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On the pathogenesis and clinical outcome of ANCA-associated vasculitis

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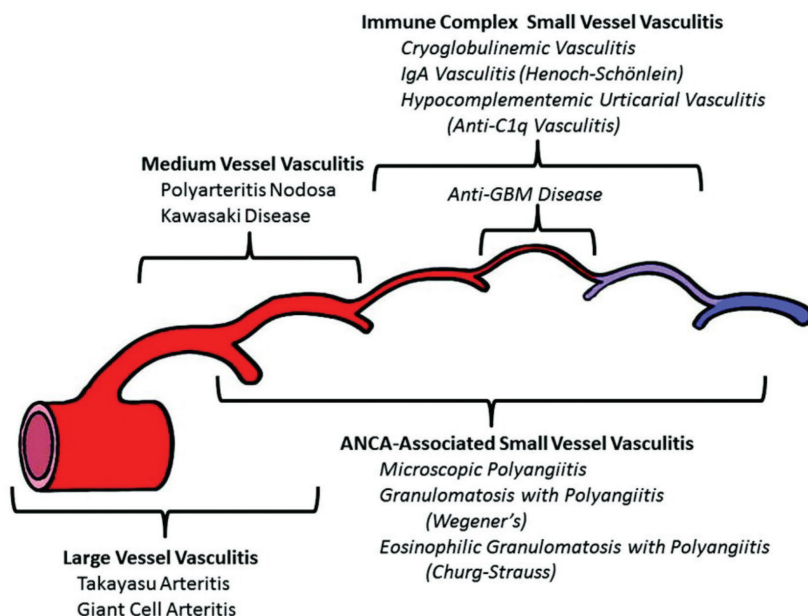
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Chapter I

General introduction and outline of this thesis

Vasculitis means inflammation of blood vessels. The International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC) provides definitions for the different vasculitides and subcategorises them into three major categories: large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis (**figure 1**).^{1, 2}

Figure 1. The International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC)



The International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC) subcategorises the different vasculitides into three major categories: large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis. The figure depicts (from left to right) aorta, large artery, medium artery, small artery/arteriole, capillary, venule, and vein. Anti-GBM = anti-glomerular basement membrane; ANCA = antineutrophil cytoplasmic antibody. Reproduced from Jennette et al.² with permission.

Large-vessel vasculitis predominantly affects the aorta and its major branches. Two important vasculitides within this category are Takayasu arteritis and giant cell arteritis. Medium-vessel vasculitis predominantly affects medium sized arteries. Kawasaki disease is an example of a medium-vessel vasculitis. Small-vessel vasculitis is further subcategorised into immune complex small-vessel vasculitis and antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis. Immune complex small-vessel vasculitis shows immunoglobulin (Ig) and complement deposits in the blood vessel walls. Two important vasculitides within this category are IgA vasculitis and anti-glomerular basement membrane

(anti-GBM) disease. ANCA-associated vasculitis is a necrotizing vasculitis with few or no immune deposits that is typically associated with ANCA-seropositivity by indirect immunofluorescence or enzyme-linked immunosorbent assay (ELISA).³

ANCA-associated vasculitis

This thesis focuses on different aspects of ANCA-associated vasculitis. ANCA first became widely recognized after a key publication in *The Lancet* in 1985 in which van der Woude *et al.* described circulating antibodies in patients with vasculitis that were in several ways similar to the granulocyte-specific antinuclear antibodies (GS-ANA) described in rheumatoid arthritis.⁴ It should however be mentioned that Davies *et al.* had already described the presence of this class of antibodies in patients with pauci-immune glomerulonephritis in 1982.⁵ Van der Woude *et al.* first named these antibodies ACPA (anticytoplasmic antibodies). This term was later replaced by ANCA, as the antibodies were found to be directed against neutrophil (and monocyte) constituents. The two most important ANCA antigens are proteinase 3 (PR3)⁶⁻⁸ and myeloperoxidase (MPO)⁹.

ANCA-associated vasculitis comprises the clinical diagnoses granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis¹⁰⁻¹²), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss Syndrome).²

GPA is a necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract.¹³ Approximately 90-95% of patients with active, generalized GPA are ANCA-positive; a small subset of GPA patients is ANCA-negative.¹⁴⁻¹⁷ Thus, the absence of ANCA does not exclude the diagnosis of GPA. About 70% of the ANCA-positive GPA patients are PR3-ANCA positive; the remainder are MPO-ANCA positive.¹⁸

MPA is characterized by a non-granulomatous necrotizing systemic vasculitis. Nearly 90% of patients with MPA are ANCA-positive.¹⁴⁻¹⁷ About 60% of the ANCA-positive MPA patients are MPO-ANCA positive; the remainder are PR3-ANCA positive.^{9, 18} Because PR3-ANCA and MPO-ANCA may occur in both GPA and MPA, these conditions cannot be distinguished on the basis of ANCA-serotype.

EGPA is an eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract. In contrast to GPA and MPA, EGPA is associated with asthma and eosinophilia. Although variable from study to study, approximately 50% of patients with EGPA are ANCA-positive.^{19, 20} About 70-90% of the ANCA-positive EGPA patients are MPO-ANCA positive.^{20, 21}

The clinical diagnoses GPA and MPA are often grouped together on the grounds of striking similarities in clinical presentation and comparable histologic

findings.^{18, 22} Nevertheless, there are marked differences between these disease entities, fuelling an ongoing debate as to whether GPA and MPA are different entities within the same disease spectrum or represent distinct auto-immune diseases.²³⁻²⁵ Although there are overlaps, a clinical diagnosis of GPA usually involves PR3-ANCA positivity whereas a clinical diagnosis of MPA usually involves MPO-ANCA positivity. In this respect, data from a large genome-wide association study (GWAS) are interesting.²⁶ These data indicate that within the spectrum of ANCA-associated vasculitis distinct subtypes may be recognized. ANCA-serotype was demonstrated to be the main indicator of these subtypes.²⁶

To date, the concept of a single disease spectrum has led to the inclusion of GPA and MPA patients in the same clinical trials and the development of similar treatment strategies for these patients.^{14-17, 27, 28} Evidence that the different entities in ANCA-associated vasculitis actually represent distinct auto-immune diseases may in the future lead to the development of more specific therapeutic strategies. The existence of different subtypes within the spectrum of ANCA-associated vasculitis and their clinical implications are further investigated in **Chapter 2** and **Chapter 3** of this thesis.

Aetiology and pathogenesis of ANCA-associated vasculitis

The pathogenesis of ANCA-associated vasculitis has not been completely elucidated. However, over the past decades, significant progress has been made in the understanding of this complex disease. Environmental exposures, genetic factors, influences of the immune system, and the intensity and duration of the injury are all hypothesized to be involved in the pathogenesis of ANCA-associated vasculitis. Interestingly, the variability in aetiological and synergistic factors that may lead to the development of ANCA-associated vasculitis is hypothesized to contribute to the clinicopathologic differences between patients.²⁹

Onset and exacerbation of ANCA-associated vasculitis occur more often during winter and spring. This points towards the existence of pathogenic, infectious or other environmental, factors that are typically present during these seasons.³⁰ Numerous studies demonstrated that pro-inflammatory factors, for example induced by infections, act synergistically in ANCA-associated vasculitis onset and exacerbation.^{31, 32} In particular, *Staphylococcus aureus* infection was linked to ANCA-associated vasculitis onset.³³⁻³⁵ Moreover, nasal carriage of *Staphylococcus aureus* was demonstrated to be an important risk factor for relapse in patients with GPA,³⁶ and treatment with trimethoprim-sulfamethoxazole was shown to aid in the induction of remission³⁷⁻⁴⁶ and the prevention of relapses³⁷. These treatment effects could be ascribed to the immunosuppressant and/or anti-staphylococcal properties of trimethoprim-sulfamethoxazole.

