renal biopsy (glomerular and interstitial) might be of value for this. Future research should focus on specifying these predictors and investigating whether the current histopathological classification of AAGN needs adaptations. These clinical and histological parameters can help the clinician in determining the right therapy.

The use of ANCA titer to predict the course of disease, i.e. disease relapse, and measure current disease activity is controversial. A biomarker which can help the clinician regarding disease course would be very helpful for the clinical management. Therefore, the search for a biomarker should continue. α-PLG, as described in this thesis, has the potential to become such a biomarker to some extent. Other potential biomarkers described in the literature that need further research are B-cells, cytokines,

complement, inflammatory mediators (CXCL13, MMP-3, TIMP-1),[94] hLAMP-2 and urinary biomarkers (MCP1, CD 164, CD 25).[95]

One of the main questions regarding renal transplantation in AAV is how long a patient should be in remission before being transplanted. Currently there is only weak evidence that we should wait for at least one year.[75] The ideal way to answer this question would be an international randomized clinical trial (RCT) of countries with the same organ donor system in which patients are transplanted after different remission times.

There has to be a balance between the risk for adverse events due to the therapy and the chance for disease relapse. The last one still being a big challenge to prevent and treat in AAV. The risk for disease relapse has not changed much for the last few decades.[41] Currently, rituximab with longterm maintenance therapy seems the best option to treat disease relapse, although more research is needed in this field with a RCT specifically focusing on patients with a severe disease relapse. It is also investigated how to taper corticosteroid therapy.

An interesting therapeutic target is the complement system. A randomized trial showed that C5a receptor inhibition with avacopan was an effective treatment in replacing high-dose glucocorticoids in treating AAV patients with newly diagnosed or relapsing disease.[96]

Based on the different genetic background of PR3-ANCA and MPO-ANCA, it remains the question whether these two are different disease modalities and whether they should be treated differently. Until now PR3-ANCA and MPO-ANCA have been treated the same way. These insights will provide us the opportunity to provide better patient-tailored therapy.

REFERENCES

1. Damoiseaux J, Csernok E, Rasmussen N, et al. Detection of antineutrophil cytoplasmic antibodies (ANCAs): a multicentre European Vasculitis Study Group (EUVAS) evaluation of the value of indirect immunofluorescence (IIF) versus antigen-specific immunoassays. *Ann Rheum Dis,* 2016;76(4): 647-653.
2. Merkel PA, Kaplan AA, Falk RJ. Initial immunosuppressive therapy in granulomatosis with polyangiitis and microscopic polyangiitis. *Up To Date,* 2019.
3. Guillevin L, Pagnoux C, Karras A, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med,* 2014;371(19): 1771-1780.
4. Karras A, Pagnoux C, Haubitz M, et al. Randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis. *Ann Rheum Dis,* 2017;76(10): 1662-1668.
5. Bautz DJ, Preston GA, Lionaki S, et al. Antibodies with dual reactivity to plasminogen and complementary PR3 in PR3-ANCA vasculitis. *J Am Soc Nephrol,* 2008;19(12): 2421-2429.
6. Berden AE, Nolan SL, Morris HL, et al. Anti-plasminogen antibodies compromise fibrinolysis and associate with renal histology in ANCA-associated vasculitis. *J Am Soc Nephrol,* 2010;21(12): 2169-2179.
7. Hao J, Wang C, Gou SJ, Zhao MH, Chen M. The association between anti-plasminogen antibodies and disease activity in ANCA-associated vasculitis. *Rheumatology (Oxford),* 2014;53(2): 300-306.
8. Zhang L, Gong Y, Grella DK, Castellino FJ, Miles LA. Endogenous plasmin converts Glu-plasminogen to Lys-plasminogen on the monocytoid cell surface. *J Thromb Haemost,* 2003;1(6): 1264-1270.
9. Claeys H, Vermylen J. Physico-chemical and proenzyme properties of NH2-terminal glutamic acid and NH2-terminal lysine human plasminogen. Influence of 6-aminohexanoic acid. *Biochim Biophys Acta,* 1974;342(2): 351-359.
10. Thorsen S, Mullertz S. Rate of activation and electrophoretic mobility of unmodified and partially degraded plasminogen. Effects of 6-aminohexanoic acid and related compounds. *Scand J Clin Lab Invest,* 1974;34(2): 167-176.
11. Violand BN, Sodetz JM, Castellino FJ. The effect of epsilon-amino caproic acid on the gross conformation of plasminogen and plasmin. *Arch Biochem Biophys,* 1975;170(1): 300-305.
12. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J Biol Chem,* 1982;257(6): 2912-2919.
13. Markus G, Evers JL, Hobika GH. Comparison of some properties of native (Glu) and modified (Lys) human plasminogen. *J Biol Chem,* 1978;253(3): 733-739.
14. Markus G, Priore RL, Wissler FC. The binding of tranexamic acid to native (Glu) and modified (Lys) human plasminogen and its effect on conformation. *J Biol Chem,* 1979;254(4): 1211-1216.
15. Pendergraft III WF, Preston GA, Shah RR, et al. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med,* 2004;10(1): 72-79.
16. Puurunen M, Manttari M, Manninen V, Palosuo T, Vaarala O. Antibodies to prothrombin crossreact with plasminogen in patients developing myocardial infarction. *Br J Haematol,* 1998;100(2): 374-379.

