



Universiteit
Leiden
The Netherlands

ANCA-associated glomerulonephritis : insights into etiology, pathogenesis, and prognosis

Lind van Wijngaarden, R.A.F. de

Citation

Lind van Wijngaarden, R. A. F. de. (2009, March 12). *ANCA-associated glomerulonephritis : insights into etiology, pathogenesis, and prognosis*. Retrieved from <https://hdl.handle.net/1887/13612>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/13612>

Note: To cite this publication please use the final published version (if applicable).



Chapter 1

General introduction



This thesis focuses on different aspects of renal involvement in anti-neutrophil cytoplasm autoantibodies (ANCA)-associated vasculitis, in particular histological lesions and clinical outcome. This chapter introduces the reader to some of the many aspects of this disease and aims to put ANCA-associated vasculitis and its various clinical and histopathological presentations into broader perspective. The paragraphs of this chapter further consider current hypotheses on pathogenesis and up-to-date views on treatment and prognosis. Considerations on etiology will be discussed in chapter 6.

Vasculitis

Classification of systemic vasculitis

Vasculitis refers to an inflammatory process in the blood vessel wall. It is a spectrum of different diseases often associated with destruction of the vessel wall and occlusion of the vascular lumen. Since any vessel in any organ can be affected, clinical manifestations can vary widely. Therefore, vasculitis is hard to diagnose, and in clinical practice, is often not recognized at first. Furthermore, within the spectrum of vasculitides, it is often not clear-cut which disease the patient is suffering from. Nevertheless, this distinction is important to establish, since the different vasculitides are treated differently and vary in prognoses ¹. There is a lack of knowledge on the etiology of most (if not all) vasculitides. Therefore, classification can not be based on the cause of the disease, but only on the similar occurrence of symptoms and signs in groups of patients. Over the last fifteen years, progress has been made in accurately classifying the different diseases, mainly by international consensus on nomenclature and serology ², but up to this day diagnosing individual patients remains hard.

In 1994, during the Chapel Hill consensus conference on the nomenclature of systemic vasculitis, an attempt was made to unify nomenclature of the different forms of vasculitis ². A classification system was made, based on the size of vessels affected by the different diseases. Distinction was made between large-vessel vasculitis, affecting the aorta and the largest arterial branches directed toward major body regions, medium-sized-vessel vasculitis, affecting the main visceral arteries and their branches, and small-vessel vasculitis, affecting small arteries, arterioles, capillaries, and venules. Of note is that all three categories of vasculitis can affect arteries. The utility of this classification for diagnosing the individual patient can be questioned. In fact, the authors claim the definitions are not classification criteria, but a guideline that can be used as a basis for classification systems in different studies ². However, the



different ANCA-associated small-vessel vasculitides might still be one disease with each patient having an individual clinical and histological profile of the disease. Moreover, these definitions are not diagnostic criteria, leaving it a difficult task to diagnose individual patients.

This thesis will only focus on small-vessel vasculitis and only on the disease-subcategories that are ANCA-related: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and renal-limited vasculitis (RLV). The Churg-Strauss syndrome (CSS) is not studied in this thesis, since histological features and clinical manifestations are different from the other ANCA-associated small-vessel vasculitides.

Clinical picture

ANCA-associated vasculitis is, with an incidence of 15-30 patients/million per year, the most frequently occurring primary small-vessel vasculitis. It is usually diagnosed between 50 and 60 years of age, but can occur at any age. Early in the course of their disease, many vasculitis patients describe a 'flu-like' syndrome. General signs and symptoms include fever, malaise, myalgias, and migratory arthralgias. Small-vessel vasculitis can involve venules, capillaries, and arterioles, but also small arteries and veins anywhere in the body². The respiratory tract, kidneys, skin, gut, skeletal muscles, and peripheral nerves, for instance, often participate in the disease process of small-vessel vasculitis. Clinically, this disease entity includes the diagnoses of WG, MPA, and CSS. WG and MPA share many histological features, such as necrotizing glomerulonephritis which often leads to progressive renal failure. The classic triad of systemic small-vessel vasculitis, granulomatous inflammation of the respiratory tract, and necrotizing glomerulonephritis is highly suggestive of a diagnosis of WG³. It can be distinguished from other vasculitides by the presence of necrotizing granulomatous inflammation in the absence of asthma. MPA has a similar spectrum of disease manifestations, but there is an absence of granulomatous inflammation². However, in WG granulomatous inflammation can often not be detected. Differentiation between these two diagnoses before starting treatment is not clinically relevant, since treatment is essentially the same. These arguments strengthen the hypothesis that WG and MPA should be regarded as two forms of a spectrum of ANCA-associated diseases, instead of two separate entities. If an ANCA-positive patient has pulmonary infiltrates with pauci-immune crescentic necrotizing glomerulonephritis on renal biopsy, treatment should not be delayed¹. RLV is

also distinguished and is characterized by the absence of extra-renal organ involvement. In CSS, three phases can be distinguished: allergic rhinitis and asthma, eosinophilic infiltrative disease resulting e.g. in gastroenteritis or eosinophilic pneumonia, and systemic small-vessel vasculitis with granulomatous inflammation ⁴⁻⁶. Neuropathy and cardiac disease are more frequent than in WG and MPA, while renal disease is less frequent ^{4,6}. CSS will not be further discussed in this thesis.

Discovery of small-vessel vasculitis

Vasculitis as it is known today was discovered through two historical pathways of investigation that eventually crossed each other's way. One sprouted from the description of necrotizing arteritis ⁷, while the other found its roots in the description of purpura ⁸⁻¹¹. Eventually, they were both considered to be part of the spectrum of systemic vasculitides.

In 1866, Kussmaul and Maier reported a patient with necrotizing arteritis with nodular inflammatory lesions that affected medium-sized and small arteries in the body, a condition which they called periarteritis nodosa (later known as polyarteritis nodosa or PAN) ¹². The first case of Wegener's granulomatosis was described by Klinger in 1931 ¹³, and later in more detail by Wegener in 1939, describing granulomatous disease ¹⁴. A related disease which could be distinguished on the basis of predominantly asthma and eosinophilia was first described in 1951 by Churg and Strauss after whom the syndrome was named ⁷. In 1948, Davson et al. described a microscopic form of PAN with renal involvement, characterized by segmental crescentic glomerulonephritis ¹⁵. Later, in 1954, Godman and Churg distinguished Wegener's granulomatosis, the 'microscopic form of periarteritis' (renamed microscopic polyangiitis in 1994 ²), and polyarteritis nodosa ³. By 1980, the term small-vessel vasculitis was well established.

Already in 1808, the first description of a symptom of small-vessel vasculitis was the distinction between infectious and non-infectious purpura, with a predilection for the lower extremities ⁸. Henoch ^{9,10} and Schönlein ¹¹, after whom the disease was later named, amongst others, reported on the spectrum of signs and symptoms associated with purpura. In the early 1900s, Osler was the first to discover that these symptoms were caused by necrotizing inflammation of the wall of small vessels ^{16,17}, designating this disease to the small-vessel vasculitides.



Immune-complex and pauci-immune small-vessel vasculitis

With the development of immunofluorescence microscopy, the identification of pathogenic antibodies in tissue and serum became part of standardized clinical practice. Examples are the discovery of antibodies directed against the glomerular basement membrane in Goodpasture's syndrome¹⁸⁻²¹, deposition of immune complexes in cryoglobulinemic vasculitis²¹, and IgA deposits in Henoch-Schönlein purpura²². These findings are supported by the concept that in the pathogenesis of some forms of small-vessel vasculitis immune-complex formation is involved. However, in a number of small-vessel vasculitides, in immunofluorescence microscopy, the presence of immune complexes is scarce, with a weak granular staining pattern of IgG, IgM, or complement^{23;24}, and often even absent. This is seen in WG, MPA, and Churg-Strauss syndrome. The condition is designated 'pauci-immune'²⁴⁻²⁶. However, animal studies have demonstrated that immune complexes are present at the site of injury in an early stage of the disease²⁷. Whether immune complexes have never been present at the site of vessel injury or whether they have been resolved by the time patients develop clinical manifestations of ANCA-associated vasculitis is still subject of debate²⁸.

ANCA and its antigens

C-ANCA and P-ANCA

A major breakthrough in the field of small-vessel vasculitis came in 1985, when WG was associated with antibodies directed against lysosomal constituents of neutrophils and monocytes: the ANCA²⁹. A technique was developed by which two distinctive patterns can be visualized by using indirect immunofluorescence (IIF), incubating ethanol-fixed neutrophils from healthy donors with patients' sera³⁰⁻³². One pattern is characterized by diffuse fine granular staining of the cytoplasm with an accentuation of staining in the central area of the cell between the nuclear lobes, which was named the cytoplasmic staining pattern (C-ANCA). This phenomenon was first described to be specific for WG by van der Woude et al.²⁹. The perinuclear staining pattern (P-ANCA) is characterized by staining of the nucleus, the perinuclear area, or both. The P-ANCA staining pattern is an artifact caused by shifting of cationic cytoplasmic proteins towards the negatively charged nuclear membrane, upon ethanol fixation³³. This pattern was later described by Falk and Jennette³⁴. The two staining patterns are visualized in Figure 1. Any positive staining not showing a clear C-ANCA or P-ANCA pattern is referred to as an atypical staining pattern.