Evidence that genetic factors contribute to the pathogenesis of ANCA-associated vasculitis comes from familial association studies,⁴⁷⁻⁵¹ differences in the prevalence of ANCA-associated vasculitis between ethnic groups,⁵² and numerous candidate gene associations studies⁵³. Quite recently, two GWAS performed by the Vasculitis Clinical Research Consortium and the European Vasculitis Genetic Consortium also identified genetic associations in ANCA-associated vasculitis.^{26, 54} Both GWAS found a strong association with a single nucleotide polymorphism (SNP) in human leukocyte antigen (*HLA*)-*DPB1*. The European Vasculitis Genetic Consortium GWAS also found a strong association between PR3-ANCA vasculitis and *PRTN3* (the gene encoding PR3) and *SERPINA1* (the gene encoding α 1-antitrypsin; a major inhibitor of PR3), and between MPO-ANCA vasculitis and *HLA-DQ*. Moreover, this GWAS demonstrated genetic distinctions between GPA and MPA patients and between PR3-ANCA positive and MPO-ANCA positive patients. The numerous candidate gene association studies and two GWAS have revealed a great number of genetic variants that possibly contribute to the pathogenesis of ANCA-associated vasculitis. In **Chapter 2** of this thesis, we conducted a meta-analysis to investigate the genetic variants that are most likely associated with ANCA-associated vasculitis. We included raw data from the European Vasculitis Genetic Consortium GWAS to increase the validity of the meta-analysis.

Are ANCA pathogenic?

In vitro and *in vivo* evidence support a pathogenic role for ANCA in the pathogenesis of ANCA-associated vasculitis.^{55, 56} *In vitro* data include experiments demonstrating that ANCA IgG activates cytokine-primed neutrophils,⁵⁷⁻⁵⁹ that ANCA-activated neutrophils induce endothelial cell injury,^{60, 61} and that ANCA-activated neutrophils activate the alternative complement pathway⁶².

The most convincing evidence that ANCA are pathogenic comes from animal models. Xiao *et al.* demonstrated that intravenous injection of purified murine anti-MPO IgG into wild type mice or immunodeficient Rag2-/- mice (these mice fail to generate mature T and B lymphocytes) induces proteinuria and haematuria in these mice. Moreover, the histopathologic lesions found in the kidneys of these mice were comparable to those in renal biopsies of patients with ANCA-associated glomerulonephritis.⁵⁶ Furthermore, Little *et al.* demonstrated that rats immunised with human MPO develop anti-human MPO-ANCA that binds these rats' neutrophils and induces pauci-immune crescentic glomerulonephritis and pulmonary haemorrhage with histologic evidence of lung vasculitis in these rats.⁶³ Thus far, it has not been possible to successfully reproduce these findings in a PR3-ANCA vasculitis animal model.^{29, 64}

Clinical evidence for the pathogenicity of ANCA includes the occurrence of pulmonary-renal syndrome in a neonate shortly after birth from a mother with MPO-ANCA positive MPA, most likely caused by transplacental transfer of maternal MPO-ANCA.^{65, 66} This clinical evidence is limited, however, in that it comprises only one case-report and until today no sequelae have been reported. Further clinical evidence for the pathogenicity of ANCA includes the induction of ANCA, particularly high titres of MPO-ANCA, by certain drugs (e.g. propylthiouracil and hydralazine⁶⁷) and the subsequent onset of disease manifestations in humans. More clinical evidence is found in the beneficial effect of plasma exchange in the treatment of ANCA-associated vasculitis.¹⁷

One observation plaguing the contention that ANCA are pathogenic was the presence of ANCA in only 90-95% of ANCA-associated vasculitis patients.¹⁴⁻¹⁷ However, the remaining 5-10% of patients that have long been assumed to be ANCA-negative based on the results of conventional clinical assays might be classified as ANCA-positive after all. Roth *et al.* demonstrated that, when using a highly sensitive epitope-excision method, ANCA-negative patients' purified IgG reacts with a specific MPO-epitope.⁶⁸ This MPO-ANCA epitope was blocked from reacting with ANCA IgG in serum because of competitive binding by ceruloplasmin, which is a natural inhibitor of MPO that is naturally present in serum.

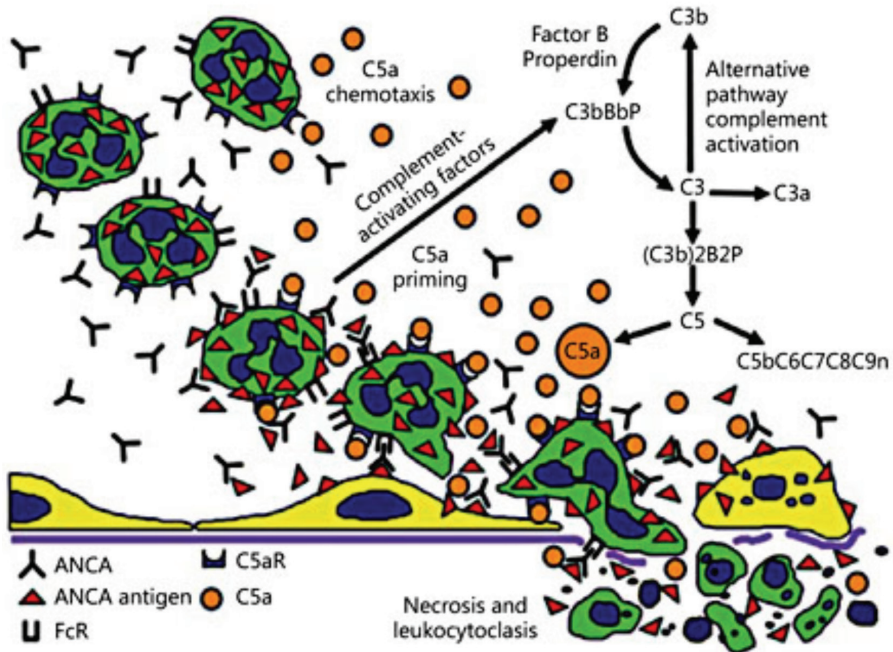
The pathogenicity of ANCA was also questioned since healthy individuals were also demonstrated to have ANCA.⁶⁹⁻⁷² Interestingly, Roth *et al.* demonstrated that compared with MPO-ANCA occurring in vasculitis, natural MPO-ANCA are present in lower titres, have lower avidity, have less subclass diversity, and are less capable of activating neutrophils *in vitro*.⁶⁸ Moreover, the epitope specificity of the repertoire of MPO-ANCA occurring in vasculitis patients was shown to differ from the epitope specificity of MPO-ANCA occurring in healthy individuals.⁶⁸ Thus, not all ANCA seem to be equal.⁷³

Presumed pathogenic sequence for acute vascular injury in ANCA-associated vasculitis

The putative pathogenic sequence inducing acute vascular inflammation in ANCA-associated vasculitis is depicted in **figure 2**.²⁹ Starting from the upper left, resting neutrophils have ANCA autoantigens (e.g. PR3 and MPO) sequestered in their cytoplasmic granules. Exposure to priming factors, e.g. cytokines induced by infection or pathogenic factors released by complement activation, leads to the exposure of ANCA-antigens on the neutrophil's surface and in the microenvironment. Circulating ANCA bind to these antigens and activate them by Fc γ receptor engagement and F(ab')₂ binding. ANCA-activated neutrophils penetrate vessel walls and release factors that initiate necrosis and

apoptosis of the neutrophils and the environment. Meanwhile, the alternative complement pathway is activated and generates C5a, a chemoattractant for neutrophils that also primes the arriving neutrophils for activation by ANCA, generating a positive feedback effect.

Figure 2. The putative pathogenic sequence inducing acute vascular inflammation in antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis.



