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## Disease-activity in ANCA-associated vasculitis

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# 1

## Introduction to the thesis

## SMALL VESSEL VASCULITIS

Since the Chapel Hill consensus conference vasculitides are classified according to the size of the involved vessels, histopathology of the lesions, and clinical findings [1]. The primary vasculitides affecting small vessels, that is small arteries, arterioles, capillaries, and venules, are Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), necrotizing crescentic glomerulonephritis (NCGN), and Churg-Strauss syndrome (CSS). WG is hallmarked by the classic triad of small-vessel vasculitis, extravascular necrotizing granulomatous inflammation, and necrotizing crescentic glomerulonephritis (NCGN). In NCGN vasculitis is limited to the kidney, and in MPA also extra-renal vessels are affected, but without the typical granulomatous inflammation present in WG. Finally, CSS is characterized by the classic triad of asthma, eosinophilia and small-vessel vasculitis. As CSS is clinically different from the other small-vessel vasculitides it is beyond the scope of this thesis.

The Chapel Hill criteria were developed for research purposes, and in clinical practice application and classification can be difficult. It can be particularly challenging to distinguish WG from MPA as granulomas are often hard to detect. Several additional differences between WG and MPA in presentation and disease progression have been identified. Among others MPA often presents with more chronic treatment resistant kidney damage [2], whereas patients with WG experience more relapses during follow-up than patients with MPA (over 50% in WG versus 30% in MPA during a follow-up of 5 year) [3].

## ANTI-NEUTROPHIL CYTOPLASMIC AUTOANTIBODIES (ANCA)

Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and necrotizing crescentic glomerulonephritis (NCGN) are associated with the presence of anti-neutrophil cytoplasmic autoantibodies (ANCA). Therefore, these vasculitides are also called ANCA-associated vasculitis (AAV). ANCA were originally detected by indirect immunofluorescence (IIF) on ethanol fixed neutrophils. Three different patterns of fluorescence are distinguished: a cytoplasmic pattern (C-ANCA), a perinuclear pattern (P-ANCA), and a more diffuse cytoplasmic staining pattern (atypical ANCA). The predominant antigens of ANCA in AAV are the constituents of neutrophilic granules proteinase 3 (PR3) and myeloperoxidase (MPO). Approximately 90% of sera that produce a C-ANCA pattern by IIF react with PR3, whereas a P-ANCA pattern is associated with MPO in approximately 70% of patients. Several lines of evidence suggest a pathogenetic role for ANCA in AAV. Key evidence is

provided by an animal model. In this mouse model introduction of anti-MPO IgG derived from MPO-immunized MPO deficient mice, into wild type or Rag2-deficient mice induces pauci-immune necrotizing crescentic glomerulonephritis [4]. The following theory has been formulated defining ANCA pathogenicity. Priming of neutrophils by cytokines induces surface expression of PR3 and MPO. Primed neutrophils can adhere to activated endothelial cells. These primed and adherent neutrophils, showing membrane expression of PR3 and MPO, can be stimulated by PR3- or MPO-specific ANCA, leading to full activation and thereby degranulation and formation of reactive oxygen species (ROS) which damages the vessel wall [5].

In clinical practice the presence of ANCA is of important diagnostic value. PR3-ANCA are predominantly associated with WG, whereas MPO-ANCA are more often associated with MPA and NCGN [6]. However, also in WG MPO-ANCA can be present, and PR3-ANCA can be associated with MPA and NCGN.

Data conflict regarding the diagnostic and prognostic potential of ANCA during follow-up. However, a rise in ANCA should caution a clinician that a relapse might be looming. As mentioned before, patients with WG are at increased risk to experience a relapse compared to patients with MPA. Thus, research efforts to identify the predictive value of ANCA rises during follow-up have focused on patients with WG. In a prospective observational single-centre study in 100 patients with WG (85 PR3-ANCA, 15 MPO-ANCA) with 2-monthly ANCA measurement, the predictive value of an increase in ANCA-titers for relapse was 71% for PR3-ANCA and 100% for MPO-ANCA (both by ELISA) [7]. However, in a recent prospective clinical trial in 156 patients with WG measuring PR3-ANCA every 3 months by capture ELISA, no relation was found between increases of PR3-ANCA levels and occurrence of relapses (adjusted hazard ratio, 0.8 (95% CI, 0.4-1.9) [8]. A different approach was used by Slot et al who measured ANCA at a fixed time point. They reported that in patients with PR3-ANCA associated vasculitis a positive C-ANCA titer by IIF after induction of remission was significantly associated with relapse (RR 2.6, 95% confidence interval 1.1-8.0; P=0.04) [9]. In chapter 3 predictive value of serial measurements of C-ANCA by IIF and PR3-ANCA by ELISA for the occurrence of clinical relapses in PR3-ANCA associated vasculitis are analyzed.