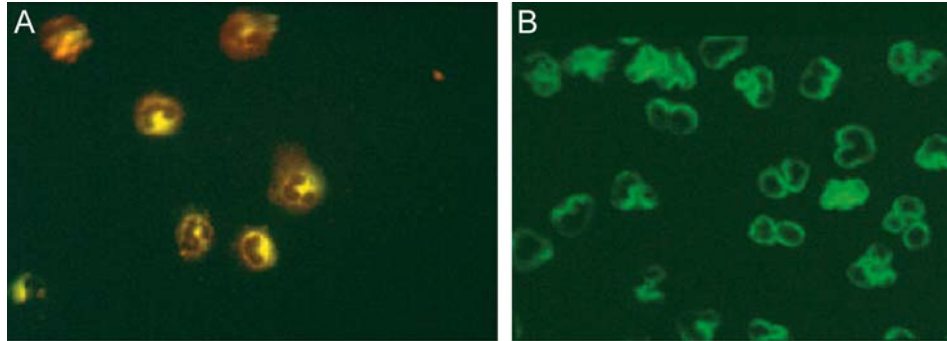


Figure 1. Cytoplasmic components of ethanol-fixed neutrophils, stained by indirect immunofluorescence. A. A characteristic granular pattern of fluorescence is seen: C-ANCA. B. This fluorescence pattern shows perinuclear staining: P-ANCA. Reprinted by permission from Macmillian Publishers Ltd: Nat Clin Pract Rheumatol 2(12): 661-70 © (2006) Kallenberg CG, Heeringa P, Stegeman C. Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides.

PR3-ANCA and MPO-ANCA

As determined by enzyme-linked immunosorbent assay (ELISA), ANCA can be directed against serine proteases such as proteinase-3 (PR3-ANCA), but also against a number of other antigens such as myeloperoxidase (MPO-ANCA), human leukocyte elastase (HLE-ANCA)³⁵, lactoferrin³⁶, cationic antimicrobial protein (CAP57)³⁷, cathepsin G³⁷ and bactericidal/permeability increasing protein (BPI-ANCA)³⁸.

ANCA detection and clinical disease

MPA is characterized by a non-granulomatous systemic vasculitis³⁹, and is often associated with ANCA directed against myeloperoxidase (MPO-ANCA)^{34;39}. WG is characterized by a granulomatous systemic vasculitis with upper airway involvement^{2;40}. ANCA are directed against proteinase-3 (PR3-ANCA) in most WG cases⁴¹. The ELISA has added substantially to diagnosing ANCA-associated vasculitis, with a diagnostic sensitivity higher than IIF⁴².

The link between ANCA pattern by ELISA or IIF and clinical diagnosis is not always clear-cut. Although classical WG is characterized by PR3-ANCA involvement and a C-ANCA pattern, this relationship is not always obvious (Table 1). Classic MPA is characterized by MPO-ANCA and a P-ANCA pattern. A P-ANCA staining pattern can also occur in many non-vasculitic diseases, for instance systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, HIV infection, and chronic liver disease. The occurrence



Table 1. Overview of different diagnoses and the occurrence of different ANCA-patterns as determined by IIF and ELISA. This table was adapted from ref 39.

Diagnosis	C-ANCA	P-ANCA	PR3-ANCA	MPO-ANCA
Wegener's granulomatosis	64%	21%	66%	24%
Microscopic polyangiitis	23%	58%	26%	58%
Renal-limited vasculitis	36%	45%	50%	64%

of the different ANCA patterns in different vasculitides was described by Hagen et al.³⁹ and is listed in Table 1.

Pathogenesis

Are ANCA pathogenic?

The question whether ANCA are pathogenic has been a subject of discussion since their discovery in 1985²⁹. Most ANCA researchers believe they are important in the pathogenesis of ANCA-associated vasculitis. There is some clinical evidence for this presumption, although this is rather suggestive than definitive. First of all, approximately 90% of patients with WG, MPA and renal limited vasculitis have circulating ANCA³⁹. Secondly, ANCA can be induced by certain drugs, such as propylthiouracil, with a concurrent onset of systemic small-vessel vasculitis. Upon cessation of these drugs, ANCA titers and clinical disease resolve (chapter 6). In addition, two recent reports from the European Vasculitis Study Group (EUVAS) have provided evidence that plasma exchange is beneficial as adjunctive treatment⁴³. Even in severe clinical and histologic renal disease, the likelihood of renal recovery was significantly improved with plasma exchange (chapter 3). However, what is responsible for ANCA production in the first place is still unknown. The most important hypotheses on the etiology of ANCA-associated vasculitis are described in chapter 6.

In vitro evidence for a pathogenetic role of ANCA in vasculitis is based on the observation of many laboratories that ANCA IgG can cause activation of MPO- and PR3-containing neutrophils and monocytes. These cells then become activated and release or express molecules that are mediators of inflammation⁴⁴⁻⁴⁸. This process is visualized in Figure 2.

In vivo evidence for pathogenicity of ANCA started with the introduction of the first successful animal model for ANCA-associated vasculitis in 1993²⁷. However in this study, MPO-ANCA did not cause disease in Brown-Norway rats immunized with human MPO. The induction of a neutrophil extract containing MPO and hydrogen peroxide did induce severe necrotizing crescentic glomerulonephritis in MPO-immunized rats²⁷. A major breakthrough

in unravelling the mystery of MPO-ANCA being pathogenic came in 2002⁴⁹. An anti-murine MPO immune response was generated in MPO^{-/-} mice by immunizing them with murine MPO. Adoptive transfer of anti-MPO-positive splenocytes into Rag2^{-/-} mice, that lack functioning T- and B-cells, led to circulating MPO-ANCA and the development of crescentic glomerulonephritis

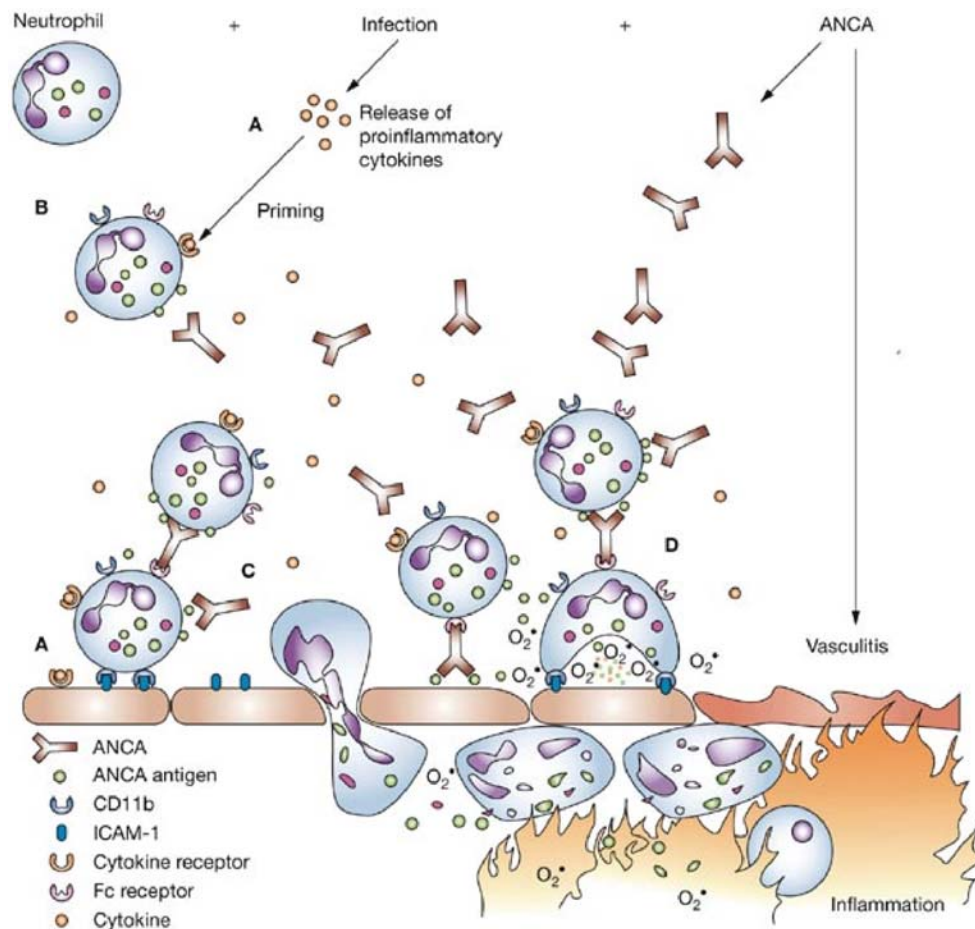


Figure 2. Schematic representation of the neutrophil responses that are putatively involved in the pathogenesis of ANCA-associated small-vessel vasculitis.