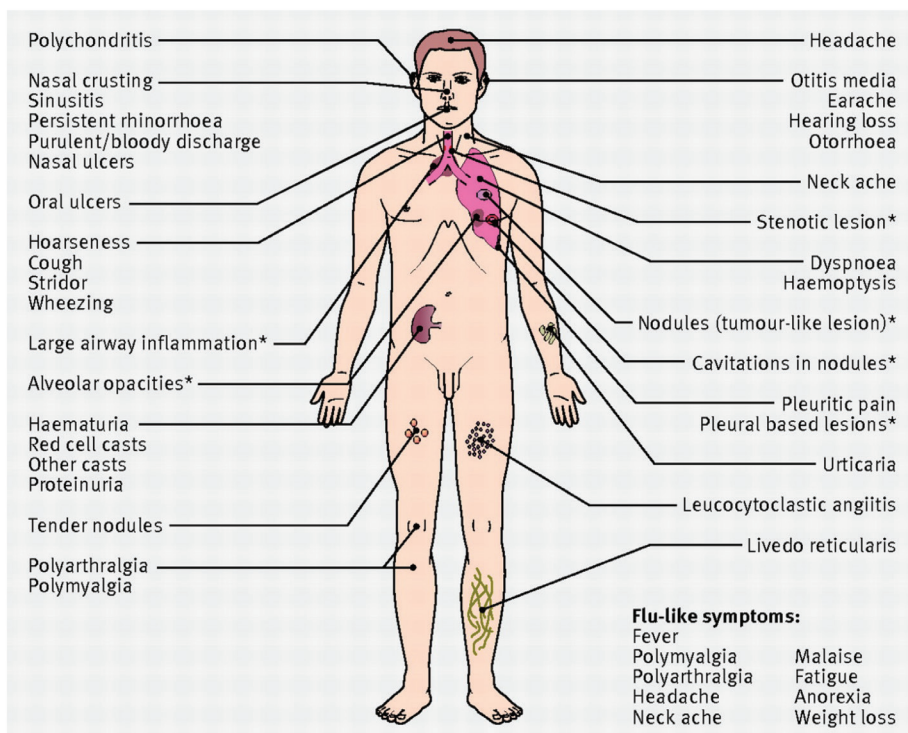
Starting from the upper left, resting neutrophils have ANCA-antigens (e.g. proteinase 3 (PR3) and myeloperoxidase (MPO)) in their cytoplasmic granules. Exposure to priming factors, e.g. cytokines induced by infection or pathogenic factors released by complement activation, leads to the exposure of the ANCA-antigens on the neutrophil's surface and in the microenvironment. Circulating ANCA bind to these antigens and activate the neutrophils. ANCA-activated neutrophils penetrate vessel walls and release factors that initiate necrosis and apoptosis of the neutrophils and the environment. Meanwhile, the alternative complement pathway is activated and generates C5a, a chemoattractant for neutrophils that also primes the arriving neutrophils for activation by ANCA, generating a positive feedback effect. Reproduced from Xiao et al.²⁹ with permission.

Disease manifestations

The peak age of ANCA-associated vasculitis onset is 65-74 years.⁷⁴ Men are more often affected than women, but when women are affected, they tend to have a younger age at disease onset than men.⁷⁵ Patients typically present with prodromal ‘flu-like’ symptoms that have been present for several weeks to months.³ These symptoms include malaise, fever, headache, polyarthralgia, polymyalgia, and unintended weight loss.^{13, 76} Presenting symptoms can be very similar to symptoms of non-vasculitic diseases such as infections, post-viral syndrome, and malignancies. It can therefore be challenging for clinicians to pinpoint the diagnosis early in the diagnostic process. A survey including 701 patients with ANCA-associated vasculitis demonstrated a lag of three to 12 months between disease onset and diagnosis, suggesting that diagnostic delay is a problem.⁷⁵ Correct diagnosis on the first visit to a physician was accomplished in only 7% of patients and 50% of patients had to visit at least four physicians before the correct diagnosis was made.

Figure 3 depicts the various manifestations of ANCA-associated vasculitis. Although disease symptoms in GPA and MPA overlap, the incidences of these symptoms can differ significantly between the two conditions. For instance, ear, nose, and throat (ENT) symptoms occur in 90% of GPA patients and in only 35% of MPA patients.^{13, 76} Large observational studies demonstrated that the airways and lung parenchyma are commonly affected, as are the kidneys, although renal involvement can be asymptomatic until renal failure occurs.⁷⁶⁻⁷⁸ Approximately half of patients develop skin manifestations.⁷⁶⁻⁷⁸ Clearly, ANCA-associated vasculitis can become manifest in virtually all organs. Therefore, thorough physical examination is important to determine the full extent of the disease.³

Figure 3. The various manifestations of antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis.



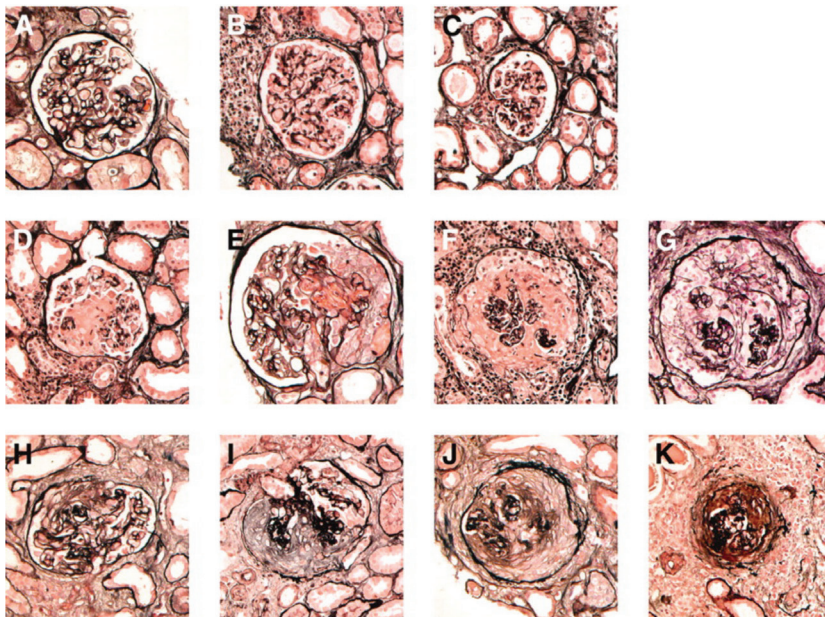
*These lesions can be seen on chest radiography and computed tomography. Reproduced from Berden et al.³ with permission.

Chapters 3 and 4 of this thesis focus on renal involvement in ANCA-associated vasculitis. Renal involvement is common and is important with respect to patient morbidity and mortality.²² The kidneys become involved in approximately 80% of GPA patients and 90% of MPA patients.¹³ A key study published in 1958 demonstrated that the main cause of death in untreated ANCA-associated vasculitis patients is uraemia due to rapidly progressive renal failure.⁷⁹

Rapidly progressive renal failure with an active sediment (i.e. red cell casts and/or proteinuria) in patients who are seropositive for ANCA is suggestive of ANCA-associated glomerulonephritis. The morphologic changes in the renal biopsy are the gold standard for establishing the diagnosis of ANCA-associated glomerulonephritis.^{80, 81} In these biopsies, light microscopy shows necrotizing and crescentic glomerulonephritis.⁸² Immunofluorescence microscopy shows little or no immunoglobulin or complement staining (the so-called pauci-immune staining pattern). By electron microscopy, subendothelial edema, microthrombosis, and degranulation of neutrophils are present, but immune deposits are absent.⁸³