## T CELLS IN AAV

Several lines of evidence support T cell involvement in AAV, including the presence of T cells in granuloma of WG patients and the subclass distribution of ANCA which suggests a T cell dependent immune-response [10]. In recent years interesting data have been generated about different CD4-positive T cell populations and their role in ANCA-associated vasculitis. CD4-positive T cell responses can be divided in three groups. First, Th1 responses, associated with cell-mediated effector immune-responses and hallmarked by production of IFN-gamma. Th1 responses seem to dominate in localized WG and MPA [11, 12]. Secondly, Th2 cells which are associated with humoral immune-responses and distinguished by the production of IL-4, IL-5, IL-10, and IL-13. Th2 markers have been suggested to dominate in generalized disease, suggesting a shift towards a Th2 response [13]. Finally, Th17 cells which have been recently identified and produce IL-17; these cells are thought to be especially important in development of auto-immunity [14]. In WG, overproduction of IL-17 in response to a local infectious stimulus, for instance *S. Aureus*, might result in priming of neutrophils, promotion of granuloma formation, and possibly ANCA production [15]. In chapter 4 we evaluated whether the T cell activation markers sIL-2R, sCD30, IL-10, and B cell activator of the TNF family (BAFF) at diagnosis and during initial follow-up are predictive for persistent or renewed ANCA-positivity and clinical relapse in PR3-ANCA associated vasculitis.

## TREATMENT OF AAV

The foremost important development regarding therapy in AAV has been the introduction of cyclophosphamide [16]. Prior to this WG was a disease with often fatal outcome [17, 18]. After the introduction of cyclophosphamide, in combination with corticosteroids, attention shifted to reducing treatment intensity and thereby toxicity. The last ten years, since the CYCAZAREM study, a staged approach of immunosuppressive therapy is standard [19]. Patients receive induction therapy with cyclophosphamide and corticosteroids, and after remission therapy is switched to less toxic immunosuppressives, ie azathioprine or methotrexate. By this scheme remission is achieved in over 90% of patients. In chapter 5 and 6 we retrospectively evaluated long-term disease-free survival in patients with AAV on cyclophosphamide maintenance therapy as compared to azathioprine maintenance.

Thus, nowadays ANCA-associated vasculitides have become chronic relapsing diseases. Apart from long-term immunosuppression, life-long intensive follow-up is warranted. The French prospective multi-center randomized open-label WEGENT study compared maintenance with oral azathioprine versus methotrexate and did not find differences regarding safety or relapses [20]. Several risk-factors for relapse have been identified in multiple studies [21, 22], these risk-factors include PR3-ANCA and lung involvement. However, up till now these risk-factors have not resulted in individualization of treatment.

## **PATHOGENESIS OF RENAL INVOLVEMENT IN AAV**

Although blood vessels in all organ systems can be affected, the kidney is regularly involved in AAV. When renal involvement is present in AAV the glomeruli will show extracapillary proliferation. In this process extrarenal inflammatory and epithelial cells proliferate into Bowman's space forming so-called crescents. First these crescents predominantly exist out of cellular components. Subsequently, during disease progression fibroblasts and macrophages will be attracted and start to reorganize the crescent composition into a fibrous crescent, leading to loss of glomerular function [23]. However, it is thought that the cellular crescent can also recover and regain normal function [24].