17. Puurunen M, Palosuo T, Lassila R, Anttila M, Vaarala O. Immunologic and hematologic properties of antibodies to prothrombin and plasminogen in a mouse model. *Lupus,* 2001;10(2): 108-115.
18. Yang CD, Hwang KK, Yan W, et al. Identification of anti-plasmin antibodies in the antiphospholipid syndrome that inhibit degradation of fibrin. *J Immunol,* 2004;172(9): 5765-5773.
19. Bajema IM. *Aspects of ANCA-associated glomerulonephritis* [PhD thesis]. Leiden, The Netherlands, Leiden University; 2000.
20. Collen A. *Fibrin matrix structure and angiogenesis* [PhD thesis]. Leiden, The Netherlands, Leiden University; 2000.
21. Jennette JC, Wilkman AS, Falk RJ. Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol,* 1989;135(5): 921-930.
22. Novak RF, Christiansen RG, Sorensen ET. The acute vasculitis of Wegener's granulomatosis in renal biopsies. *Am J Clin Pathol,* 1982;78(3): 367-371.
23. Serra A, Cameron JS, Turner DR, et al. Vasculitis affecting the kidney: presentation, histopathology and long-term outcome. *Q J Med,* 1984;53(210): 181-207.
24. Channing AA, Kasuga T, Horowitz RE, Dubois EL, Demopoulos HB. An ultrastructural study of spontaneous lupus nephritis in the NZB-BL-NZW mouse. *Am J Pathol,* 1965;47(4): 677-694.
25. Silva FG, Hoyer JR, Pirani CL. Sequential studies of glomerular crescent formation in rats with antiglomerular basement membrane-induced glomerulonephritis and the role of coagulation factors. *Lab Invest,* 1984;51(4): 404-415.
26. Vassalli P, McCluskey RT. The Pathogenic Role of the Coagulation Process in Rabbit Masugi Nephritis. *Am J Pathol,* 1964;45: 653-677.
27. Booth AD, Almond MK, Burns A, et al. Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am J Kidney Dis,* 2003;41(4): 776-784.
28. Harper L, Morgan MD, Walsh M, et al. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated vasculitis: long-term follow-up. *Ann Rheum Dis,* 2012;71(6): 955-960.
29. Hogan SL, Falk RJ, Chin H, et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. *Ann Intern Med,* 2005;143(9): 621-631.
30. Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med,* 2003;349(1): 36-44.
31. Koldingsnes W, Nossent JC. Baseline features and initial treatment as predictors of remission and relapse in Wegener's granulomatosis. *J Rheumatol,* 2003;30(1): 80-88.
32. Kyndt X, Reumaux D, Bridoux F, et al. Serial measurements of antineutrophil cytoplasmic autoantibodies in patients with systemic vasculitis. *Am J Med,* 1999;106(5): 527-533.
33. Lionaki S, Blyth ER, Hogan SL, et al. Classification of antineutrophil cytoplasmic autoantibody vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. *Arthritis Rheum,* 2012;64(10): 3452-3462.
34. Pagnoux C, Hogan SL, Chin H, et al. Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis: comparison of two independent cohorts. *Arthritis Rheum,* 2008;58(9): 2908-2918.

35. Salmela A, Tornroth T, Poussa T, Ekstrand A. Prognostic Factors for Survival and Relapse in ANCA-Associated Vasculitis with Renal Involvement: A Clinical Long-Term Follow-Up Study. *Int J Nephrol,* 2018: 6369814.
36. Stegeman CA, Cohen Tervaert JW, Sluiter WJ, Manson WL, De Jong PE, Kallenberg CGM. Association of chronic nasal carriage of Staphylococcus aureus and higher relapse rates in Wegener granulomatosis. *Ann Intern Med,* 1994;120(1): 12-17.
37. Walsh M, Flossmann O, Berden A, et al. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum,* 2012;64(2): 542-548.
38. Bajema IM, Hagen EC, Hermans J, et al. Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int,* 1999;56(5): 1751-1758.
39. Hauer HA, Bajema IM, Van Houwelingen HC, et al. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int,* 2002;62(5): 1732-1742.
40. Wester Trejo MAC, Flossmann O, Westman KW, et al. Renal relapse in antineutrophil cytoplasmic autoantibody-associated vasculitis: unpredictable, but predictive of renal outcome. *Rheumatology (Oxford),* 2019;58(1): 103-109.
41. Rhee RL, Hogan SL, Poulton CJ, et al. Trends in Long-Term Outcomes Among Patients With Antineutrophil Cytoplasmic Antibody-Associated Vasculitis With Renal Disease. *Arthritis Rheumatol,* 2016;68(7): 1711-1720.
42. de Joode AA, Sanders JS, Stegeman CA. Renal survival in proteinase 3 and myeloperoxidase ANCA-associated systemic vasculitis. *Clin J Am Soc Nephrol,* 2013;8(10): 1709-1717.
43. Norby GE, Strom EH, Midtvedt K, et al. Recurrent lupus nephritis after kidney transplantation: a surveillance biopsy study. *Ann Rheum Dis,* 2010;69(8): 1484-1487.
44. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol,* 2004;15(2): 241-250.
45. Mark EJ, Flieder DB, Matsubara O. Treated Wegener's granulomatosis: distinctive pathological findings in the lungs of 20 patients and what they tell us about the natural history of the disease. *Hum Pathol,* 1997;28(4): 450-458.
46. Voswinkel J, Mueller A, Kraemer JA, et al. B lymphocyte maturation in Wegener's granulomatosis: a comparative analysis of VH genes from endonasal lesions. *Ann Rheum Dis,* 2006;65(7): 859-864.
47. Westman KWA, Bygren PG, Olsson H, Ranstam J, Wieslander J. Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J Am Soc Nephrol,* 1998;9(5): 842-852.
48. Bacon PA. The spectrum of Wegener's granulomatosis and disease relapse. *N Engl J Med,* 2005;352(4): 330-332.
49. Lyons PA, Rayner TF, Trivedi S, et al. Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med,* 2012;367(3): 214-223.
50. Rahmattulla C, Mooyaart AL, van Hooven D, et al. Genetic variants in ANCA-associated vasculitis: a meta-analysis. *Ann Rheum Dis,* 2015;75: 1687-1692.
51. Aasarod K, Bostad L, Hammerstrom J, Jorstad S, Iversen BM. Renal histopathology and clinical course in 94 patients with Wegener's granulomatosis. *Nephrol Dial Transplant,* 2001;16(5): 953-960.