Reprinted from Trends Immunol 26: 561–564 © (2005) Heeringa P, Huugen D, Tervaert JW. Anti-neutrophil cytoplasmic autoantibodies and leukocyte–endothelial interactions: a sticky connection?, by permission from Elsevier Ltd. and reprinted by permission from Macmillian Publishers Ltd: Nat Clin Pract Rheumatol 2(12): 661–70 © (2006) Kallenberg CG, Heeringa P, Stegeman C. Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides.



in these mice. In a separate experiment, Rag2^{-/-} and wild-type mice were injected with purified anti-MPO IgG inducing pauci-immune focal necrotizing crescentic glomerulonephritis, more outspoken in the Rag2^{-/-} mice⁴⁹. These experiments led to the conclusion that ANCA can induce vasculitis, even in the absence of T- and B-cells^{50;51}. Furthermore, human MPO immunized WKY rats developed anti-human MPO antibodies cross-reacting with rat MPO and inducing pauci-immune necrotizing crescentic glomerulonephritis over time⁵². Attempts at creating an animal model for vasculitic disease associated with PR3-ANCA have not yet been successful. Recently, it was demonstrated that mice and rats immunised with chimeric human/mouse PR3 produced autoantibodies to mouse PR3⁵³. However, this did not result in any gross pathology in the kidneys nor the lungs of these animals. A possible explanation as to why these experiments have failed might be that murine PR3 is more similar to murine and human neutrophil elastase than to human PR3. This might result in an absence of expression of murine PR3 at the neutrophil membrane of the mouse under inflammatory conditions⁵⁴. Another explanation might be that there are differences in ionic strength between murine MPO, human PR3, and murine PR3, which could explain differences in tissue retention and could account for different pathophysiological effects^{55;56}.

The pathogenesis of fibrinoid necrosis

One of the histologic hallmarks in renal biopsies in ANCA-associated vasculitis is a lesion named fibrinoid necrosis. It is assumed to be a lesion containing necrotic material with a fibrin-like structure, however the exact components of the lesion have not been identified yet⁵⁷. This lesion can not only be found in glomeruli, but also in vessel walls. The pathogenic pathway of fibrinoid necrosis is still a subject of discussion. Nowadays, the most common pathogenic theory (as visualized in Figure 2) is that proinflammatory cytokines and chemokines prime neutrophils and monocytes, causing upregulation of neutrophil adhesion molecules (e.g. CD11b) and translocation of the ANCA antigens from the lysosomal compartments to the cell surface⁵⁰. Proinflammatory cytokines also upregulate endothelial cell adhesion molecules, such as selectins, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1)⁵⁸. Dimer formation between neutrophils takes place by the interaction of the F(ab')₂ portion of ANCA with ANCA antigens on the endothelial cell surface and of the Fc part of the antibody with the Fc receptors on the neutrophil. These interactions activate neutrophils,

promoting the adherence to and transmigration through the vessel wall. Furthermore, ANCA-mediated neutrophil activation causes these cells to undergo a respiratory burst with the release of reactive oxygen species as well as degranulation of azurophilic granules, and the release of pro-inflammatory cytokines⁵⁹⁻⁶². This release of proteolytic enzymes, including the ANCA antigens PR3 and MPO, leads to endothelial cell damage and vasculitis.

A recent hypothesis postulated that PR3 and MPO not only bind to endothelial cells, but are also internalized in these cells⁵⁰. Internalization of PR3 causes endothelial cell apoptosis, whereas internalization of MPO causes generation of oxygen radicals^{50;63}. The encounter of ANCA with its antigens, located on the surface of endothelial cells of tissue matrix, results in the formation of focal immune complexes. These immune complexes recruit neutrophils which dissolve the immune complexes. The presence of the transient immune complexes together with the activation of the alternative pathway of the complement system following ANCA-neutrophil interaction probably initiate an inflammatory amplification loop, enhancing the recruitment of more neutrophils, eventually leading to necrotizing vasculitis^{50;46}.

Another hypothesis that parallels the former, postulated that after the endothelial cells are damaged and vasculitis is initiated, more mononuclear leucocytes are assembled which augments the vascular inflammation and hereby the damage. After this event, the neutrophils (in self-limited inflammation) normally go into apoptosis and are cleared by macrophages⁶⁵⁻⁶⁸. This, however, is interfered with by the activation of ANCA accelerating apoptosis and secondary necrosis in cytokine-primed neutrophils^{60;69-70}, inhibiting clearance of apoptotic cells by macrophages⁶⁹. This finally results in the formation of fibrinoid necrosis.

The involvement of T-cells

Increasing evidence accumulates that next to neutrophils, endothelial cells, and macrophages, T-cells play an important role in the pathogenesis of ANCA-associated vasculitis^{46;71}. Controversially, experiments in animal models have demonstrated the induction of vasculitis by MPO-ANCA in the absence of T-cells. This demonstrates that these cells are not needed for the induction of vasculitis (in animals), but this does not rule out a role in a more downstream process in the pathogenesis of the disease. However, there is evidence for a role for T-cells in early stages of vasculitic disease, but much of their function is still undiscovered.



The help of T-cells is required in the ANCA production ⁷². The predominance of the subclasses IgG1, IgG3, and IgG4 suggests an antigen-driven T-cell dependent immune response ^{72;73}. T-cells were also found to be oligoclonically expanded in systemic necrotizing vasculitis ⁷⁴. In WG, serum levels of sIL-2R, sCD4, sCD8, and sCD30 are elevated, indicating T-cell activation, and are correlated with disease activity ⁷⁵⁻⁷⁸. However, even in remission and despite treatment, activation markers on T-cells are upregulated ^{79;80}. In the presence of PR3 and MPO, T-cells proliferate ^{81;82}. They also infiltrate vasculitic and granulomatous lesions ⁸³⁻⁸⁶, and in the kidney T-cell accumulation is correlated with renal impairment ⁸⁵. Treatment with monoclonal antibodies directed against T-cell markers is effective in inducing remission ⁸⁷⁻⁹⁰.

The theory of autoantigen complementarity

A recent, remarkable finding was that patients with ANCA directed against PR3 not only harbor antibodies to PR3, but also to the peptide translated from the antisense DNA strand of PR3 (complementary PR3, cPR3), as visualized in Figure 3. Complementary portions of the PR3 encoding gene PRTN3 were found in a number of microbial and fungal organisms, including *Staphylococcus aureus* and Ross River virus ⁹¹. The role of *S. aureus* as an etiological factor in ANCA-associated vasculitis is discussed in chapter 6. The theory of autoantigen complementarity encompasses autoimmunity as a consequence of an immune response to a protein whose amino acid sequence is complementary to that of a self protein ⁹¹. In vasculitis patients, the idiotypic antibody directed against cPR3 evokes a second immune response with newly-formed antibodies directed against the active binding of the idiotypic antibodies. The binding site of these newly-formed antibodies (PR3-ANCA) is complimentary to PR3 and these antibodies can react with PR3. This theory was also tested *in vivo*. Mice immunized with cPR3 developed anti-cPR3 and anti-PR3 antibodies ⁹¹. Whether therapies directed against the anti-idiotypic antibody are helpful remains to be seen. The observation that intravenous immunoglobulins (IVIg) inhibit ANCA-induced neutrophil activation ^{92;93} and that IVIg reduces disease activity in persistent ANCA-associated vasculitis may be based on this concept ⁹⁴. The theory of autoantigen complementarity provides new clues to the thought of the concept of autoimmunity, including its etiology.