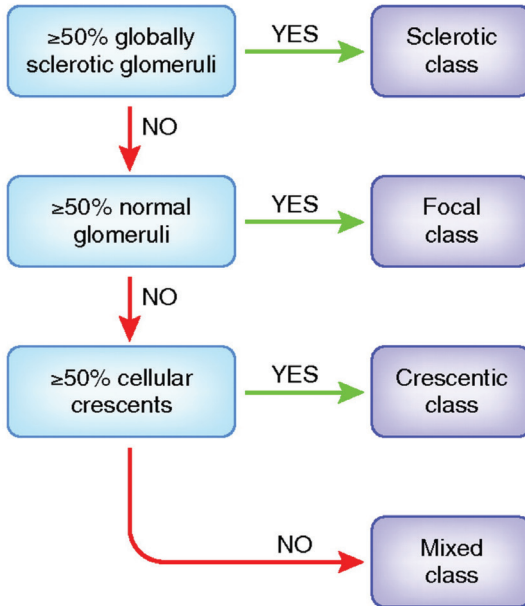
Several studies have demonstrated strong associations between histopathological parameters in the renal biopsy and renal outcome. The most consistent findings were associations between the percentage of normal glomeruli and favorable renal outcome and between the percentage of sclerotic glomeruli and poor renal outcome.⁸⁴⁻⁸⁷ Moreover, the presence of active lesions such as cellular crescents was found to be positively associated with renal recovery under immunosuppressive treatment.^{84, 85} In 2010, the histopathological classification of ANCA-associated glomerulonephritis was devised within the collaboration of the European Vasculitis Society (EUVAS) with the aim of further adding to the prognostication of patients with ANCA-associated glomerulonephritis.⁸⁸ This classification system is built around glomerular pathology and distinguishes four classes: focal class biopsies contain $\geq 50\%$ normal glomeruli; crescentic class biopsies contain $\geq 50\%$ glomeruli with cellular crescents; sclerotic class biopsies contain $\geq 50\%$ sclerotic glomeruli; and biopsies without predominant lesions are assigned to the mixed class. Examples of glomerular lesions typically for each of the four classes are depicted in **figure 4**. The classification flowchart is depicted in **figure 5**.

Figure 4. Examples of glomerular lesions that can be observed in the four classes of the histopathological classification of antineutrophil cytoplasmic antibodies (ANCA)-associated glomerulonephritis.



A-C show examples of normal glomeruli; D-G show examples of cellular crescents; H-J show examples of fibrous crescents; and K shows an example of global glomerulosclerosis. Reproduced from Berden et al.⁸⁸ with permission.

Figure 5. The classification flowchart for the histopathological classification of ANCA-associated glomerulonephritis.



Reproduced from Berden *et al.*⁸⁸ with permission.

The study by Berden *et al.* wherein the classification system was proposed incorporated a validation study including 100 patients.⁸⁸ Multiple regression analysis showed that baseline renal function and the histopathological classification were the only independent predictors of renal outcome. The histopathological classification of ANCA-associated glomerulonephritis has been validated in various cohorts.⁸⁹⁻¹⁰⁹ The outcomes of these validation studies, points of consideration, and future perspectives are discussed in **Chapter 4** of this thesis.

Treatment of ANCA-associated vasculitis

Early diagnosis of ANCA-associated vasculitis is of major importance as prompt instigation of treatment is essential to prevent progressive organ damage, particularly end-stage renal disease, and death.⁷⁹ In the last 20 years, a significant number of large, international trials were performed with the aim of improving the treatment modalities in ANCA-associated vasculitis.^{14-17, 27, 28, 110-112}

Treatment of ANCA-associated vasculitis traditionally consists of two phases.¹¹³ Phase I consists of aggressive remission-inducing immunosuppression classically consisting of cyclophosphamide and corticosteroids for the duration of three to six months. Phase II consists of remission maintenance treatment with,

amongst others, azathioprine or methotrexate while corticosteroids are tapered and if possible stopped.¹¹⁴ The choice of treatment depends on disease severity and organ manifestations. Recently, rituximab was introduced for both induction and remission maintenance treatment in ANCA-associated vasculitis.^{27, 28, 115}

Induction treatment

Induction treatment of ANCA-associated vasculitis consists of cyclophosphamide treatment until complete clinical remission is achieved, which is often after three to six months. The CYCLOPS trial demonstrated that, while achieving similar remission rates, intravenous pulsed cyclophosphamide treatment results in a lower cumulative cyclophosphamide dose than oral cyclophosphamide treatment.¹⁵ However, long-term analysis of this cohort demonstrated a higher relapse risk in the intravenous group than in the oral group.¹¹⁶ The increased frequency of relapses in the intravenous group was not associated with increased mortality or renal damage.

Two randomised controlled trials, RITUXVAS²⁷ and RAVE²⁸, investigated rituximab – a chimeric monoclonal antibody directed against pre-B cells and mature B lymphocytes – in the treatment of ANCA-associated vasculitis. RITUXVAS included only newly diagnosed patients, whereas in RAVE both new and relapsing patients were included. In both trials, rituximab was non-inferior to cyclophosphamide for remission induction. Moreover, both trials demonstrated no significant difference between the number of adverse events in the rituximab group and the cyclophosphamide group. However, concerns were raised about a possible higher malignancy rate in patients treated with rituximab.^{117, 118} **Chapter 5** of this thesis reports the first 10-year follow-up study investigating malignancy risk in patients with ANCA-associated vasculitis treated with cyclophosphamide according to current treatment regimens. **Chapter 6** reports the first study to compare the long-term malignancy risks between rituximab-based treatment and cyclophosphamide-based treatment in ANCA-associated vasculitis.

The NORAM trial investigated methotrexate induction treatment in patients with ANCA-associated vasculitis with early systemic disease without significant renal involvement.¹⁴ Methotrexate was demonstrated to have similar efficacy to cyclophosphamide for remission induction, but was associated with a higher relapse rate. Long-term follow-up of these patients demonstrated that methotrexate treatment is associated with less effective disease control and prolonged use of steroids.¹¹⁹

Evidence from a retrospective study¹²⁰ and a prospective pilot trial¹²¹ indicates that mycophenolate mofetil is effective in the induction treatment of ANCA-associated vasculitis. An advantage of mycophenolate mofetil over cyclophosphamide includes a more favourable safety profile in terms of toxicity. The MYCYC trial compares mycophenolate mofetil to cyclophosphamide

for induction treatment in ANCA-associated vasculitis (ClinicalTrials.gov NCT00414128). The MYCYC trial results are expected in 2018.

Maintenance treatment

Maintenance treatment aims at the prevention of relapses. The landmark EUVAS trial CYCAZAREM demonstrated that cyclophosphamide exposure in patients with ANCA-associated vasculitis can be safely reduced by the substitution of cyclophosphamide by azathioprine shortly after remission achievement.¹⁶ Relapse risk and severe adverse event occurrence were similar between the cyclophosphamide group and the azathioprine group. Currently, azathioprine, alongside with methotrexate, is the first choice for maintenance treatment in ANCA-associated vasculitis.¹²²

Methotrexate can be used as an alternative to azathioprine for remission maintenance in patients with ANCA-associated vasculitis in whom renal function is not severely impaired. The WEGENT trial demonstrated similar remission maintenance and adverse events rates between methotrexate and azathioprine maintenance treatment.¹¹⁴

Rituximab was recently introduced for remission maintenance treatment in ANCA-associated vasculitis patients who relapsed on other maintenance therapies or who are at high risk of relapse. The MAINRITSAN trial demonstrated a superiority of rituximab over azathioprine as maintenance treatment after cyclophosphamide induction treatment.¹¹⁵ Adverse events rates were similar in the rituximab group and the azathioprine group. The ongoing RITAZERAM trial compares rituximab to azathioprine as maintenance treatment in relapsing patients who achieved remission following rituximab induction treatment (ClinicalTrials.gov NCT01697267).

The IMPROVE trial demonstrated that mycophenolate mofetil is less effective than azathioprine in remission maintenance after remission induction with cyclophosphamide.¹¹¹ Therefore, mycophenolate mofetil maintenance treatment is only considered in ANCA-associated vasculitis patients in whom azathioprine and methotrexate are contraindicated.