It is not well known what influences the typical organ distribution of AAV. Probably an interaction between the immune system and the vessel wall, that is endothelial cells, is essential. The endothelium in the kidney stands out because of its fenestrated nature, essential for its role as filter. In a pro-inflammatory environment the endothelial cells will express adhesion molecules as selectins, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1. Activated neutrophils and monocytes will be able to adhere and transmigrate through the vessel wall. As mentioned before, primed neutrophils will express the ANCA-antigens MPO and PR3 on their surface and when adhered to endothelial cells binding by ANCA will result in a respiratory burst as well as degranulation and release of proteolytic enzymes. One class of these enzymes are the matrix metalloproteinases (MMPs).

## **MMPS IN VASCULITIS**

The MMPs are a family of over 25 proteases able to degrade extracellular matrix components [25]. One of five subgroups is the basement membrane-degrading MMPs or gelatinases

(MMP-2 and -9). MMP activity is tightly regulated at three different levels: gene expression, activation and inactivation. Gene expression of MMP-2 is constitutive, while MMP-9 production is induced by inflammatory cytokines such as IL-1 and TNF- $\alpha$ . MMPs are produced as inactive pro-enzymes, and activation involves multiple steps. Four endogenous tissue inhibitors of matrix metalloproteinases (TIMPs) are present in excess and neutralize the detrimental actions of MMPs [26]. The balance between MMPs and their inhibitors TIMPS will determine either net degradation or production of collagens, and might thereby discriminate inflammation from fibrosis.

MMPs are essential in renal homeostasis and inflammation [27]. By degrading extracellular matrix MMPs facilitate influx of inflammatory cells and release of pro-inflammatory products from the extracellular matrix. Thereby MMPs probably have an important pathogenetic role in the initial phase of crescentic glomerulonephritis. In Kawasaki disease patients, elevated expression, activity and protein levels of MMP-9 have been detected [28]. In giant cell arteritis, MMP-9 participates in the degradation of elastic tissue and is associated with intimal hyperplasia, subsequent luminal narrowing, and neoangiogenesis [29]. Chapter 7 analyzes the presence of MMPs and TIMPs in kidney biopsies of patients with AAV in relation to renal disease activity and damage. Consecutively, in chapter 8 urinary and serum levels of MMP-2 and -9 and TIMP-1 are measured and related to renal levels and disease activity.

## AIM OF THE THESIS

This thesis focuses on monitoring disease activity in AAV. Part one focuses on prediction and occurrence of relapses in AAV. First, in chapter 2 we review risk factors for relapse in AAV, their potential pathogenic implications, and their possible role in preventive strategies and adaptations of current treatment policies. Then, in chapter 3 the predictive value of serial measurements of C- and PR3-ANCA for clinical relapses in PR3-AAV are analyzed. In chapter 4 we evaluated whether the T cell activation markers sIL-2R, sCD30, IL-10, and B cell activator of the TNF family (BAFF) at diagnosis and during initial follow-up are predictive for persistent or renewed ANCA-positivity and clinical relapse in PR3-AAV. In chapter 5 and 6 we retrospectively evaluated long-term disease-free survival in patients with AAV on cyclophosphamide maintenance therapy as compared to azathioprine maintenance. Then in part two of the thesis focus is on renal production of MMPs and TIMPs in AAV. Chapter 7 analyzes the presence of MMPs and TIMPs in kidney biopsies of patients with

AAV in relation to renal disease activity and damage. Consecutively, in chapter 8 urinary and serum levels of MMP-2 and -9 and TIMP-1 are measured and related to renal levels and disease activity. Finally, in chapter 9 the results of our studies are summarized and put into perspective.