52. Haroun MK, Stone JH, Nair R, Racusen L, Hellmann DB, Eustace JA. Correlation of percentage of normal glomeruli with renal outcome in Wegener's granulomatosis. *Am J Nephrol,* 2002;22(5-6): 497-503.
53. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol,* 2010;21(10): 1628-1636.
54. Chen YX, Xu J, Pan XX, et al. Histopathological Classification and Renal Outcome in Patients with Antineutrophil Cytoplasmic Antibodies-associated Renal Vasculitis: A Study of 186 Patients and Metaanalysis. *J Rheumatol,* 2017;44(3): 304-313.
55. Huang S, Shen Q, Yang R, Lai H, Zhang J. An evaluation of the 2010 histopathological classification of anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis: a Bayesian network meta-analysis. *Int Urol Nephrol,* 2018;50(10): 1853-1861.
56. Watts RA, Lane SE, Scott DG, et al. Epidemiology of vasculitis in Europe. *Ann Rheum Dis,* 2001;60(12): 1156-1157.
57. Watts RA, Scott DG, Jayne DR, et al. Renal vasculitis in Japan and the UK--are there differences in epidemiology and clinical phenotype? *Nephrol Dial Transplant,* 2008;23(12): 3928-3931.
58. de Lind van Wijngaarden RAF, Hauer HA, Wolterbeek R, et al. Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: a prospective analysis of 100 patients with severe renal involvement. *J Am Soc Nephrol,* 2006;17(8): 2264-2274.
59. Berden AE, Jones RB, Erasmus DD, et al. Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. *J Am Soc Nephrol,* 2012;23(2): 313-321.
60. Brix SR, Noriega M, Tennstedt P, et al. Development and validation of a renal risk score in ANCA-associated glomerulonephritis. *Kidney Int,* 2018;94(6): 1177-1188.
61. Ford SL, Polkinghorne KR, Longano A, et al. Histopathologic and clinical predictors of kidney outcomes in ANCA-associated vasculitis. *Am J Kidney Dis,* 2014;63(2): 227-235.
62. Hogan SL, Nachman PH, Wilkman AS, Jennette JC, Falk RJ, Network GDC. Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol,* 1996;7(1): 23-32.
63. Levi C, Meas-Yedid V, Daniliuc C, et al. Computerized Instersitial Fibrosis Is the Most Powerful Histological Predictor of Renal Outcome in ANCA-Associated Vasculitis. *J Am Soc Nephrol,* 2012(23): 710A-711A.
64. Muso E, Endo T, Itabashi M, et al. Evaluation of the newly proposed simplified histological classification in Japanese cohorts of myeloperoxidase-anti-neutrophil cytoplasmic antibody-associated glomerulonephritis in comparison with other Asian and European cohorts. *Clin Exp Nephrol,* 2013;17(5): 659-662.
65. Muso E, Endo T, Yumura W, Joh K. Need of Interstitial Fibrosis Parameter on the Newly Proposed Simplified Glomerular Histological Classification to Predict the Longterm Outcome in Japanese Cohort of MPO-ANCA Associated RPGN. *J Am Soc Nephrol,* 2012(23): 532A-532A.
66. Quintana LF, Perez NS, De SE, et al. ANCA serotype and histopathological classification for the prediction of renal outcome in ANCA-associated glomerulonephritis. *Nephrol Dial Transplant,* 2014;29(9): 1764-1769.

67. Tanna A, Guarino L, Tam FW, et al. Long-term outcome of anti-neutrophil cytoplasm antibody-associated glomerulonephritis: evaluation of the international histological classification and other prognostic factors. *Nephrol Dial Transplant,* 2015;30(7): 1185-1192.
68. Goceroglu A, Berden AE, Fiocco M, et al. ANCA-Associated Glomerulonephritis: Risk Factors for Renal Relapse. *PLoS One,* 2016;11(12): e0165402.
69. Little MA, Nazar L, Farrington K. Outcome in glomerulonephritis due to systemic small vessel vasculitis: effect of functional status and non-vasculitic co-morbidity. *Nephrol Dial Transplant,* 2004;19(2): 356-364.
70. Slot MC, Cohen Tervaert JW, Franssen CFM, Stegeman CA. Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int,* 2003;63(2): 670-677.
71. Cecka JM. The OPTN/UNOS Renal Transplant Registry. *Clin Transpl.* 2005;19:1-16.
72. Geetha D, Eirin A, True K, et al. Renal transplantation in antineutrophil cytoplasmic antibody-associated vasculitis: a multicenter experience. *Transplantation,* 2011;91(12): 1370-1375.
73. Gera M, Griffin MD, Specks U, Leung N, Stegall MD, Fervenza FC. Recurrence of ANCA-associated vasculitis following renal transplantation in the modern era of immunosupression. *Kidney Int,* 2007;71(12): 1296-1301.
74. Gondos A, Dohler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation,* 2013;95(2): 267-274.
75. Little MA, Hassan B, Jacques S, et al. Renal transplantation in systemic vasculitis: when is it safe? *Nephrol Dial Transplant,* 2009;24(10): 3219-3225.
76. Shen J, Gill J, Shangguan M, Sampaio MS, Bunnapradist S. Outcomes of renal transplantation in recipients with Wegener's granulomatosis. *Clin Transplant,* 2011;25(3): 380-387.
77. Stel VS, van de Luijtgaarden MWM, Wanner C, Jager KJ, Investigators ERR. The 2008 ERA-EDTA Registry Annual Report-a precis. *NDT Plus,* 2011;4(1): 1-13.
78. Tang W, Bose B, McDonald SP, et al. The Outcomes of Patients with ESRD and ANCA-Associated Vasculitis in Australia and New Zealand. *Clin J Am Soc Nephrol,* 2013;8: 773-780.
79. Buttigieg J, Henderson L, Kidder D. Outcome of Kidney Transplant in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis. *Exp Clin Transplant,* 2017;15(5): 509-515.
80. Wallace ZS, Wallwork R, Zhang Y, et al. Improved survival with renal transplantation for end-stage renal disease due to granulomatosis with polyangiitis: data from the United States Renal Data System. *Ann Rheum Dis,* 2018;77(9): 1333-1338.
81. Allen A, Pusey C, Gaskin G. Outcome of renal replacement therapy in antineutrophil cytoplasmic antibody-associated systemic vasculitis. *J Am Soc Nephrol,* 1998;9(7): 1258-1263.
82. Fogazzi GB, Banfi G, Allegri L, Bignardi L. Late recurrence of systemic vasculitis after kidney transplantation involving the kidney allograft. *Adv Exp Med Biol,* 1993;336: 503-506.
83. Hadaya K, Marangon N, Moll S, Ferrari-Lacraz S, Villard J. Early relapse of autoimmune glomerulonephritis after kidney transplantation despite antibody induction and triple-drug-based immunosuppression. *Transplantation,* 2010;89(6): 767-769.