Prognosis of patients with ANCA-associated vasculitis

As stated previously, the natural history of untreated ANCA-associated vasculitis is that of a rapidly progressive, usually fatal disease.⁷⁹ The introduction of immunosuppressive therapy in the 1960s has dramatically reduced the 1-year mortality rate of patients with ANCA-associated vasculitis from 82% to 10%.^{32, 79, 123} Nevertheless, patients continue to have an increased mortality risk compared to the general population.¹²³

A large, prospective 5-year follow-up study that included 535 patients demonstrated that ANCA-associated vasculitis patients have a 2.6 times increased mortality risk compared to the general population.¹²³ Infection (48%) and active vasculitis (19%) were the main causes of death during the first year after diagnosis. This finding emphasizes the importance of finding the right balance between disease control and immunosuppressive treatment. After the first year of diagnosis, patients continued to have a 1.3 times increased mortality risk.¹²³ Cardiovascular disease (26%), malignancy (22%), and infection (20%) accounted for the majority of these deaths.

About 14% of patients will experience at least one major cardiovascular event within 5 years after ANCA-associated vasculitis diagnosis.¹²⁴ Patients with GPA are reported to have a 3.6 times increased myocardial infarction risk compared to the general population.¹²⁵ In addition, when matched for renal function and other traditional risk factors, cardiovascular risk is still doubled in patients with ANCA-associated vasculitis.¹²⁶ PR3-ANCA positivity, older age, and diastolic hypertension were shown to be independent determinants of poor cardiovascular outcome.¹²⁴ Factors contributing to the increased cardiovascular risk in vasculitis include the chronic inflammatory state, endothelial dysfunction, renal dysfunction, and the use of corticosteroids, which accelerates the development of hypertension, dyslipidaemia, and diabetes.^{127, 128}

Malignancies were demonstrated to be the second leading cause of death after the first year of ANCA-associated vasculitis diagnosis.¹²³ A number of studies, using data from retrospective monocentre cohorts,^{76, 129-131} prospective multicentre clinical trials,^{132, 133} and nationwide registry linkage,¹³⁴ demonstrated that patients with ANCA-associated vasculitis have an increased malignancy risk compared to the general population.¹³⁵ In particular, the risks of leukaemia, lymphoma, bladder cancer, and non-melanoma skin cancer (NMSC) were increased. Except for one 7-year follow-up study published in 1992,⁷⁶ average follow-up in these studies was at most five years¹²⁹⁻¹³². Moreover, the observation period in most studies was 1960-1990. Thus, the patients included in these studies were treated with treatment regimens that are outdated and, consequently, the findings of these studies do not represent current malignancy risks.^{76, 129, 131, 134}

As explained previously, treatment regimens in ANCA-associated vasculitis have changed significantly in recent years based on efforts to reduce cumulative cyclophosphamide exposure.¹⁴⁻¹⁷ Moreover, rituximab has emerged as a promising substitute for cyclophosphamide in the treatment of ANCA-associated vasculitis.^{27,}

^{28, 136, 137} **Chapter 5** of this thesis reports the first 10-year follow-up study that investigates malignancy risk in ANCA-associated vasculitis patients treated with cyclophosphamide according to current treatment regimens. **Chapter 6** reports the first study to compare the long-term malignancy risks between rituximab-based treatment and cyclophosphamide-based treatment in ANCA-associated vasculitis.

Several factors have been hypothesized to contribute to the increased malignancy risk in patients with ANCA-associated vasculitis. Firstly, immunosuppressive treatment may decrease the immune system's ability to recognize and eradicate malignant cells. The importance of a well-functioning immune system in the prevention of malignancies is well-demonstrated by the increased malignancy risk observed in HIV-positive patients.¹³⁸ Moreover, immunosuppressive therapy itself may have direct mutagenic properties, as, for example, demonstrated in cyclophosphamide-induced bladder cancer.¹³⁹⁻¹⁴³ Furthermore, long-standing immune activation per se may be oncogenic; for example, long-standing immune activation is hypothesized to contribute to the increased lymphoma risk seen in a number of chronic autoimmune rheumatic conditions.¹⁴⁴

The European Vasculitis Society and the European Vasculitis Genetics Consortium

Research in ANCA-associated vasculitis is hindered by the low incidence of this complex disease. Therefore, in 1994 the EUVAS was founded with the objective of uniting vasculitis researchers and clinicians and promoting the study of vasculitis. The apparent need for clinical trials was demonstrated by the wide heterogeneity in treatment, poor patient outcomes, and high levels of treatment-related toxicity.¹²² Thus far, the EUVAS has conducted a significant number of clinical trials with the aim of optimizing the treatment of ANCA-associated vasculitis.^{14-17, 27, 110, 111, 145} Long-term follow-up studies of the first four EUVAS trials (CYCAZAREM,¹⁶ NORAM,¹⁴ MEPEX,¹⁷ and CYCLOPS¹⁵) have been published and longer follow-up is pending. The aim of these studies was to investigate several long-term outcomes in ANCA-associated vasculitis, including long-term patient survival,¹²³ disease relapse,¹⁴⁶ cardiovascular events,¹²⁴ malignancies,¹³² disease-related damage,¹⁴⁷⁻¹⁴⁹ and severe adverse events¹⁵⁰. To harmonize the clinical trials, scoring systems were created for both presenting clinical manifestations¹⁵¹⁻¹⁵³ and renal histology¹⁵⁴. Other studies conducted by the EUVAS include ANCA assay standardization studies^{18, 155, 156} and renal histopathological studies^{84, 85, 88, 157-162}. The study presented in **Chapter 3** of this thesis was performed within the collaboration of the EUVAS. The European Vasculitis Genetics Consortium has grown out of EUVAS activity and focuses on the genetics of ANCA-associated vasculitis. In 2010, the European Vasculitis Genetic Consortium published the first GWAS in ANCA-associated vasculitis.²⁶ The study presented in **Chapter 2** of this thesis was performed within the collaboration of the European Vasculitis Genetic Consortium.

Outline of this thesis

The role of genetic variants in ANCA-associated vasculitis is explored in **Chapter 2** of this thesis. The numerous candidate gene association studies and two GWAS have revealed a great number of genetic variants that could contribute to the pathogenesis of ANCA-associated vasculitis. In **Chapter 2** we report the results of a meta-analysis investigating the genetic variants that are most likely associated with ANCA-associated vasculitis. We included raw data from the European Vasculitis Genetic Consortium GWAS to increase the validity of the meta-analysis.²⁶ Moreover, in the light of the ongoing debate as to whether the different subtypes within ANCA-associated vasculitis represent different entities within the same disease spectrum or are distinct auto-immune diseases, we investigated whether these subtypes have distinct genetic backgrounds. In **Chapter 3** we investigated whether ENT involvement represents a separate phenotype in ANCA-associated vasculitis by exploring the relationship between ENT manifestations and renal outcome.

As discussed previously, renal disease is a common and severe manifestation of ANCA-associated vasculitis that can lead to end-stage renal disease and death. **Chapter 4** reviews the outcomes of studies validating the histopathological classification of ANCA-associated glomerulonephritis and discusses points of consideration and future perspectives.

The prognosis of patients with ANCA-associated vasculitis has improved dramatically in recent years, shifting attention towards the long-term complications these patients experience. In **Chapter 5** the malignancy risk in patients with ANCA-associated vasculitis treated with current immunosuppressive regimens was investigated and its relationship with cyclophosphamide treatment was explored. Recently, rituximab was introduced for both induction and remission maintenance treatment in ANCA-associated vasculitis with the promise of, amongst others, further reducing malignancy risk. **Chapter 6** reports the first study to date to compare the long-term malignancy risks between rituximab-based treatment and cyclophosphamide-based treatment in ANCA-associated vasculitis.