## REFERENCES

1. Jennette JC, Falk RJ, Andrassy K et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994; 37(2):187-192.
2. Franssen CF, Stegeman CA, Kallenberg CG et al. Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int* 2000; 57(6):2195-2206.
3. Sanders JS, Stassen PM, van Rossum AP, Kallenberg CG, Stegeman CA. Risk factors for relapse in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis: tools for treatment decisions? *Clin Exp Rheumatol* 2004; 22(6 Suppl 36):S94-101.
4. Xiao H, Heeringa P, Hu P et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 2002; 110(7):955-963.
5. van Rossum AP, Limburg PC, Kallenberg CG. Activation, apoptosis, and clearance of neutrophils in Wegener's granulomatosis. *Ann N Y Acad Sci* 2005; 1051:1-11.
6. 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(3):743-753.
7. Boomsma MM, Stegeman CA, van der Leij MJ et al. Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. *Arthritis Rheum* 2000; 43(9):2025-2033.
8. Finkelmann JD, Merkel PA, Schroeder D et al. Antiproteinase 3 antineutrophil cytoplasmic antibodies and disease activity in Wegener granulomatosis. *Ann Intern Med* 2007; 147(9):611-619.
9. Slot MC, Cohen Tervaert JW, Boomsma MM, Stegeman CA. Positive classic antineutrophil cytoplasmic antibody (C-ANCA) titer at switch to azathioprine therapy associated with relapse in proteinase 3-related vasculitis. *Arthritis Rheum* 2004; 51(2):269-273.
10. Sanders JS, Stegeman CA, Kallenberg CG. The Th1 and Th2 paradigm in ANCA-associated vasculitis. *Kidney Blood Press Res* 2003; 26(4):215-220.
11. Muller A, Trabandt A, Gloeckner-Hofmann K et al. Localized Wegener's granulomatosis: predominance of CD26 and IFN-gamma expression. *J Pathol* 2000; 192(1):113-120.
12. Schonermarck U, Csernok E, Trabandt A, Hansen H, Gross WL. Circulating cytokines and soluble CD23, CD26 and CD30 in ANCA-associated vasculitides. *Clin Exp Rheumatol* 2000; 18(4):457-463.
13. Lamprecht P, Erdmann A, Mueller A et al. Heterogeneity of CD4 and CD8+ memory T cells in localized and generalized Wegener's granulomatosis. *Arthritis Res Ther* 2003; 5(1):R25-R31.



14. Fouser LA, Wright JF, Dunussi-Joannopoulos K, Collins M. Th17 cytokines and their emerging roles in inflammation and autoimmunity. *Immunol Rev* 2008; 226:87-102.
15. Abdulahad WH, Stegeman CA, Kallenberg CG. Review article: The role of CD4(+) T cells in ANCA-associated systemic vasculitis. *Nephrology (Carlton)* 2009; 14(1):26-32.
16. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 1983; 98(1):76-85.
17. Hollander D, Manning RT. The use of alkylating agents in the treatment of Wegener's granulomatosis. *Ann Intern Med* 1967; 67(2):393-398.
18. Walton EW. Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *Br Med J* 1958; 2(5091):265-270.
19. Jayne D, Rasmussen N, Andrassy K et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 2003; 349(1):36-44.
20. Pagnoux C, Mahr A, Hamidou MA et al. Azathioprine or methotrexate maintenance for ANCA-associated vasculitis. *N Engl J Med* 2008; 359(26):2790-2803.
21. Hogan SL, Falk RJ, Chin H et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. *Ann Intern Med* 2005; 143(9):621-631.
22. Pagnoux C, Hogan SL, Chin H et al. Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis: Comparison of two independent cohorts. *Arthritis Rheum* 2008; 58(9):2908-2918.
23. Hricik DE, Chung-Park M, Sedor JR. Glomerulonephritis. *N Engl J Med* 1998; 339(13):888-899.
24. Atkins RC, Nikolic-Paterson DJ, Song Q, Lan HY. Modulators of crescentic glomerulonephritis. *J Am Soc Nephrol* 1996; 7(11):2271-2278.
25. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. *J Biol Chem* 1999; 274(31):21491-21494.
26. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol* 1997; 74(2):111-122.
27. Lenz O, Elliot SJ, Stetler-Stevenson WG. Matrix metalloproteinases in renal development and disease. *J Am Soc Nephrol* 2000; 11(3):574-581.
28. Senzaki H. The pathophysiology of coronary artery aneurysms in Kawasaki disease: role of matrix metalloproteinases. *Arch Dis Child* 2006; 91(10):847-851.
29. Rodriguez-Pla A, Bosch-Gil JA, Rossello-Urgell J, Huguete-Redecilla P, Stone JH, Vilardell-Tarres M. Metalloproteinase-2 and -9 in giant cell arteritis: involvement in vascular remodeling. *Circulation* 2005; 112(2):264-269.