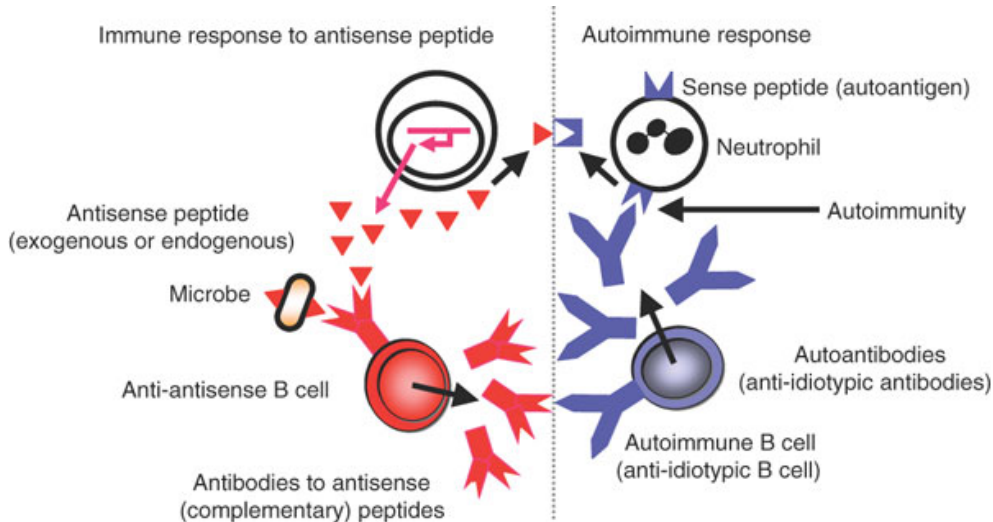


Figure 3. The theory of autoantigen complementarity, a new mechanism for the development of autoimmunity whereby proteins complementary to autoantigens are initiators of disease. Autoimmunity is a consequence of an immune response to a protein whose amino acid sequence is complementary to that of a self-protein. The immunogen, which elicits the initial immune response (idiotypic response), is complementary in amino acid sequence to the autoantigen. This idiotype antibody elicits a second immune response (anti-idiotypic response) in which anti-idiotypic antibodies or autoanti-idiotypes are produced. The anti-idiotypic antibodies are now autoantibodies that react with self-antigen, resulting in autoimmunity. Reprinted with permission from Macmillian Publishers Ltd: Nat Med 10: 72-79 © (2004) Pendergraft WF, III, Preston GA, Shah RR, Tropsha A, Carter CW, Jr., Jennette JC, Falk RJ. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3.

Histopathology of renal lesions

In biopsies of kidneys affected by ANCA-associated vasculitis, a variety of glomerular and tubulo-interstitial lesions can be found. Although the glomerular lesions have been studied extensively, the involvement of the tubules and the interstitium in the disease process may be of importance too, especially with regard to predicting renal outcome. This is discussed in chapter 2, 3, and 4. A hallmark in renal histopathology in patients suffering from ANCA-associated vasculitis is fibrinoid necrosis. This lesion is considered to be caused by vasculitis of the capillary tuft⁹⁵ and can be revealed for instance as red spots in fibrin Lendrum staining (Figure 4A). Because of its fibrin-like structure upon microscopical examination⁹⁶, this lesion was called fibrinoid, however it is not composed of fibrin or fibrinogen alone, but also of extracellular matrix molecules⁵⁷. It has been suggested that this lesion is the result of a disturbed



interaction of endothelium with ANCA-antigens, expressed by TNF- α primed neutrophils⁹⁷. Damage of the vessel wall leads to an intracapillary thrombotic process accumulating clotting factors and eventually fibrin, resulting in disintegration of the mesangium⁹⁸ and rupture of the capillary tuft⁹⁹. Others claim that glomerular thrombosis plays a primary pathogenetic role in the development of ANCA-associated glomerulonephritis²⁶. A hypothesis we propose is that fibrinoid necrosis is part of a healing process, founded by the discovery that fibrinoid necrotic lesions of C-ANCA patients contain the ED-B-positive fibronectin isoform¹⁰⁰. This isoform is involved in the process of angiogenesis and plays a role in tissue repair mechanisms and scar formation similar to what has been described for wound healing^{101;102}. Also the upregulation of integrin $\alpha 6 \beta 1$, and the downregulation of $\alpha 5 \beta 1$ in fibrinoid necrotic lesions of ANCA-associated vasculitis patients could well reflect tissue repair reactions¹⁰³. This hypothesis is further supported by the fact that fibrinoid necrosis bears regenerative capability¹⁰⁴ and its presence is a predictor of better renal outcome in patients presenting with mild to moderate renal involvement¹⁰⁵. Another histopathological hallmark is extracapillary proliferation, a lesion seen in a number of glomerular diseases, such as anti-GBM glomerulonephritis, lupus nephritis, and IgA nephropathy. Extracapillary proliferation refers to cells of epithelial and inflammatory origin proliferating into Bowman's space (Figure 4B). This process which is also referred to as crescent formation, finds its origin in fibrin leakage from the glomerular tuft through the damaged glomerular basement membrane⁹⁸. Consequently, fibroblasts migrating from the interstitium through defects in the glomerular capsule are held responsible for the formation of scar tissue following fibrotic changes¹⁰⁶. The deposition of fibrin attracts macrophages by chemotaxis¹⁰⁷, exerting pro-coagulant activity. In its turn, this leads to intraglomerular fibrin deposition¹⁰⁸, completing the vicious circle of extracapillary proliferation and compressing the glomerular tuft, resulting in a decrease of filtration surface. In parallel with this process, predominantly cellular crescents (Figure 4C) that may still recover to normal glomeruli, surpass a point at which these become irreversibly damaged. Fibroblasts enter Bowman's space with the evolvement of a fibrocellular crescent^{107;109;110}. Consequent collagen accumulation will lead to the formation of fibrous crescents (Figure 4D) and eventually glomerulosclerosis (Figure 4E). Crescents can be distinguished by those that cover less than 50% of the glomerular surface in one cross-section, referred to as segmental crescents (Figure 4C), and those that cover more than 50% of the surface, referred to as

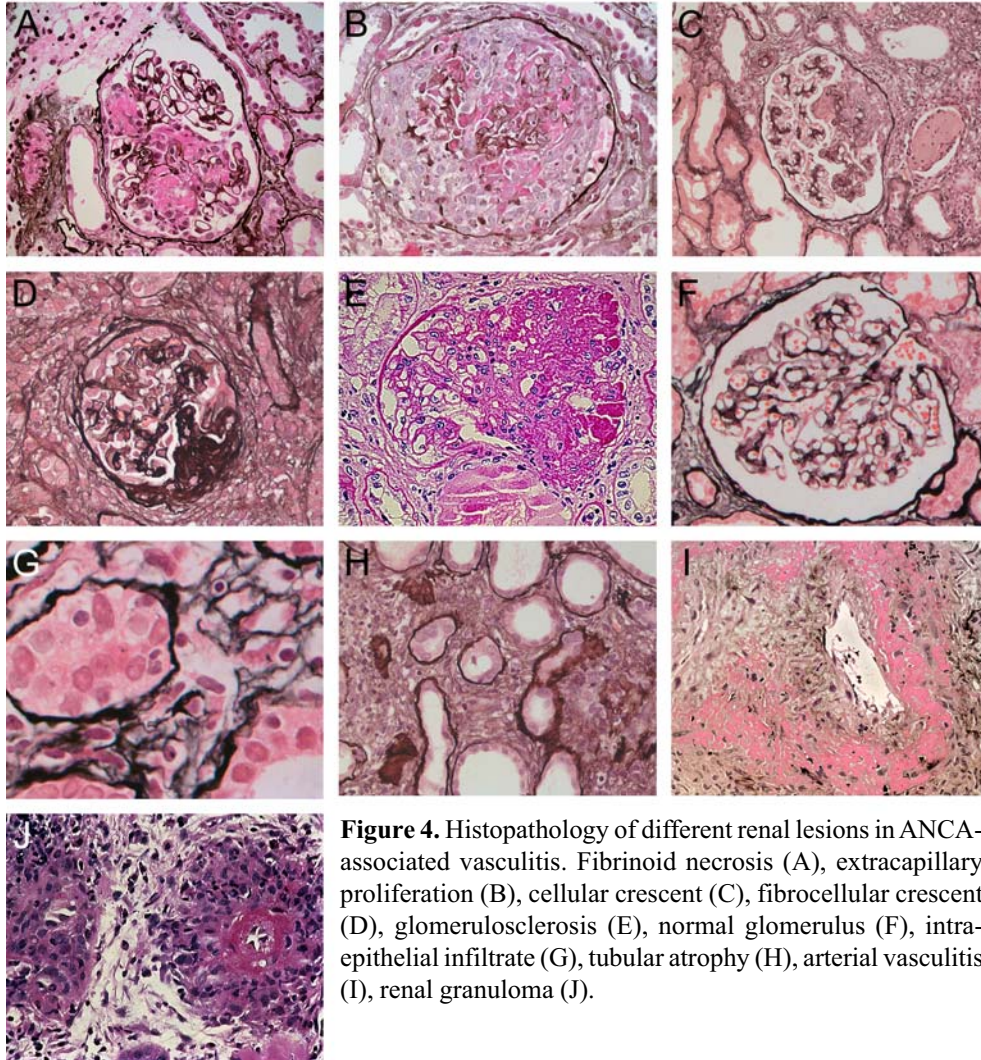


Figure 4. Histopathology of different renal lesions in ANCA-associated vasculitis. Fibrinoid necrosis (A), extracapillary proliferation (B), cellular crescent (C), fibrocellular crescent (D), glomerulosclerosis (E), normal glomerulus (F), intra-epithelial infiltrate (G), tubular atrophy (H), arterial vasculitis (I), renal granuloma (J).

circumferential crescents (Figure 4B). Due to the focal nature of the disease, also normal glomeruli can be found in the biopsy (Figure 4F).