The findings of this thesis will be summarized and placed in a more general perspective in **Chapter 7**.

References

- Jennette JC, Falk RJ, Andrassy K, *et al.* Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187-92.
- Jennette JC, Falk RJ, Bacon PA, *et al.* 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1-11.
- Berden A, Goceroglu A, Jayne D, *et al.* Diagnosis and management of ANCA associated vasculitis. *BMJ* 2012;344:e26.
- van der Woude FJ, Rasmussen N, Lobatto S, *et al.* Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985;1:425-9.
- Davies DJ, Moran JE, Niall JF, *et al.* Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed)* 1982;285:606.
- Goldschmeding R, van der Schoot CE, ten Bokkel Huinink D, *et al.* Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 1989;84:1577-87.
- Niles JL, McCluskey RT, Ahmad MF, *et al.* Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. *Blood* 1989;74:1888-93.
- Jennette JC, Hoidal JR, Falk RJ. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood* 1990;75:2263-4.
- 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;318:1651-7.
- Falk RJ, Gross WL, Guillevin L, *et al.* Granulomatosis with polyangiitis (Wegener's): an alternative name for Wegener's granulomatosis. *J Am Soc Nephrol* 2011;22:587-8.
- Falk RJ, Gross WL, Guillevin L, *et al.* Granulomatosis with polyangiitis (Wegener's): an alternative name for Wegener's granulomatosis. *Ann Rheum Dis* 2011;70:704.
- Falk RJ, Gross WL, Guillevin L, *et al.* Granulomatosis with polyangiitis (Wegener's): an alternative name for Wegener's granulomatosis. *Arthritis Rheum* 2011;63:863-4.
- Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med* 1997;337:1512-23.
- 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;52:2461-9.
- 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;150:670-80.
- 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.
- Jayne DR, Gaskin G, Rasmussen N, *et al.* Randomized trial of plasma exchange or high-dose methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol* 2007;18:2180-8.
- 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;53:743-53.

19. Keogh KA, Specks U. Churg-Strauss syndrome: clinical presentation, antineutrophil cytoplasmic antibodies, and leukotriene receptor antagonists. *Am J Med* 2003;115:284-90.
20. Sable-Fourtassou R, Cohen P, Mahr A, *et al.* Antineutrophil cytoplasmic antibodies and the Churg-Strauss syndrome. *Ann Intern Med* 2005;143:632-8.
21. Sinico RA, Di Toma L, Maggiore U, *et al.* Renal involvement in Churg-Strauss syndrome. *Am J Kidney Dis* 2006;47:770-9.
22. 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;67:1004-10.
23. Hogan SL, Falk RJ, Nachman PH, *et al.* Various forms of life in antineutrophil cytoplasmic antibody-associated vasculitis. *Ann Intern Med* 2006;144:377-8; author reply 8-9.
24. Hoffman GS, Langford CA. Are there different forms of life in the antineutrophil cytoplasmic antibody universe? *Ann Intern Med* 2005;143:683-5.
25. Linder R, Orth I, Hagen EC, *et al.* Differentiation between Wegener's granulomatosis and microscopic polyangiitis by an artificial neural network and by traditional methods. *J Rheumatol* 2011;38:1039-47.
26. Lyons PA, Rayner TF, Trivedi S, *et al.* Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med* 2012;367:214-23.
27. Jones RB, Tervaert JW, Hauser T, *et al.* Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010;363:211-20.
28. Stone JH, Merkel PA, Spiera R, *et al.* Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010;363:221-32.
29. Xiao H, Hu P, Falk RJ, *et al.* Overview of the Pathogenesis of ANCA-Associated Vasculitis. *Kidney Dis (Basel)* 2016;1:205-15.
30. 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;3:237-52.
31. Pinching AJ, Rees AJ, Pussell BA, *et al.* Relapses in Wegener's granulomatosis: the role of infection. *Br Med J* 1980;281:836-8.
32. Fauci AS, Haynes BF, Katz P, *et al.* Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 1983;98:76-85.
33. Boudewyns A, Verbelen J, Koekelkoren E, *et al.* Wegener's granulomatosis triggered by infection? *Acta Otorhinolaryngol Belg* 2001;55:57-63.
34. Subra JF, Michelet C, Laporte J, *et al.* The presence of cytoplasmic antineutrophil cytoplasmic antibodies (C-ANCA) in the course of subacute bacterial endocarditis with glomerular involvement, coincidence or association? *Clin Nephrol* 1998;49:15-8.
35. Hellmich B, Ehren M, Lindstaedt M, *et al.* Anti-MPO-ANCA-positive microscopic polyangiitis following subacute bacterial endocarditis. *Clin Rheumatol* 2001;20:441-3.
36. Stegeman CA, Tervaert JW, Sluiter WJ, *et al.* Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. *Ann Intern Med* 1994;120:12-7.
37. Stegeman CA, Tervaert JW, de Jong PE, *et al.* 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.
38. Axelson JA, Clark RH, Ancerewicz S. Wegener granulomatosis and trimethoprim-sulfamethoxazole. *Ann Intern Med* 1987;107:600.

39. DeRemee RA. The treatment of Wegener's granulomatosis with trimethoprim/sulfamethoxazole: illusion or vision? *Arthritis Rheum* 1988;31:1068-74.
40. DeRemee RA, McDonald TJ, Weiland LH. Wegener's granulomatosis: observations on treatment with antimicrobial agents. *Mayo Clin Proc* 1985;60:27-32.
41. George J, Levy Y, Kallenberg CG, *et al.* Infections and Wegener's granulomatosis--a cause and effect relationship? *QJM* 1997;90:367-73.
42. Valeriano-Marcet J, Spiera H. Treatment of Wegener's granulomatosis with sulfamethoxazole-trimethoprim. *Arch Intern Med* 1991;151:1649-52.
43. West BC, Todd JR, King JW. Wegener granulomatosis and trimethoprim-sulfamethoxazole. Complete remission after a twenty-year course. *Ann Intern Med* 1987;106:840-2.
44. Yuasa K, Tokitsu M, Goto H, *et al.* Wegener's granulomatosis: diagnosis by transbronchial lung biopsy, evaluation by gallium scintigraphy and treatment with sulfamethoxazole/trimethoprim. *Am J Med* 1988;84:371-2.
45. Zycinska K, Wardyn KA, Zielonka TM, *et al.* Co-trimoxazole and prevention of relapses of PR3-ANCA positive vasculitis with pulmonary involvement. *Eur J Med Res* 2009;14 Suppl 4:265-7.
46. Ohtake T, Kobayashi S, Honjou Y, *et al.* Generalized Wegener's granulomatosis responding to sulfamethoxazole-trimethoprim monotherapy. *Intern Med* 2001;40:666-70.
47. Franssen CF, ter Maaten JC, Hoorntje SJ. Brother and sister with myeloperoxidase associated autoimmune disease. *Ann Rheum Dis* 1994;53:213.
48. Hay EM, Beaman M, Ralston AJ, *et al.* Wegener's granulomatosis occurring in siblings. *Br J Rheumatol* 1991;30:144-5.
49. Hull CM, Couser WG, Knostman JD. A familial case of P-ANCA glomerulonephritis presenting in a father and daughter. *Am J Kidney Dis* 2000;35:E23.
50. Muniain MA, Moreno JC, Gonzalez Campora R. Wegener's granulomatosis in two sisters. *Ann Rheum Dis* 1986;45:417-21.
51. Nowack R, Lehmann H, Flores-Suarez LF, *et al.* Familial occurrence of systemic vasculitis and rapidly progressive glomerulonephritis. *Am J Kidney Dis* 1999;34:364-73.
52. Piram M, Maldini C, Mahr A. Effect of race/ethnicity on risk, presentation and course of connective tissue diseases and primary systemic vasculitides. *Curr Opin Rheumatol* 2012;24:193-200.
53. Bonatti F, Reina M, Neri TM, *et al.* Genetic Susceptibility to ANCA-Associated Vasculitis: State of the Art. *Front Immunol* 2014;5:577.
54. Xie G, Roshandel D, Sherva R, *et al.* Association of granulomatosis with polyangiitis (Wegener's) with HLA-DPB1*04 and SEMA6A gene variants: evidence from genome-wide analysis. *Arthritis Rheum* 2013;65:2457-68.
55. Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat Rev Rheumatol* 2014;10:463-73.
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;110:955-63.
57. Falk RJ, Terrell RS, Charles LA, *et al.* Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A* 1990;87:4115-9.
58. Huugen D, Xiao H, van Esch A, *et al.* Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-alpha. *Am J Pathol* 2005;167:47-58.
59. Schreiber A, Xiao H, Jennette JC, *et al.* C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol* 2009;20:289-98.