84. Marco H, Mirapeix E, Arcos E, et al. Long-term outcome of antineutrophil cytoplasmic antibody-associated small vessel vasculitis after renal transplantation. *Clin Transplant,* 2013;27(3): 338-347.
85. Moroni G, Torri A, Gallelli B, et al. The long-term prognosis of renal transplant in patients with systemic vasculitis. *Am J Transplant,* 2007;7(9): 2133-2139.
86. Deegens JK, Artz MA, Hoitsma AJ, Wetzels JF. Outcome of renal transplantation in patients with pauci-immune small vessel vasculitis or anti-GBM disease. *Clin Nephrol,* 2003;59(1): 1-9.
87. Nachman PH, Segelmark M, Westman K, et al. Recurrent ANCA-associated small vessel vasculitis after transplantation: a pooled analysis. *Kidney Int,* 1999;56(4): 1544-1550.
88. Geetha D, Lee SM, Shah S, Rahman HM. Relevance of ANCA positivity at the time of renal transplantation in ANCA associated vasculitis. *J Nephrol,* 2017;30(1): 147-153.
89. Haubitz M, Kliem V, Koch KM, et al. Renal transplantation for patients with autoimmune diseases: single-center experience with 42 patients. *Transplantation,* 1997;63(9): 1251-1257.
90. Nyberg G, Akesson P, Norden G, Wieslander J. Kidney transplantation in patients with systemic vasculitis. *Transplant Proc,* 1997;29(1-2): 235.
91. Nyberg G, Akesson P, Norden G, Wieslander J. Systemic vasculitis in a kidney transplant population. *Transplantation,* 1997;63(9): 1273-1277.
92. Rostaing L, Modesto A, Oksman F, Cisterne JM, Le Mao G, Durand D. Outcome of patients with antineutrophil cytoplasmic autoantibody-associated vasculitis following cadaveric kidney transplantation. *Am J Kidney Dis,* 1997;29(1): 96-102.
93. Briganti EM, Russ GR, McNeil JJ, Atkins RC, Chadban SJ. Risk of renal allograft loss from recurrent glomerulonephritis. *N Engl J Med,* 2002;347(2): 103-109.
94. Monach PA, Warner RL, Tomasson G, et al. Serum proteins reflecting inflammation, injury and repair as biomarkers of disease activity in ANCA-associated vasculitis. *Ann Rheum Dis,* 2013;72(8): 1342-1350.
95. Tedesco M, Gallieni M, Pellegata F, Cozzolino M, Alberici F. Update on ANCA-associated vasculitis: from biomarkers to therapy. *J Nephrol,* 2019;32(6): 871-882.
96. Jayne DR, Bruchfeld AN, Harper L, et al. Randomized Trial of C5a Receptor Inhibitor Avacopan in ANCA-Associated Vasculitis. *J Am Soc Nephrol,* 2017;28(9): 2756-2767.

8

NEDERLANDSE SAMENVATTING EN DISCUSSIE

Vasculitis is een verzamelnaam van verschillende ziekten gekenmerkt door ontsteking van bloedvaten. Afhankelijk van de soort vasculitis kunnen dit grote en/of kleine bloedvaten zijn. Vasculitis betekent vertaald ontsteking ('=itis') van bloedvaten. Dit proefschrift gaat over één specifiek soort vasculitis, namelijk ANCA-geassocieerde vasculitis (AAV). Bij deze ziekte zijn vooral de kleine bloedvaten betrokken.

ANCA staat voor Anti-Neutrofiel Cytoplasmatische Autoantilichamen en de aanwezigheid hiervan in het bloed is een kenmerk van AAV. ANCA's zijn afweerstoffen die zich richten tegen twee eiwitten: proteïnase 3 (PR3) en myeloperoxidase (MPO). Beide eiwitten bevinden zich in bepaalde soorten witte bloedcellen: neutrofielen en monocyten.

AAV kan verder onderverdeeld worden in drie subtypen:

- Granulomatosis met polyangiitis (GPA, voorheen bekend als de ziekte van Wegener)
- Microscopische polyangiitis (MPA)
- Eosinofiele granulomatosis met polyangiitis (EGPA, voorheen bekend als de ziekte van Churg-Strauss)

Dit proefschrift gaat over de eerste twee subtypen, namelijk GPA en MPA.

AAV is een zeldzame ziekte. De ziekte wordt in de Westerse wereld per jaar bij ongeveer twintig op de één miljoen mensen gediagnosticeerd. AAV komt het meest voor bij mensen tussen de 65 en 74 jaar en wordt vaker bij mannen gezien. Hoe vaak de aandoening precies voorkomt verschilt per land en per ras.

Patiënten met deze ziekte presenteren zich in het begin met griepachtige verschijnselen zoals koorts, hoofdpijn, spierpijn, gewrichtspijn en verminderde eetlust. Deze 'vage' klachten kunnen weken tot maanden aanhouden en komen ook bij veel andere ziekten voor. Hierdoor is het voor een arts in het begin heel moeilijk om de ziekte te herkennen. Meestal duurt het tussen de drie en twaalf maanden voordat de ziekte herkend wordt. Vaak wordt een patiënt door meerdere artsen gezien voordat de diagnose wordt gesteld. Naarmate de ziekte vordert, begint de ziekte zich steeds duidelijker te uiten. AAV kan in één orgaan beginnen en gedurende het beloop kunnen meer organen betrokken raken. Afhankelijk van de betrokken organen, worden de klachten steeds specifieker. De meest betrokken organen zijn: keel, neus, oren, nieren, longen, huid, ogen en het zenuwstelsel. De diagnose wordt uiteindelijk gesteld op basis van

de klachten, bloedonderzoek en weefselonderzoek. In dit proefschrift richten wij ons vooral op nierbetrokkenheid.