The combination of fibrinoid necrosis, extracapillary proliferation and the absence of immune deposits in indirect immunofluorescence (IIF) techniques, is known as pauci-immune crescentic necrotizing glomerulonephritis, the histological diagnosis of kidney disease in ANCA-associated vasculitis.

The damage in the tubulointerstitium has often been considered as secondary in histopathological studies on ANCA-associated vasculitis, however these turn out to be very important when it comes to predicting renal outcome over



time in patients presenting with severe renal involvement (chapter 2, 3, and 4). With regard to tubulointerstitial lesions a distinction can be made between acute and chronic lesions. Intraepithelial infiltrates -often referred to as tubulitis- (Figure 4G), tubular necrosis, interstitial edema, and interstitial infiltrates are associated with active disease. Tubular atrophy (Figure 4H) and interstitial fibrosis are associated with chronic damage.

Vasculitis of arteries and arterioles is detected in approximately 25% of renal biopsies from patients with ANCA-associated vasculitis (Figure 4I). This low number can be explained by the small size of the biopsy and the focal nature of the disease, and consequently, the relatively low chance of an affected small vessel in the biopsy specimen being present. Alternatively, renal blood vessels may be less prone to vasculitis, since not all vessels in the body are equally involved.

In approximately 5% of renal biopsies from patients suffering from Wegener's granulomatosis, renal granulomas are observed ¹¹¹ (Figure 4J). However, these can also be found in other vasculitides and a number of other inflammatory diseases, such as sarcoidosis, tuberculosis, hypersensitivity, and around foreign bodies ¹¹², and should therefore not be regarded as pathognomonic of the disease.

Treatment and prognosis

Untreated, generalised ANCA-associated vasculitis follows a progressive course to vital organ failure with a fatal outcome ¹¹³. In the early 1970's, oral administration of the toxic cyclophosphamide in combination with corticosteroids was already appreciated as an effective treatment for ANCA-associated vasculitis ¹¹⁴. Seventy-five percent of patients achieved complete remission, but a major point of concern was that 50% of these relapsed within 5 years ¹¹⁵. Another point of concern was the adverse effects associated with cyclophosphamide and steroid treatment, especially cyclophosphamide toxicity. Hemorrhagic cystitis, bladder cancer, and opportunistic infection during leukopenia are clinical manifestations of these adverse effects ^{58;115-117}. Other adverse effects include nausea, vomiting, neutropenia, alopecia, male and female infertility, leukaemia, and lymphoma. In the CYCAZAREM (cyclophosphamide versus azathioprine for maintenance of remission) trial ran by the European Vasculitis Study Group (EUVAS), cyclophosphamide was shown to be safely replaced by azathioprine after induction of remission in patients with mild to moderate renal disease ($200 \mu\text{mol/L} \leq \text{serum creatinine} < 500 \mu\text{mol/L}$). A similar number of patients relapsed within 18 months after

diagnosis, but less toxicity was observed in the patients who switched to azathioprine treatment during remission (10% vs. 18%)¹¹⁸. To reduce cyclophosphamide toxicity, different ways of administration of cyclophosphamide for the induction of remission were studied by the EUVAS¹¹⁹. Although in patients treated with daily oral cyclophosphamide (2 mg/kg/day) the cumulative dose was twice as high as in those treated with intravenous pulse cyclophosphamide (15 mg/kg every 2 to 3 weeks), no differences were observed between cumulative survival, time to remission, time to relapse, or disease-free periods¹¹⁹.

Although cyclophosphamide and corticosteroids are effective in treating ANCA-associated vasculitis patients with mild to moderate disease, patients suffering from life-threatening vasculitis with organ failure require a more aggressive therapeutic regimen. In the MEPEX (intravenous methyl prednisolone versus plasma exchange as adjunctive treatment) study, performed by the EUVAS, patients with severe renal involvement (serum creatinine > 500 $\mu\text{mol/L}$) were treated with a standard therapy of cyclophosphamide and corticosteroids, in addition of which they received adjunctive treatment consisting of either intravenous methyl prednisolone or plasma exchange. Although a similar percentage of patients died in both groups (about 25% within the first year after diagnosis), plasma exchange proved to be superior to intravenous methyl prednisolone as adjunct to standard therapy with regard to dialysis-free survival after one year (80% versus 57%, respectively). In this thesis, renal histology and clinical aspects of this particular patient group with severe renal disease are studied in detail in chapter 2, 3, and 4.

Antibody therapy

Etanercept, which inhibits tumor necrosis factor alpha ($\text{TNF}\alpha$), was studied by the Wegener's Granulomatosis Etanercept Trial (WGET) Group¹²⁰. No beneficial effect was seen with the addition of etanercept, but there was a threefold increase in solid malignancies¹²¹. Infliximab, another anti- $\text{TNF}\alpha$ biological, has proved to be efficient in suppressing disease activity in a small number of patients with refractory vasculitis¹²², however another pilot study contradicts this beneficial effect¹²³. For patients with persistent disease activity, a single course of intravenous immunoglobulin (IVIg) treatment (2 g/kg) was proven to be effective in reducing disease activity, although the effect was of short duration¹²⁴. The immunosuppressant gusperimus (deoxyspergualin), which inhibits growth of activated naïve CD4^+ T-cells, has also been shown to



have a high level of efficacy in remission induction in refractory or relapsing Wegener's granulomatosis ¹²⁵. Rituximab, a B-cell depleting drug targeted against the B-cell surface antigen CD20, has been effective in patients with active severe ANCA-associated vasculitis not tolerating or not responding to cyclophosphamide treatment ¹²⁶. Long term analysis of rituximab treatment is promising: in patients with chronically relapsing WG it prohibited relapse as long as B-lymphocytes were undetectable, and long term B cell depletion was well tolerated ^{127;128}. With two randomized controlled clinical trials underway studying the effects of rituximab in ANCA-associated vasculitis, this might be a promising drug for the future ¹²⁵.

An international vasculitis network: EUVAS

Since ANCA-associated vasculitis is a rare disease, for performing clinical trials in uniform groups of patients that are large enough to make statistically significant conclusions, there is a need for investigators to work together. This led to the initiative of founding a group, later known as the European Vasculitis Study Group (EUVAS) in 1993. Its aim was to standardize current treatment regimens and test new treatment regimens. Physicians with an interest in the field of ANCA-associated vasculitis joined efforts to start a number of multicenter randomized controlled clinical trials. To harmonize these clinical trials, validated scoring systems were created for both clinical manifestation ¹²⁹⁻¹³¹ and histology ¹³². The latter system was used in the studies described in chapter 2, 3, and 4 in this thesis. Within the EUVAS, efforts have been joined to standardize the analysis of serologic ANCA ^{39;133;134}. The EUVAS stimulates and facilitates top-level scientific and clinical collaboration concerning primary ANCA-associated vasculitis. Most of the work, described in this thesis originated from this collaborative group.

Outline of the thesis

This thesis commences with a focus on the patients with ANCA-associated vasculitis presenting with rapidly progressive renal disease in chapter 2. Clinical and histological determinants of renal and patient outcome one year after diagnosis are extensively described. Hereafter, attention is focused on patients who present with dialysis dependent ANCA-associated glomerulonephritis in chapter 3. Treatment decisions are discussed, taking into account the chance of recovery and that of dying from therapy. Both patients with mild to moderate and severe renal involvement at diagnosis were followed for five years, after

which long term outcome was analyzed. For these patients chances of recovery, dialysis dependency, and death were studied. Predictors of five-year outcome are extensively described in chapter 4. Moreover, a model is proposed to calculate the chance on each of these outcomes after one year and after five years.

An overview of the most common hypotheses on the etiology of ANCA-associated vasculitis is given, in chapter 6. Many potential initiating factors have been launched over the past two decades, but no single factor has been proven to be exclusively responsible for the induction of vasculitic disease. Most current views explain the disease by a combination of different environmental factors that superimpose upon a genetic susceptibility for developing this disease. Finally, the results of all these studies are summarized and more extensively discussed in chapter 7.

References

1. Jennette JC, Falk RJ: Small-vessel vasculitis. *N Engl J Med* 337: 1512-1523, 1997
2. Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CG: Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 37: 187-192, 1994
3. Godman G.C, Churg J: Wegener's granulomatosis. Pathology and review of literature. *Arch Pathol* 58: 533-553, 1954.
4. Lhote F, Guillevin L: Polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome. Clinical aspects and treatment. *Rheum Dis Clin North Am* 21: 911-947, 1995
5. Lanham JG, Elkon KB, Pusey CD, Hughes GR: Systemic vasculitis with asthma and eosinophilia: a clinical approach to the Churg-Strauss syndrome. *Medicine (Baltimore)* 63: 65-81, 1984
6. Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY: The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum* 33: 1094-1100, 1990
7. Churg J, Strauss L: Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. *Am J Pathol* 27: 277-294, 1951.