60. Ewert BH, Jennette JC, Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int* 1992;41:375-83.
61. Savage CO, Gaskin G, Pusey CD, *et al.* Myeloperoxidase binds to vascular endothelial cells, is recognized by ANCA and can enhance complement dependent cytotoxicity. *Adv Exp Med Biol* 1993;336:121-3.
62. Xiao H, Schreiber A, Heeringa P, *et al.* Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007;170:52-64.
63. Little MA, Smyth CL, Yadav R, *et al.* Anti-neutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood* 2005;106:2050-8.
64. Little MA. L7. Animal models of PR3-ANCA vasculitis: approaches and controversies. *Presse Med* 2013;42:512-5.
65. 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.
66. Schlieben DJ, Korbet SM, Kimura RE, *et al.* Pulmonary-renal syndrome in a newborn with placental transmission of ANCAs. *Am J Kidney Dis* 2005;45:758-61.
67. Choi HK, Merkel PA, Walker AM, *et al.* Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titers of antimyeloperoxidase antibodies. *Arthritis Rheum* 2000;43:405-13.
68. Roth AJ, Ooi JD, Hess JJ, *et al.* Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. *J Clin Invest* 2013;123:1773-83.
69. Olson SW, Owshalimpur D, Yuan CM, *et al.* Relation between asymptomatic proteinase 3 antibodies and future granulomatosis with polyangiitis. *Clin J Am Soc Nephrol* 2013;8:1312-8.
70. Cui Z, Zhao MH, Segelmark M, *et al.* Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int* 2010;78:590-7.
71. Olson SW, Arbogast CB, Baker TP, *et al.* Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. *J Am Soc Nephrol* 2011;22:1946-52.
72. Xu PC, Cui Z, Chen M, *et al.* Comparison of characteristics of natural autoantibodies against myeloperoxidase and anti-myeloperoxidase autoantibodies from patients with microscopic polyangiitis. *Rheumatology (Oxford)* 2011;50:1236-43.
73. Jennette JC, Falk RJ. L1. Pathogenesis of ANCA-associated vasculitis: observations, theories and speculations. *Presse Med* 2013;42:493-8.
74. Watts RA, Lane SE, Bentham G, *et al.* Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. *Arthritis Rheum* 2000;43:414-9.
75. 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;29:309-16.
76. Hoffman GS, Kerr GS, Leavitt RY, *et al.* Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med* 1992;116:488-98.
77. 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;43:1021-32.
78. Stone JH, Wegener's Granulomatosis Etanercept Trial Research Group. Limited versus severe Wegener's granulomatosis: baseline data on patients in the Wegener's granu-

- lomatosis etanercept trial. *Arthritis Rheum* 2003;48:2299-309.
79. Walton EW. Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *Br Med J* 1958;2:265-70.
80. Jayne DR, Marshall PD, Jones SJ, *et al.* Auto-antibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 1990;37:965-70.
81. Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164-77.
82. Bajema IM, Hagen EC, Ferrario F, *et al.* Immunopathological aspects of systemic vasculitis. *Springer Semin Immunopathol* 2001;23:253-65.
83. 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;12:277-91.
84. Bajema IM, Hagen EC, Hermans J, *et al.* Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int* 1999;56:1751-8.
85. 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;62:1732-42.
86. Aasarod K, Bostad L, Hammerstrom J, *et al.* Renal histopathology and clinical course in 94 patients with Wegener's granulomatosis. *Nephrol Dial Transplant* 2001;16:953-60.
87. Haroun MK, Stone JH, Nair R, *et al.* Correlation of percentage of normal glomeruli with renal outcome in Wegener's granulomatosis. *Am J Nephrol* 2002;22:497-503.
88. Berden AE, Ferrario F, Hagen EC, *et al.* Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 2010;21:1628-36.
89. 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;9:1684-91.
90. Khalighi MA, Wang S, Henriksen KJ, *et al.* Pauci-immune glomerulonephritis in children: a clinicopathologic study of 21 patients. *Pediatr Nephrol* 2015;30:953-9.
91. Bjornekleit R, Sriskandarajah S, Bostad L. Prognostic Value of Histologic Classification of ANCA-Associated Glomerulonephritis. *Clin J Am Soc Nephrol* 2016;11:2159-67.
92. Kristensen T, Gregersen JW, Krag SR, *et al.* The relation between histopathological classification and renal outcome, ANCA subtype and treatment regimens in ANCA-associated vasculitis. *Clin Exp Rheumatol* 2016;34:S105-10.
93. 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;52:119-27.
94. 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;30:1185-92.
95. 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;29:1764-9.
96. Cordova-Sanchez BM, Mejia-Vilet JM, Morales-Buenrostro LE, *et al.* Clinical presentation and outcome prediction of clinical, serological, and histopathological classification schemes in ANCA-associated vasculitis with renal involvement. *Clin Rheumatol* 2016;35:1805-16.
97. 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;44:304-13.
98. Nohr E, Girard L, James M, *et al.* Validation of a histopathologic classification scheme for antineutrophil cytoplasmic antibody-associated glomerulonephritis. *Hum Pathol* 2014;45:1423-9.
99. Unlu M, Kiremitci S, Ensari A, *et al.* Pauci-immune necrotizing crescentic glomerulonephritis with crescentic and full moon extracapillary proliferation: clinico-pathologic correlation and follow-up study. *Pathol Res Pract* 2013;209:75-82.
100. Ford SL, Polkinghorne KR, Longano A, *et al.* Histopathologic and clinical predictors of kidney outcomes in ANCA-associated vasculitis. *Am J Kidney Dis* 2014;63:227-35.
101. 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;24:300-3.
102. Ellis CL, Manno RL, Havill JP, *et al.* 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.
103. Chang DY, Wu LH, Liu G, *et al.* Re-evaluation of the histopathologic classification of ANCA-associated glomerulonephritis: a study of 121 patients in a single center. *Nephrol Dial Transplant* 2012;27:2343-9.
104. 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;17:659-62.
105. 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.
106. Hilhorst M, Wilde B, van Breda Vriesman P, *et al.* Estimating renal survival using the ANCA-associated GN classification. *J Am Soc Nephrol* 2013;24:1371-5.
107. Naidu GS, Sharma A, Nada R, *et al.* Histopathological classification of pauci-immune glomerulonephritis and its impact on outcome. *Rheumatol Int* 2014;34:1721-7.
108. Moroni G, Binda V, Leoni A, *et al.* Predictors of renal survival in ANCA-associated vasculitis. Validation of a histopathological classification schema and review of the literature. *Clin Exp Rheumatol* 2015;33:S-56-63.
109. Li X, Liang S, Zheng C, *et al.* Clinicopathological characteristics and outcomes of pediatric patients with systemic small blood vessel vasculitis. *Pediatr Nephrol* 2014;29:2365-71.
110. Schmitt WH, Hagen EC, Neumann I, *et al.* Treatment of refractory Wegener's granulomatosis with antithymocyte globulin (ATG): an open study in 15 patients. *Kidney Int* 2004;65:1440-8.
111. 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;304:2381-8.
112. Specks U, Merkel PA, Seo P, *et al.* Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med* 2013;369:417-27.
113. Hellmich B, Lamprecht P, Gross WL. Advances in the therapy of Wegener's granulomatosis. *Curr Opin Rheumatol* 2006;18:25-32.
114. Pagnoux C, Mahr A, Hamidou MA, *et al.* Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med* 2008;359:2790-803.
115. Guillevin L, Pagnoux C, Karras A, *et al.* Rituximab versus azathioprine for maintenance