Zonder behandeling overlijdt 82% van de patiënten binnen één jaar en 90% binnen twee jaar. De meest voorkomende doodsoorzaken zijn nier- en/of longfalen ten gevolge van de ziekte. Dankzij de huidige behandeling met medicijnen is het een chronische ziekte geworden die zich kenmerkt door perioden van rust en opvlammingen van de ziekte. Medicijnen richten zich vooral op het onderdrukken van het afweersysteem of 'immuunsysteem'. De behandeling is langdurig: het is bekend dat te kort behandelen een groter risico op opvlammingen geeft. Een van de grote uitdagingen van AAV is dat ondanks een goede behandeling, de ziekte toch kan opvlammen: ongeveer de helft van de patiënten heeft binnen vijf jaar een of meer opvlammingen doorgemaakt.

DE PATIËNT

Onderzoek laat zien dat artsen anders naar de ziekte en de impact daarvan kijken dan patiënten. Om deze reden krijgt het perspectief van de patiënt steeds meer aandacht in het onderzoek dat verricht wordt naar AAV. Meerdere studies laten zien dat patiënten met AAV een verslechtering van hun kwaliteit van leven ervaren. Dit wordt vooral bepaald door de fysieke belasting van de ziekte. Patiënten geven aan het meeste last te hebben van klachten als moeheid, verlies van energie en pijn aan gewrichten.

HET ZIEKTEPROCES

Het is onbekend hoe de ziekte precies ontstaat. Waarschijnlijk spelen de eerder genoemde witte bloedcellen -neutrofielen en monocyten- een belangrijke rol. Deze witte bloedcellen bevatten de eiwitten PR3 en MPO. Bij AAV worden deze eiwitten door afweerstoffen – de ANCA's – herkend. ANCA's worden geproduceerd door een ander type witte bloedcel: de B-cellen. Op het moment waarop ANCA's zich binden aan PR3 of MPO ontstaat er een reactie welke leidt tot ontsteking van bloedvaten. Dit is een zichzelf versterkend proces waardoor de ontstekingen van de bloedvaten in het lichaam steeds heviger worden. Om deze reden is het belangrijk dat de behandeling tijdig wordt gestart.

AFWEERSTOFFEN

ANCA's gericht tegen PR3 en MPO zijn de belangrijkste afweerstoffen bij de ziekte AAV. De aanwezigheid van ANCA's in het bloed kan gebruikt worden voor het diagnosticeren van de ziekte.

Hoewel de aanwezigheid van ANCA's in het bloed een belangrijk kenmerk is van AAV, laten studies tegenstrijdige resultaten zien als het gaat om de relatie tussen de hoeveelheid ANCA's in het bloed en de activiteit van de ziekte. Een patiënt kan bijvoorbeeld inactieve ziekte hebben en toch aantoonbaar ANCA's in het bloed. Aan de andere kant zijn er wel aanwijzingen dat bij patiënten met nierbetrokkenheid een stijging van het aantal ANCA's in het bloed kan duiden op toename van ziekteactiviteit. Verder kan het zijn dat een stijging bij de ene patiënt meer voorspellende waarde heeft voor een opvlamming dan bij de andere patiënt. In ieder geval is het bepalen van de ANCA-waarde in het bloed alleen onvoldoende om behandelkeuzes te maken.

Naast ANCA's zijn nog andere afweerstoffen beschreven bij patiënten met AAV welke mogelijk een bijdragende rol spelen. Voorbeelden zijn afweerstoffen tegen plasminogeen en human lysosomal-associated membrane protein-2 (hLAMP-2). Beide afweerstoffen zijn gericht tegen eiwitten die zich van nature in het menselijk lichaam bevinden. In dit proefschrift wordt in hoofdstuk 2 een onderzoek beschreven waarin we kijken naar het bepalen van afweerstoffen tegen plasminogeen in het bloed van patiënten. Plasminogeen is een eiwit dat een rol speelt bij het stollen van bloed.

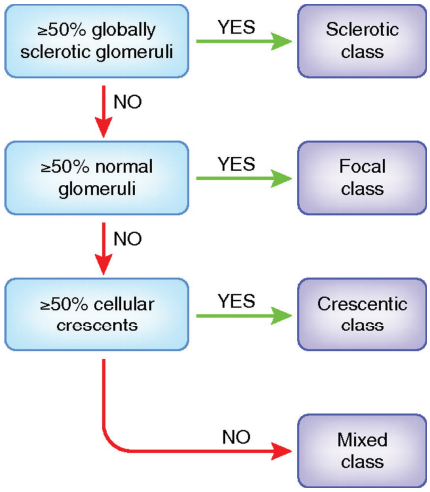
Samengevat wordt in hoofdstuk 2 een geoptimaliseerde methode gepresenteerd om de aanwezigheid van afweerstoffen tegen plasminogeen in het bloed van AAV-patiënten te onderzoeken. We hebben verschillende methodes uit eerdere studies onderzocht en samengevoegd. Met onze verbeterde methode zagen we in 14.3% van de patiënten met ANCA's tegen MPO ook afweerstoffen tegen plasminogeen. Deze anti-plasminogeen afweerstoffen zagen we in geen van de patiënten met ANCA's tegen PR3. Deze uitkomst verschilt met de eerdere studies. Een mogelijke verklaring is dat onze methode verschilt van de eerder gebruikte methoden. Een andere mogelijke verklaring voor het verschil in aantonen of 'detectie' van afweerstoffen is dat er wellicht verschillende soorten afweerstoffen tegen plasminogeen bestaan, welke tegen een ander stukje van het plasminogeen reageren. Dit moet verder onderzocht worden, omdat het hebben van deze afweerstoffen tegen plasminogeen mogelijk relevant kan zijn voor de patiënt. Een eerdere studie toonde aan dat de aanwezigheid van afweerstoffen tegen plasminogeen bij patiënten gepaard ging met meer schade in het nierbiopt en een slechtere uitkomst van de nierfunctie na één jaar.