8. Willan R: Purpura. In: On cutaneous diseases, 1 edn., Philadelphia, Kimber & Conrad, 1808, pp 452-471
9. Henoch E: Über den Zusammenhang von Purpura und Intestinalstörungen. *Berl Klin Wochenschr* 5: 517-519, 1868
10. Henoch EH: Lectures on diseases of children: a handbook for physicians and students. New York, W. Wood, 1882
11. Schönlein JL: Allgemeine und specielle Pathologie und Therapie, 3 edn. Herisau, Switzerland, Literatur-Comptoir, 1837, p 48
12. Kussmaul A, Maier R: Ueber eine bisher nicht beschriebene eigenthümliche Arterienerkrankung (Periarteritis nodosa), die mit Morbus Brightii und rapid fortschreitender allgemeiner Muskellähmung einhergeht. *Deutsches Arch klin Med* 1: 484-518, 1866
13. Klinger H: Grenzformen der Periarteriitis nodosa. *Frankfurt Z Pathol* 42: 455-480, 1932
14. Wegener F: Über eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystem und der Nieren. *Beitr Pathol Anat* 102: 36-68, 1939
15. Davson J, Ball J, Platt R: The Kidney in Periarteritis Nodosa. *Q J Med* 17: 175, 1948
16. Osler W: On the visceral complications of erythema exudativum multiforme. *Am J Med Sci* 110: 629-646, 1895
17. Osler W: The visceral lesions of purpura and allied conditions. *BMJ* 1: 517-525, 1914
18. Scheer RL, Grossman MA: Immune aspects of the glomerulonephritis associated with pulmonary hemorrhage. *Ann Intern Med* 60: 1009-1021, 1964
19. Sturgill BC, Westerfelt FB: Immunofluorescence studies in a case of Goodpasture's syndrome. *JAMA* 194: 914-916, 1965
20. Lerner RA, Glassock RJ, Dixon FJ: The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med* 126: 989-1004, 1967
21. Meltzer M, Franklin EC, Elias K, Mccluskey RT, Cooper N: Cryoglobulinemia—a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 40: 837-856, 1966



Chapter 1

22. Faille-Kuyber EH, Kater L, Kooiker CJ, Dorhout Mees EJ: IgA-deposits in cutaneous blood-vessel walls and mesangium in Henoch-Schonlein syndrome. *Lancet* 1: 892-893, 1973
23. Jennette JC, Wilkman AS, Falk RJ: Anti-Neutrophil Cytoplasmic Autoantibody-Associated Glomerulonephritis and Vasculitis. *Am J Pathol* 135: 921-930, 1989
24. Ronco P, Verroust P, Mignon F, Kourilsky O, Vanhille P, Meyrier A, Mery JP, Morel-Maroger L: Immunopathological studies of polyarteritis nodosa and Wegener's granulomatosis: a report of 43 patients with 51 renal biopsies. *Q J Med* 52: 212-223, 1983
25. Leatherman JW, Sibley RK, Davies SF: Diffuse intrapulmonary hemorrhage and glomerulonephritis unrelated to anti-glomerular basement membrane antibody. *Am J Med* 72: 401-410, 1982
26. Weiss MA, Crissman JD: Renal biopsy findings in Wegener's granulomatosis: segmental necrotizing glomerulonephritis with glomerular thrombosis. *Hum Pathol* 15: 943-956, 1984
27. Brouwer E, Huitema MG, Klok PA, de Weerd H, Tervaert JW, Weening JJ, Kallenberg CG: Antimyeloperoxidase-associated proliferative glomerulonephritis: an animal model. *J Exp Med* 177: 905-914, 1993
28. van der Woude FJ: Complexes or no complexes? a study into factors affecting immune complex formation and elimination in glomerulonephritis and vasculitis in man. Thesis, 1985
29. van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA, van der GM, van der Hem GK, The TH: Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1: 425-429, 1985
30. Wiik A: Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. *APMIS Suppl* 6: 12-13, 1989
31. Wiik A, Rasmussen N, Wieslander J: Methods to detect autoantibodies to neutrophilic granulocytes. *Man Biol Markers Dis* A9: 1-14, 1993
32. Bajema IM, Hagen EC: Evolving concepts about the role of antineutrophil cytoplasm autoantibodies in systemic vasculitides. *Curr Opin Rheumatol* 11: 34-40, 1999



33. Pryzwansky KB, Martin LE, Spitznagel JK: Immunocytochemical localization of myeloperoxidase, lactoferrin, lysozyme and neutral proteases in human monocytes and neutrophilic granulocytes. *J Reticuloendothel Soc* 24: 295-310, 1978
34. 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* 318: 1651-1657, 1988
35. Goldschmeding R, van der Schoot CE, ten Bokkel HD, Hack CE, van den Ende ME, Kallenberg CG, dem Borne AE: Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 84: 1577-1587, 1989
36. Skogh T, Dahlgren C, Holmgren K, Peen E, Stendahl O: Anti-granulocyte antibodies (C-ANCA, P-ANCA, GS-ANA) studied by confocal scanning laser fluorescence microscopy, ELISA, and chemiluminescence techniques. *Scand J Immunol* 34: 137-145, 1991
37. Lesavre P: Antineutrophil cytoplasmic autoantibodies antigen specificity. *Am J Kidney Dis* 18: 159-163, 1991
38. Zhao MH, Jones SJ, Lockwood CM: Bactericidal/permeability-increasing protein (BPI) is an important antigen for anti-neutrophil cytoplasmic autoantibodies (ANCA) in vasculitis. *Clin Exp Immunol* 99: 49-56, 1995
39. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, Lesavre P, Ludemann J, Rasmussen N, Sinico RA, Wiik A, van der Woude FJ: Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int* 53: 743-753, 1998
40. Falk RJ, Jennette JC: ANCA small-vessel vasculitis. *J Am Soc Nephrol* 8: 314-322, 1997
41. Ludemann J, Utecht B, Gross WL: Anti-cytoplasmic antibodies in Wegener's granulomatosis are directed against proteinase 3. *Adv Exp Med Biol* 297: 141-150, 1991
42. Westman KW, Selga D, Bygren P, Segelmark M, Baslund B, Wiik A, Wieslander J: Clinical evaluation of a capture ELISA for detection of proteinase-3 antineutrophil cytoplasmic antibody. *Kidney Int* 53: 1230-1236, 1998

43. Jayne DR, Gaskin G, Rasmussen N, Abramowicz D, Ferrario F, Guillevin L, Mirapeix E, Savage CO, Sinico RA, Stegeman CA, Westman KW, van der Woude FJ, de Lind van Wijngaarden RA, Pusey CD: Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol* 18: 2180-2188, 2007
44. Jennette JC, Falk RJ: New insight into the pathogenesis of vasculitis associated with antineutrophil cytoplasmic autoantibodies. *Curr Opin Rheumatol* 20: 55-60, 2008
45. Jennette JC, Xiao H, Falk RJ: Pathogenesis of vascular inflammation by anti-neutrophil cytoplasmic antibodies. *J Am Soc Nephrol* 17: 1235-1242, 2006
46. Day CJ, Hewins P, Savage CO: New developments in the pathogenesis of ANCA-associated vasculitis. *Clin Exp Rheumatol* 21: S35-S48, 2003
47. Rarok AA, Limburg PC, Kallenberg CG: Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. *J Leukoc Biol* 74: 3-15, 2003
48. Williams JM, Kamesh L, Savage CO: Translating basic science into patient therapy for ANCA-associated small vessel vasculitis. *Clin Sci (Lond)* 108: 101-112, 2005
49. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, Jennette JC: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 110: 955-963, 2002
50. van Paassen P, Cohen Tervaert JW, Heeringa P: Mechanisms of vasculitis: how pauci-immune is ANCA-associated renal vasculitis? *Nephron Exp Nephrol* 105: e10-e16, 2007
51. Heeringa P, Huugen D, Tervaert JW: Anti-neutrophil cytoplasmic autoantibodies and leukocyte-endothelial interactions: a sticky connection? *Trends Immunol* 26: 561-564, 2005
52. Little MA, Smyth CL, Yadav R, Ambrose L, Cook HT, Nourshargh S, Pusey CD: Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood* 106: 2050-2058, 2005
53. Van Der Geld YM, Hellmark T, Selga D, Heeringa P, Huitema MG, Limburg PC, Kallenberg CG: Rats and mice immunised with chimeric human/mouse proteinase 3 produce autoantibodies to mouse Pr3 and rat granulocytes. *Ann Rheum Dis* 66: 1679-1682, 2007