- in ANCA-associated vasculitis. *N Engl J Med* 2014;371:1771-80.
116. 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;71:955-60.
117. Karassa FB. Rituximab or cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010;363:2073; author reply -4.
118. Falk RJ, Jennette JC. Rituximab in ANCA-associated disease. *N Engl J Med* 2010;363:285-6.
119. Faurschou M, Westman K, Rasmussen N, *et al.* Brief Report: long-term outcome of a randomized clinical trial comparing methotrexate to cyclophosphamide for remission induction in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2012;64:3472-7.
120. Draibe J, Poveda R, Fulladosa X, *et al.* Use of mycophenolate in ANCA-associated renal vasculitis: 13 years of experience at a university hospital. *Nephrol Dial Transplant* 2015;30 Suppl 1:i132-7.
121. Silva F, Specks U, Kalra S, *et al.* Mycophenolate mofetil for induction and maintenance of remission in microscopic polyangiitis with mild to moderate renal involvement--a prospective, open-label pilot trial. *Clin J Am Soc Nephrol* 2010;5:445-53.
122. Gopaluni S, Jayne D. Clinical Trials in Vasculitis. *Curr Treatm Opt Rheumatol* 2016;2:161-77.
123. Flossmann O, Berden A, de Groot K, *et al.* Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis* 2011;70:488-94.
124. 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;63:588-96.
125. Faurschou M, Mellemkjaer L, Sorensen IJ, *et al.* Increased morbidity from ischemic heart disease in patients with Wegener's granulomatosis. *Arthritis Rheum* 2009;60:1187-92.
126. 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;60:3493-500.
127. Raza K, Thambyrajah J, Townend JN, *et al.* Suppression of inflammation in primary systemic vasculitis restores vascular endothelial function: lessons for atherosclerotic disease? *Circulation* 2000;102:1470-2.
128. Booth AD, Jayne DR, Kharbanda RK, *et al.* Infliximab improves endothelial dysfunction in systemic vasculitis: a model of vascular inflammation. *Circulation* 2004;109:1718-23.
129. Westman KW, Bygren PG, Olsson H, *et al.* Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J Am Soc Nephrol* 1998;9:842-52.
130. Faurschou M, Sorensen IJ, Mellemkjaer L, *et al.* Malignancies in Wegener's granulomatosis: incidence and relation to cyclophosphamide therapy in a cohort of 293 patients. *J Rheumatol* 2008;35:100-5.
131. Holle JU, Gross WL, Latza U, *et al.* Improved outcome in 445 patients with Wegener's granulomatosis in a German vasculitis center over four decades. *Arthritis Rheum* 2011;63:257-66.
132. 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;70:1415-21.
133. Silva F, Seo P, Schroeder DR, *et al.* Solid malignancies among etanercept-treated patients with granulomatosis with polyangiitis (Wegener's): long-term followup of a multicenter longitudinal cohort. *Arthritis Rheum* 2011;63:2495-503.

134. Knight A, Askling J, Ekbom A. Cancer incidence in a population-based cohort of patients with Wegener's granulomatosis. *Int J Cancer* 2002;100:82-5.
135. Mahr A, Heijl C, Le Guenno G, *et al.* ANCA-associated vasculitis and malignancy: current evidence for cause and consequence relationships. *Best Pract Res Clin Rheumatol* 2013;27:45-56.
136. Specks U, Fervenza FC, McDonald TJ, *et al.* Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy. *Arthritis Rheum* 2001;44:2836-40.
137. Keogh KA, Ytterberg SR, Fervenza FC, *et al.* Rituximab for refractory Wegener's granulomatosis: report of a prospective, open-label pilot trial. *Am J Respir Crit Care Med* 2006;173:180-7.
138. Grulich AE, van Leeuwen MT, Falster MO, *et al.* Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 2007;370:59-67.
139. Pedersen-Bjergaard J, Specht L, Larsen SO, *et al.* Risk of therapy-related leukaemia and pre-leukaemia after Hodgkin's disease. Relation to age, cumulative dose of alkylating agents, and time from chemotherapy. *Lancet* 1987;2:83-8.
140. Pedersen-Bjergaard J, Ersboll J, Hansen VL, *et al.* Carcinoma of the urinary bladder after treatment with cyclophosphamide for non-Hodgkin's lymphoma. *N Engl J Med* 1988;318:1028-32.
141. Travis LB, Curtis RE, Glimelius B, *et al.* Bladder and kidney cancer following cyclophosphamide therapy for non-Hodgkin's lymphoma. *J Natl Cancer Inst* 1995;87:524-30.
142. Worth PH. Cyclophosphamide and the bladder. *Br Med J* 1971;3:182.
143. Grosse Y, Baan R, Straif K, *et al.* A review of human carcinogens--Part A: pharmaceuticals. *Lancet Oncol* 2009;10:13-4.
144. Zintzaras E, Voulgarelis M, Moutsopoulos HM. The risk of lymphoma development in autoimmune diseases: a meta-analysis. *Arch Intern Med* 2005;165:2337-44.
145. 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.
146. Walsh M, Flossmann O, Berden A, *et al.* Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2012;64:542-8.
147. Robson J, Doll H, Suppiah R, *et al.* Damage in the anca-associated vasculitides: long-term data from the European vasculitis study group (EUVAS) therapeutic trials. *Ann Rheum Dis* 2015;74:177-84.
148. Martinez Del Pero M, Walsh M, Luqmani R, *et al.* Long-term damage to the ENT system in Wegener's granulomatosis. *Eur Arch Otorhinolaryngol* 2011;268:733-9.
149. Suppiah R, Hadden RD, Batra R, *et al.* Peripheral neuropathy in ANCA-associated vasculitis: outcomes from the European Vasculitis Study Group trials. *Rheumatology (Oxford)* 2011;50:2214-22.
150. 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;69:1036-43.
151. Luqmani RA, Bacon PA, Moots RJ, *et al.* Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM* 1994;87:671-8.
152. 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;40:371-80.
153. 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 Vascu-

- litides (INSSYS). *Arthritis Rheum* 2001;44:912-20.
154. 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;11:1989-95.
155. 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.
156. Hagen EC, Andrassy K, Chernok E, *et al.* The value of indirect immunofluorescence and solid phase techniques for ANCA detection. A report on the first phase of an international cooperative study on the standardization of ANCA assays. EEC/BCR Group for ANCA Assay Standardization. *J Immunol Methods* 1993;159:1-16.
157. Hauer HA, Bajema IM, Hagen EC, *et al.* Long-term renal injury in ANCA-associated vasculitis: an analysis of 31 patients with follow-up biopsies. *Nephrol Dial Transplant* 2002;17:587-96.
158. Hauer HA, Bajema IM, van Houwelingen HC, *et al.* Renal histology in ANCA-associated vasculitis: differences between diagnostic and serologic subgroups. *Kidney Int* 2002;61:80-9.
159. Vergunst CE, van Gurp E, Hagen EC, *et al.* An index for renal outcome in ANCA-associated glomerulonephritis. *Am J Kidney Dis* 2003;41:532-8.
160. 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;17:2264-74.
161. de Lind van Wijngaarden RA, Hauer HA, Wolterbeek R, *et al.* Chances of renal recovery for dialysis-dependent ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 2007;18:2189-97.
162. 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;23:313-21.