DE DIAGNOSE

Voor de diagnose AAV wordt gekeken naar de combinatie van klachten en aanwezigheid van ANCA's in het bloed. Daarbij moet uitgesloten zijn dat een andere ziekte de verklaring is voor de klachten. Uiteindelijk moet de diagnose (het liefst) met een

biopt (stukje weefsel voor microscopisch onderzoek) bevestigd worden. Een biopt is de gouden standaard voor het diagnosticeren van nierbetrokkenheid bij AAV. Verschillende studies hebben laten zien dat de afwijkingen die in het biopt van de nier worden gezien voorspellend kunnen zijn voor het beloop of 'de prognose' van de nier. In hoofdstuk 3 wordt een studie beschreven waarin voor het eerst wordt gekeken naar het voorspellen van terugkeer van de ziekte in de nier middels de afwijkingen in het nierbiopt. Het is belangrijk opvlammingen van de ziekte in de nieren te voorkomen of snel te herkennen, aangezien ze negatief van invloed zijn op de nierfunctie en de kans op nierfalen vergroten.

Gebaseerd op een aantal van deze afwijkingen in de nier is er in 2010 een classificatiesysteem geïntroduceerd welke nierbiopten afgenomen op het moment van diagnose indeelt in één van de vier groepen: de 'focal', 'crescentic', 'mixed' en 'sclerotic' groep (figuur 1). De 'focal' groep bevat nierbiopten die weinig afwijkingen laten zien; de 'crescentic' groep bevat nierbiopten waarin actieve ontstekingen worden gezien; de 'sclerotic' groep bevat voornamelijk verlittekening en de 'mixed' groep laat een mengbeeld zien van de andere drie groepen. Meerdere studies lieten een relatie zien tussen de groep waarin het biopt was ingedeeld en de nierfunctie na één en vijf jaar. Omdat de gevonden relatie in de verschillende studies niet helemaal eenduidig was, hebben we een groot internationaal onderzoek gedaan naar de voorspellende waarde van de classificatie. De resultaten uit dit onderzoek staan in hoofdstuk 4 beschreven.



Figuur 1 | Classificatie die nierbiopten afgenomen op het moment van de diagnose AAV indeelt in één van de vier groepen afhankelijk van de afwijkingen die in het biopt worden gezien.
AAV= ANCA-geassocieerde vasculitis. Gebruikt uit Berden *et al.* (J Am Soc Nephrol 2010;21:1628-1636) met toestemming.

In hoofdstuk 4 wordt bevestigd dat de 'focale' groep de beste en de 'sclerotic' groep de slechtste uitkomst hebben als het gaat om de nier. Tussen de 'crescentic' en 'mixed' groep vonden we geen verschil. Dit is verschillend van de studie waarin de classificatie voor het eerst is gepresenteerd, maar komt overeen met latere studies die door andere onderzoeksgroepen zijn verricht. Er is een aantal mogelijke verklaringen voor de verschillen tussen deze studies. Zo kunnen de onderzochte populaties van elkaar verschillen. Hierbij kan het ook zijn dat de verschillende populaties op een verschillende manier zijn behandeld. Daarnaast wordt in dit hoofdstuk aangetoond dat verschillende pathologen net op een andere manier naar hetzelfde nierbiopt kijken; dit kan ook deels de verschillen tussen de studies verklaren.

Verder is het zo dat het classificatiesysteem niet alle afwijkingen meeneemt die men in een nierbiopt bij AAV kan zien. Het is mogelijk dat het toevoegen van andere afwijkingen (bijvoorbeeld ontstekingen aan nierbuisjes of de hoeveelheid ontstekingscellen in het nierweefsel) de classificatie verfijnt. Echter moet wel rekening gehouden worden met het feit dat toevoegen van extra onderdelen de classificatie complexer maakt, waarbij de kans op verschillen tussen de beoordeling van nierbiopten door pathologen toeneemt. Momenteel wordt onderzocht of en hoe de classificatie verfijnd kan worden.

DE BEHANDELING

Als iemand zich presenteert met actieve ziekte en de diagnose AAV wordt gesteld, dan moet er gestart worden met de behandeling. Om de ziekte te onderdrukken start men met prednison met cyclofosfamide of rituximab (inductietherapie). Als de ziekte onderdrukt is, wat meestal na 3 tot 6 maanden het geval is, begint men een behandeling om de ziekte onderdrukt te houden (onderhoudstherapie). De cyclofosfamide wordt dan gewisseld naar azathioprine, methotrexaat of rituximab. Indien rituximab wordt gegeven als inductietherapie, kan deze gecontinueerd worden als onderhoudstherapie. Tijdens de onderhoudstherapie wordt de prednison afgebouwd. De onderhoudstherapie kan langdurig worden gegeven, echter is nog niet bekend hoe lang dit precies moet zijn; dit wordt nog onderzocht. Wel weten we al dat één jaar onderhoudstherapie te kort is en dat bijvoorbeeld 18-24 maanden beter is. Op de inductie- en onderhoudstherapie zijn kleine variaties mogelijk, afhankelijk van de eigenschappen van de patiënt en manifestaties van de ziekte (bijv. soort klachten en betrokken organen). Zo kan bijvoorbeeld bij mildere ziekte methotrexaat of mycofenolaat mofetil als inductietherapie gegeven worden. Bij levensbedreigende situaties kan de behandeling uitgebreid worden met prednison via het infuus of kan het bloed schoongemaakt worden met een machine (dit heet 'plasmaferese' en is een vorm

van dialyse). Echter is er recent een grote studie gepubliceerd, welke geen toegevoegde waarde laat zien van plasmaferese; dit in tegenstelling tot een eerdere grote studie.