54. Wiesner O, Litwiller RD, Hummel AM, Viss MA, McDonald CJ, Jenne DE, Fass DN, Specks U: Differences between human proteinase 3 and neutrophil elastase and their murine homologues are relevant for murine model experiments. *FEBS Lett* 579: 5305-5312, 2005
55. Jenne DE, Frohlich L, Hummel AM, Specks U: Cloning and functional expression of the murine homologue of proteinase 3: implications for the design of murine models of vasculitis. *FEBS Lett* 408: 187-190, 1997
56. Kao RC, Wehner NG, Skubitz KM, Gray BH, Hoidal JR: Proteinase 3. A distinct human polymorphonuclear leukocyte proteinase that produces emphysema in hamsters. *J Clin Invest* 82: 1963-1973, 1988
57. Bajema IM, Bruijn JA: What stuff is this! A historical perspective on fibrinoid necrosis. *J Pathol* 191: 235-238, 2000
58. Kallenberg CG, Heeringa P, Stegeman CA: Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides. *Nat Clin Pract Rheumatol* 2: 661-670, 2006
59. 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* 87: 4115-4119, 1990
60. Harper L, Savage CO: Pathogenesis of ANCA-associated systemic vasculitis. *J Pathol* 190: 349-359, 2000
61. Charles LA, Falk RJ, Jennette JC: Reactivity of antineutrophil cytoplasmic autoantibodies with mononuclear phagocytes. *J Leukoc Biol* 51: 65-68, 1992
62. Falk RJ, Jennette JC: ANCA Are Pathogenic-Oh Yes They Are! *J Am Soc Nephrol* 13: 1977-1979, 2002
63. Yang JJ, Preston GA, Pendergraft WF, Segelmark M, Heeringa P, Hogan SL, Jennette JC, Falk RJ: Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am J Pathol* 158: 581-592, 2001
64. 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* 170: 52-64, 2007
65. Zimmerman GA, Prescott SM, McIntyre TM: Endothelial cell interactions with granulocytes: tethering and signaling molecules. *Immunol Today* 13: 93-100, 1992

66. Hughes J, Liu Y, Van Damme J, Savill J: Human glomerular mesangial cell phagocytosis of apoptotic neutrophils: mediation by a novel CD36-independent vitronectin receptor/thrombospondin recognition mechanism that is uncoupled from chemokine secretion. *J Immunol* 158: 4389-4397, 1997
67. Savill J, Smith J, Sarraf C, Ren Y, Abbott F, Rees A: Glomerular mesangial cells and inflammatory macrophages ingest neutrophils undergoing apoptosis. *Kidney Int* 42: 924-936, 1992
68. Savill J, Haslett C: Granulocyte clearance by apoptosis in the resolution of inflammation. *Semin Cell Biol* 6: 385-393, 1995
69. Harper L, Cockwell P, Adu D, Savage CO: Neutrophil priming and apoptosis in anti-neutrophil cytoplasmic autoantibody-associated vasculitis. *Kidney Int* 59: 1729-1738, 2001
70. Hebert MJ, Takano T, Holthofer H, Brady HR: Sequential morphologic events during apoptosis of human neutrophils. Modulation by lipoxygenase-derived eicosanoids. *J Immunol* 157: 3105-3115, 1996
71. Lamprecht P: Off balance: T-cells in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides. *Clin Exp Immunol* 141: 201-210, 2005
72. Mellbye OJ, Mollnes TE, Steen LS: IgG subclass distribution and complement activation ability of autoantibodies to neutrophil cytoplasmic antigens (ANCA). *Clin Immunol Immunopathol* 70: 32-39, 1994
73. Mulder AH, Stegeman CA, Kallenberg CG: Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis: a predominant role for the IgG3 subclass of ANCA. *Clin Exp Immunol* 101: 227-232, 1995
74. Giscombe R, Grunewald J, Nityanand S, Lefvert AK: T cell receptor (TCR) V gene usage in patients with systemic necrotizing vasculitis. *Clin Exp Immunol* 101: 213-219, 1995
75. Schmitt WH, Heesen C, Csernok E, Rautmann A, Gross WL: Elevated serum levels of soluble interleukin-2 receptor in patients with Wegener's granulomatosis. Association with disease activity. *Arthritis Rheum* 35: 1088-1096, 1992
76. Stegeman CA, Tervaert JW, Huitema MG, Kallenberg CG: Serum markers of T cell activation in relapses of Wegener's granulomatosis. *Clin Exp Immunol* 91: 415-420, 1993



77. Wang G, Hansen H, Tatsis E, Csernok E, Lemke H, Gross WL: High plasma levels of the soluble form of CD30 activation molecule reflect disease activity in patients with Wegener's granulomatosis. *Am J Med* 102: 517-523, 1997
78. D'Cruz D, Direskeneli H, Khamashta M, Hughes GR: Lymphocyte activation markers and von Willebrand factor antigen in Wegener's granulomatosis: potential markers for disease activity. *J Rheumatol* 26: 103-109, 1999
79. Popa ER, Stegeman CA, Bos NA, Kallenberg CG, Tervaert JW: Differential B- and T-cell activation in Wegener's granulomatosis. *J Allergy Clin Immunol* 103: 885-894, 1999
80. Christensson M, Pettersson E, Sundqvist KG, Christensson B: T cell activation in patients with ANCA-associated vasculitis: inefficient immune suppression by therapy. *Clin Nephrol* 54: 435-442, 2000
81. Ballieux BE, van der Burg SH, Hagen EC, van der Woude FJ, Melief CJ, Daha MR: Cell-mediated autoimmunity in patients with Wegener's granulomatosis (WG). *Clin Exp Immunol* 100: 186-193, 1995
82. Griffith ME, Coulthart A, Pusey CD: T cell responses to myeloperoxidase (MPO) and proteinase 3 (PR3) in patients with systemic vasculitis. *Clin Exp Immunol* 103: 253-258, 1996
83. Gephardt GN, Ahmad M, Tubbs RR: Pulmonary vasculitis (Wegener's granulomatosis). Immunohistochemical study of T and B cell markers. *Am J Med* 74: 700-704, 1983
84. Cunningham MA, Huang XR, Dowling JP, Tipping PG, Holdsworth SR: Prominence of cell-mediated immunity effectors in "pauci-immune" glomerulonephritis. *J Am Soc Nephrol* 10: 499-506, 1999
85. Brouwer E, Tervaert JWC, Weening JJ, Kallenberg CGM: Immunohistopathology of Renal Biopsies in Wegeners Granulomatosis (Wg) - Clues to Its Pathogenesis. *Kidney Int* 39: 1055-1056, 1991
86. Weidner S, Carl M, Riess R, Rupprecht HD: Histologic analysis of renal leukocyte infiltration in antineutrophil cytoplasmic antibody-associated vasculitis: importance of monocyte and neutrophil infiltration in tissue damage. *Arthritis Rheum* 50: 3651-3657, 2004
87. Mathieson PW, Cobbold SP, Hale G, Clark MR, Oliveira DB, Lockwood CM, Waldmann H: Monoclonal-antibody therapy in systemic vasculitis. *N Engl J Med* 323: 250-254, 1990

88. Lockwood CM, Thiru S, Isaacs JD, Hale G, Waldmann H: Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. *Lancet* 341: 1620-1622, 1993
89. Schmitt WH, Hagen EC, Neumann I, Nowack R, Flores-Suarez LF, van der Woude FJ: Treatment of refractory Wegener's granulomatosis with antithymocyte globulin (ATG): an open study in 15 patients. *Kidney Int* 65: 1440-1448, 2004
90. Hagen EC, de Keizer RJ, Andrassy K, van Boven WP, Bruijn JA, van Es LA, van der Woude FJ: Compassionate treatment of Wegener's granulomatosis with rabbit anti-thymocyte globulin. *Clin Nephrol* 43: 351-359, 1995
91. Pendergraft WF, III, Preston GA, Shah RR, Tropsha A, Carter CW, Jr., Jennette JC, Falk RJ: Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med* 10: 72-79, 2004
92. Rossi F, Jayne DR, Lockwood CM, Kazatchkine MD: Anti-idiotypes against anti-neutrophil cytoplasmic antigen autoantibodies in normal human polyspecific IgG for therapeutic use and in the remission sera of patients with systemic vasculitis. *Clin Exp Immunol* 83: 298-303, 1991
93. Brooks CJ, King WJ, Radford DJ, Adu D, McGrath M, Savage CO: IL-1 beta production by human polymorphonuclear leucocytes stimulated by anti-neutrophil cytoplasmic autoantibodies: relevance to systemic vasculitis. *Clin Exp Immunol* 106: 273-279, 1996
94. Jayne DR, Chapel H, Adu D, Misbah S, O'Donoghue D, Scott D, Lockwood CM: Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. *QJM* 93: 433-439, 2000
95. Serra A, Cameron JS, Turner DR, Hartley B, Ogg CS, Neild GH, Williams DG, Taube D, Brown CB, Hicks JA: Vasculitis affecting the kidney: presentation, histopathology and long-term outcome. *Q J Med* 53: 181-207, 1984
96. Lendrum AC, Fraser DS, Slidders W, HENDERSON R: Studies on the character and staining of fibrin. *J Clin Pathol* 15: 401-413, 1962
97. Jennette JC, Falk RJ: Pathogenesis of the vascular and glomerular damage in ANCA-positive vasculitis. *Nephrol Dial Transplant* 13 Suppl 1: 16-20, 1998
98. Watanabe T, Yoshikawa Y, Toyoshima H: Morphological and clinical features of the kidney in Wegener's granulomatosis. A survey of 28 autopsies in Japan. *Nippon Jinzo Gakkai Shi* 23: 921-930, 1981