Een grote uitdaging van de behandeling is het evenwicht tussen hoe lang doorbehandeld moet worden met een bepaald medicijn en de kans op bijwerkingen van het medicijn. Een belangrijk voorbeeld van bijwerkingen is de hogere kans op infecties of verschillende vormen van kanker (vooral huidkanker) bij het langdurig gebruik van cyclofosfamide. De kans op infecties wordt nog eens vergroot door het simultaan gebruiken van prednison. Het liefst wil de arts zo lang mogelijk doorbehandelen om de ziekte te onderdrukken, echter betekent een langere behandeling meer kans op bijwerkingen. Dit is een delicaat evenwicht waar veel onderzoek naar gedaan wordt. Het huidig advies is om in ieder geval twee jaar te behandelen.

Een andere grote uitdaging bij AAV is de kans op een nieuwe opvlamming van de ziekte, ondanks dat de patiënt medicijnen krijgt. Het liefst wil de arts de patiënten met een grotere kans op een opvlamming van de ziekte herkennen op het moment van de diagnose. Zo kunnen deze patiënten intensiever worden behandeld. Studies zijn nodig om deze 'voorspellers' te ontdekken. In hoofdstuk 3 van dit proefschrift wordt gekeken naar risicofactoren en voorspellers van een opvlamming van de ziekte in de nier, ondanks adequate behandeling. Op het moment lijkt rituximab de beste behandeling te zijn bij een opvlamming van de ziekte.

DE NIEREN EN NIERTRANSPLANTATIE

In 80-90% van de patiënten met AAV raken de nieren tijdens de ziekte betrokken. Van deze groep verliest uiteindelijk 20-40% hun nierfunctie ten gevolge van de ziekte. Deze patiënten komen dan in aanmerking voor een niertransplantatie, waarbij ze een nier ontvangen van een donor. Na vijf jaar functioneert nog circa 70% van de donornieren. De kans op terugkeer van de ziekte in de donornier is 1-2% per jaar. Deze getallen komen uit verschillende kleine studies. In hoofdstuk 5 van dit proefschrift wordt middels een grote landelijke studie gekeken naar de uitkomst van niertransplantaties bij patiënten met AAV. Hierbij hebben we specifiek gekeken naar de invloed van terugkeer van de ziekte in de donornier en het risico op het verliezen van de donornier hierdoor.

Samengevat laat onze studie zien dat de vijfjaars overleving van de donornieren 82.8% is. Dit is gelijk aan de overleving van donornieren bij andere ziektes. De kans op terugkeer van de ziekte in de donornier is 2.8% per jaar. We zagen dat terugkeer van de ziekte in de donornier een belangrijke oorzaak was van het verliezen van die nier: in 36% van alle gevallen waarbij AAV is terug gekeerd in de donornier, zijn de

donornieren verloren gegaan ten gevolge van de terugkeer van de ziekte. De terugkeer van de ziekte in de donornier kan op elk moment na de niertransplantatie gebeuren; van dagen tot jaren na de niertransplantatie. Om de kans op terugkeer zo klein mogelijk te houden, wordt bij voorkeur een niertransplantatie uitgevoerd op het moment dat de ziekte inactief is. Het lijkt erop dat patiënten met inactieve ziekte en enkel een positieve ANCA in het bloed wel een niertransplantatie kunnen ondergaan zonder verhoogd risico op verlies van de donornier. Hoe lang de ziekte rustig moet zijn moet nog verder onderzocht worden. Een niertransplantatie is dus een goede behandeling bij AAV patiënten met nierfalen, maar op het moment dat de ziekte in de donornier terugkeert, moet de arts zich bewust zijn van een verhoogd risico op verlies van de donornier.

VRAGEN VOOR DE TOEKOMST

Er zijn nog genoeg uitdagingen in AAV waarnaar verder onderzoek gedaan moet worden.

Zo willen we meer inzicht krijgen in welke patiënten wel of geen opvlammingen van de ziekte krijgen, ondanks behandeling. Er zijn in verschillende studies factoren beschreven die de kans op een opvlamming van de ziekte vergroten, zoals bijvoorbeeld de aanwezigheid van PR3-ANCA en long- en/of cardiovasculaire betrokkenheid. Onderzoek is nodig om dit soort factoren verder te specificeren. Hierbij moet ook gekeken worden naar afwijkingen in het nierbiopt die voorspellend kunnen zijn. Zo kan de arts in de toekomst patiënten met een grotere kans op een opvlamming eerder herkennen en intensiever behandelen.

Het gebruik van de ANCA-concentratie in het bloed om daarmee de huidige situatie van ziekte te meten of om een opvlamming te voorspellen blijft een punt van discussie. Het is goed mogelijk dat patiënten met AAV andere stoffen of 'biomarkers' in het bloed hebben, die beter samenhangen met ziekteactiviteit. Biomarkers kunnen de arts helpen bij besluitvorming over de behandeling. Er zijn een aantal potentiële biomarkers beschreven, waaronder ook anti-plasminogeen afweerstoffen.

Het is nog niet goed uitgezocht hoe lang een AAV-patiënt inactieve ziekte moet hebben voordat een niertransplantatie uitgevoerd kan worden met zo min mogelijk risico's erna. Er is - weliswaar zwak- bewijs dat één jaar inactieve ziekte mogelijk een goede termijn is voorafgaand aan een niertransplantatie. Op dit punt is verder onderzoek nodig.

Het blijft een uitdaging om een goede balans te vinden tussen behandelen met medicijnen om een opvlamming te voorkomen en de kans op bijwerkingen. De afgelopen decennia is het risico voor een opvlamming niet sterk veranderd. Er moet meer onderzoek komen naar nieuwe medicijnen die de ziekte goed behandelen en de kans op een opvlamming verkleinen. Een potentieel middel hiervoor is avacopan, een recent ontwikkeld medicijn dat ingrijpt op een specifiek proces binnen ons afweersysteem. Momenteel is de beste behandeling voor een opvlamming van de ziekte rituximab met hierna langdurige onderhoudstherapie.

Momenteel wordt ook veel onderzoek gedaan naar genetica en AAV. Eerdere studies lieten zien dat er een genetische variatie is tussen patiënten met ANCA's tegen PR3 en patiënten met ANCA's tegen MPO. Dit wekt de vraag op of we hier te maken hebben met twee verschillende ziektes die verschillend behandeld moeten worden. Tot nu toe zijn PR3-AAV en MPO-AAV altijd op dezelfde manier behandeld. Dit soort inzichten in de verschillen tussen PR3-AAV en MPO-AAV geven ons de kans om betere behandelingen meer gericht op de individuele patiënt te ontwikkelen en te geven.

A

APPENDICES

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B CURRICULUM VITAE

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CURRICULUM VITAE

Arda Göçeroğlu was born on the 23rd of January 1989 in Lindenfels, Germany. At the age of six, he and his parents moved from Germany to the Netherlands. In 2007, he graduated with honours from Ashram College in Alphen aan den Rijn. During his secondary school period, he also participated in the Leiden Advanced Pre-university Programme for Top students at Leiden University. After graduation, he started medical school at Leiden University. In the second year of medical school, he participated in an intra-curricular exchange with Karolinska Institutet in Stockholm, Sweden. Shortly after this exchange, he started his research on ANCA-associated vasculitis under supervision of dr. I.M. Bajema, dr. A.E. Berden and prof. dr. J.A. Bruijn at the Pathology department of the Leiden University Medical Center (LUMC). In 2009, he was awarded a research scholarship within the Excellent Student Programme (MD/PhD-programme) of the LUMC. After receiving his bachelor degree with honours in 2010 and finishing the preclinical phase of medical school in 2011, he started working as a fulltime PhD fellow at the Pathology department of the LUMC (head: prof. dr. G.J. Fleuren and since 1st of May 2013 prof. dr. V.H.T.B.M. Smit) during two years for which he was granted a scholarship within the MD/PhD-programme. During his research, he received several awards and (travel) grants. In 2011, Arda also received the ECHO award for academic education for his study results and extra-curricular activities. With this award he attended a summer course at the University of California Los Angeles (UCLA). During his research period, he was a member of the editorial board of Vascuzine, the magazine of the Dutch Vasculitis Patient Foundation, and he was a member of the European Vasculitis Society (EUVAS). He graduated from medical school with honours in 2015 and worked during 7 months at the Surgery and Plastic, Reconstructive and Hand Surgery department of the Erasmus Medical Center (EMC) in Rotterdam. In October 2016, he started as a resident for Plastic, Reconstructive and Hand Surgery at the EMC (mentor: dr. A.J.M. Luijsterburg). Currently, he finished the preparatory training at Franciscus Gasthuis en Vlietland (mentor: dr. T.M.A.L. Klem) and works as a resident at the Plastic, Reconstructive and Hand Surgery department of the EMC.

BIBLIOGRAPHY

Diagnosis and management of ANCA associated vasculitis.
Berden A, **Göçeroğlu A**, Jayne D, Luqmani R, Rasmussen N, Bruijn JA, Bajema I.
British Medical Journal 2012;344:e26. doi: 10.1136/bmj.e26.

Renal function and ear, nose, throat involvement in anti-neutrophil cytoplasmic antibody-associated vasculitis: prospective data from the European Vasculitis Society clinical trials.
Rahmattulla C, de Lind van Wijngaarden RA, Berden AE, Hauer HA, Floßmann O, Jayne DR, Gaskin G, Rasmussen N, Noël LH, Ferrario F, Waldherr R, Wolterbeek R, **Göçeroğlu A**, Pusey CD, Hagen EC, Bruijn JA, Bajema IM; European Vasculitis Study Group (EUVAS).
Rheumatology (Oxford) 2015;54:899-907. doi: 10.1093/rheumatology/keu357.

The Dutch transplantation in vasculitis (DUTRAVAS) study: outcome of renal transplantation in antineutrophil cytoplasmic antibody-associated glomerulonephritis.
Göçeroğlu A, Rahmattulla C, Berden AE, Reinders ME, Wolterbeek R, Steenbergen EJ, Hilbrands LB, Noorlander I, Berger SP, Peutz-Kootstra CJ, Christiaans MH, van Dijk MC, de Joode AA, Goldschmeding R, van Zuilen AD, Harper L, Little MA, Hagen EC, Bruijn JA, Bajema IM.
Transplantation 2016;100:916-24. doi: 10.1097/TP.0000000000000910.

ANCA-associated glomerulonephritis: risk factors for renal relapse.
Göçeroğlu A, Berden AE, Fiocco M, Floßmann O, Westman KW, Ferrario F, Gaskin G, Pusey CD, Hagen EC, Noël LH, Rasmussen N, Waldherr R, Walsh M, Bruijn JA, Jayne DR, Bajema IM; European Vasculitis Society (EUVAS).
PLoS One 2016;11:e0165402. doi: 10.1371/journal.pone.0165402.

Anti-plasminogen antibodies in ANCA-associated vasculitis: an optimized anti-plasminogen assay.
Göçeroğlu A, Grenmyr E, Berden AE, Hagen EC, Bunch D, Sommarin Y, Bruijn JA, Bajema IM, Wieslander J.
PLoS One 2018;13:e0207064. doi: 10.1371/journal.pone.0207064.

Histopathological classification of ANCA-associated glomerulonephritis: interobserver variability and clinical outcome.
Göçeroğlu A, van Daalen EE, Ferrario F, Joh K, Noël L-H, Ogawa Y, Wilhelmus S, Ball MJF, Honsova E, Hruskova Z, Kain R, Kimura T, Kollar M, Kronbichler A, Lindhard K, Puéchal X, Salvatore S, Szpirt W, Tesar V, Rahmattulla C, Hagen EC, Oosting J, Berden AE, Bruijn JA, Bajema IM.
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