99. Weiss MA, Crissman JD: Renal Pathologic Features of Wegeners Granulomatosis - A Review. *Semin Respir Med* 10: 141-148, 1989
100. Bajema IM, Hagen EC, de Heer E, Bruijn JA: The PAF-Halmi staining distinguishes two types glomerular fibrinoid necrosis in systemic vasculitis. *J Am Soc Nephrol* 9: A2717, 2003
101. Van Vliet AI, Baelde HJ, Vleming LJ, de Heer E, Bruijn JA: Distribution of fibronectin isoforms in human renal disease. *J Pathol* 193: 256-262, 2001
102. Brown LF, Dubin D, Lavigne L, Logan B, Dvorak HF, Vandewater L: Macrophages and Fibroblasts Express Embryonic Fibronectins During Cutaneous Wound-Healing. *Am Journal Pathol* 142: 793-801, 1993
103. Hauer HA, Bajema IM, Vergunst CE, de Heer E, Bruijn JA: Involvement of integrins in the pathogenesis of fibrinoid necrosis in ANCA-associated vasculitis. Renal disease in ANCA-associated vasculitis: considerations on pathogenesis, disease manifestations, and prognosis. The Hague, Pasmans, 2002
104. Hauer HA, Bajema IM, de Heer E, Hermans J, Hagen EC, Bruijn JA: Distribution of renal lesions in idiopathic systemic vasculitis: A three-dimensional analysis of 87 glomeruli. *Am J Kidney Dis* 36: 257-265, 2000
105. Hauer HA, Bajema IM, van Houwelingen HC, Ferrario F, Noel LH, Waldherr R, Jayne DR, Rasmussen N, Bruijn JA, Hagen EC: Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int* 62: 1732-1742, 2002
106. Mathieson PW: The ins and outs of glomerular crescent formation. *Clin Exp Immunol* 110: 155-157, 1997
107. 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* 51: 404-415, 1984
108. Savage CO: The biology of the glomerulus: endothelial cells. *Kidney Int* 45: 314-319, 1994
109. Downer G, Phan SH, Wiggins RC: Analysis of renal fibrosis in a rabbit model of crescentic nephritis. *J Clin Invest* 82: 998-1006, 1988
110. Striker LMM, Killen PD, Chi E, Striker GE: The Composition of Glomerulosclerosis .1. Studies in Focal Sclerosis, Crescentic Glomerulonephritis, and Membranoproliferative Glomerulonephritis. *Lab Invest* 51: 181-192, 1984

111. 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* 129: 17-22, 1997
112. Bajema IM, Hagen EC, Ferrario F, Waldherr R, Noel LH, Hermans J, van der Woude FJ, Bruijn JA: Renal granulomas in systemic vasculitis. EC/BCR Project for ANCA-Assay Standardization. *Clin Nephrol* 48: 16-21, 1997
113. Walton EW: Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *Br Med J* 2: 265-270, 1958
114. Fauci AS, Wolff SM, Johnson JS: Effect of cyclophosphamide upon the immune response in Wegener's granulomatosis. *N Engl J Med* 285: 1493-1496, 1971
115. Hoffman GS, Kerr GS, Leavitt RY, Hallahan CW, Lebovics RS, Travis WD, Rottem M, Fauci AS: Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med* 116: 488-498, 1992
116. 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* 9: 842-852, 1998
117. Stillwell TJ, Benson RC, Jr., DeRemee RA, McDonald TJ, Weiland LH: Cyclophosphamide-induced bladder toxicity in Wegener's granulomatosis. *Arthritis Rheum* 31: 465-470, 1988
118. Jayne D, Rasmussen N, Andrassy K, Bacon P, Tervaert JW, Dadoniene J, Ekstrand A, Gaskin G, Gregorini G, de Groot K, Gross W, Hagen EC, Mirapeix E, Pettersson E, Siegert C, Sinico A, Tesar V, Westman K, Pusey C: A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 349: 36-44, 2003
119. de Groot K, Jayne DRW, Tesar V, Savage CO, for the European Vasculitis Study Group: European, multicenter randomised controlled trial of daily oral versus pulse cyclophosphamide for induction of remission in ANCA-associated systemic vasculitis. *J Am Soc Nephrol* 16: 7A, 2005
120. Etanercept plus standard therapy for Wegener's granulomatosis. *N Engl J Med* 352: 351-361, 2005
121. Stone JH, Holbrook JT, Marriott MA, Tibbs AK, Sejismundo LP, Min YI, Specks U, Merkel PA, Spiera R, Davis JC, St Clair EW, McCune WJ, Ytterberg SR, Allen NB, Hoffman GS: Solid malignancies among patients in the Wegener's Granulomatosis Etanercept Trial. *Arthritis Rheum* 54: 1608-1618, 2006



122. Booth AD, Jefferson HJ, Ayliffe W, Andrews PA, Jayne DR: Safety and efficacy of TNFalpha blockade in relapsing vasculitis. *Ann Rheum Dis* 61: 559, 2002
123. Morgan MD, Harper L: Infliximab as additional therapy in anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis. *J Am Soc Nephrol* 18: 2007
124. Jayne DR, Chapel H, Adu D, Misbah S, O'Donoghue D, Scott D, Lockwood CM: Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. *QJM* 93: 433-439, 2000
125. Flossmann O, Jones RB, Jayne DR, Luqmani RA: Should rituximab be used to treat antineutrophil cytoplasmic antibody associated vasculitis? *Ann Rheum Dis* 65: 841-844, 2006
126. Keogh KA, Ytterberg SR, Fervenza FC, Carlson KA, Schroeder DR, Specks U: Rituximab for refractory Wegener's granulomatosis: report of a prospective, open-label pilot trial. *Am J Respir Crit Care Med* 173: 180-187, 2006
127. Golbin JM, Fervenza FC, Keogh KA, Ytterberg SR, Specks U: Long-term use of Rituximab in patients with chronically relapsing Wegener Granulomatosis (WG). *J Am Soc Nephrol* 18: 2008
128. Wong CF: Rituximab and antineutrophil cytoplasmic antibody-associated vasculitis: granulomatous disease more resistant than vasculitis disease? *J Clin Rheumatol* 14: 61-64, 2008
129. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, Savage C, Adu D: Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM* 87: 671-678, 1994
130. Stone JH, Hoffman GS, Merkel PA, Min YI, Uhlfelder ML, Hellmann DB, Specks U, Allen NB, Davis JC, Spiera RF, Calabrese LH, Wigley FM, Maiden N, Valente RM, Niles JL, Fye KH, McCune JW, St Clair EW, Luqmani RA: 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* 44: 912-920, 2001
131. Exley AR, Bacon PA, Luqmani RA, Kitas GD, Gordon C, Savage CO, Adu D: Development and initial validation of the Vasculitis Damage Index for the standardized clinical assessment of damage in the systemic vasculitides. *Arthritis Rheum* 40: 371-380, 1997
132. Bajema IM, Hagen EC, Hansen BE, Hermans J, Noel LH, Waldherr R, Ferrario F, van der Woude FJ, Bruijn JA: The renal histopathology in systemic vasculitis: an international survey study of inter- and intra-observer agreement. *Nephrol Dial Transplant* 11: 1989-1995, 1996



Chapter 1

133. Hagen EC, Andrassy K, Chernok E, Daha MR, Gaskin G, Gross W, Lesavre P, Ludemann J, Pusey CD, Rasmussen N: 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* 159: 1-16, 1993
134. Hagen EC, Andrassy K, Csernok E, Daha MR, Gaskin G, Gross WL, Hansen B, Heigl Z, Hermans J, Jayne D, Kallenberg CG, Lesavre P, Lockwood CM, Ludemann J, Mascart-Lemone F, Mirapeix E, Pusey CD, Rasmussen N, Sinico RA, Tzioufas A, Wieslander J, Wiik A, van der Woude FJ: 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* 196: 1-15, 1996

