On the pathogenesis and clinical outcome of ANCA-associated vasculitis

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Chapter VII

Summary and general discussion

More than 150 years after the publication of the first case-report of a patient with, what is now considered to be, antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, considerable progress has been made in the understanding of the pathogenesis, the diagnostic approach, and the treatment of this disease.[1] Research in ANCA-associated vasculitis has come a long way. However, the heart of the matter is still unsolved, as the exact nature of this complex disease and, consequently, the most appropriate disease classification system and optimal treatment strategies are still not unravelled. In this thesis, several aspects of ANCA-associated vasculitis concerning genetics, clinical and histopathological classification, and long-term outcome were investigated.

Genetics and disease classification

Despite the considerable progress made in recent decades, the pathophysiology of ANCA-associated vasculitis is still not fully unravelled. Amongst others, genetic factors are believed to contribute to the pathogenesis of this complex disease.[2] 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. To increase the validity of our meta-analysis, we included raw data from a large genome-wide association study (GWAS).[3] This study provides the first complete and comprehensive overview of all genetic variants investigated in ANCA-associated vasculitis in at least two independent studies.

Thirty-three genetic variants, located in or near 15 genes, were found to be associated with ANCA-associated vasculitis. The genetic variants were located in or near the following genes: *CD226, CTLA-4, FCGR2A, HLA-B, HLA-DP, HLA-DQ, HLA-DR, HSD17B8, IRF5, PTPN22, RING1/RXRB, RXRB, STAT4, SERPINA1,* and *TLR9.* The results of this meta-analysis confirm that genetic factors contribute to the pathogenesis of ANCA-associated vasculitis. In particular, they support a role for the major histocompatibility complex (MHC), the innate and adaptive immune system, and several inflammatory processes in the pathogenesis of this complex disease; the identified genetic factors could potentially result in altered HLA mediated antigen presentation, inadequate T- and B-lymphocyte activation, and abnormal target antigen structure and/or function. The mechanisms whereby and the extent to which these genetic variants directly cause disease requires further investigation.

Most importantly, the results of our meta-analysis provide clear evidence of a genetic susceptibility that differed between granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) patients and between proteinase 3 (PR3)-ANCA positive and myeloperoxidase (MPO)-ANCA positive patients. Moreover, the associations were primary aligned with ANCA-serotype rather than with the clinically defined syndromes: in 76% of the genetic variants,

subdivision based on ANCA-serotype resulted in higher odds ratios than subdivision based on clinical diagnosis.

Considerable debate surrounds the classification of ANCA-associated vasculitis. GPA and MPA have overlapping clinical features, and while patients with typical characteristics are easy to classify, many patients are allocated inconsistently even by experienced clinicians. Moreover, even though the Chapel Hill Consensus Conference (CHCC) and European Medicines Agency (EMA) classification systems use identical names for the ANCA-associated vasculitis disease categories, a study performed by Lionaki *et al.* demonstrated substantial discrepancies between the two classification systems in the allocation of patients to these categories: 78% of patients classified as having MPA using the CHCC system were considered to have GPA by the EMA system.[4] The authors argued that this resulted in part from differences in disease definitions. For instance, the EMA system over-represents the diagnostic category of GPA because, according to the American College of Rheumatology criteria, essentially any upper respiratory involvement is considered to be GPA.[5] Overall, the inconsistencies between clinicians and between the classification systems and the lack of diagnostic criteria make the use of the terms GPA and MPA less accurate and less predictive of outcome.

Interestingly, a number of clinical studies demonstrated that disease course, treatment response, and patient outcome align more closely with ANCA-serotype than with clinical diagnosis. Lionaki *et al.* demonstrated that ANCA-serotype is closely associated with clinical features and the relative frequency of organ system involvement.[4] ANCA-serotype was shown to be more strongly associated with relapse than clinical diagnosis, and patients with PR3-ANCA positive GPA were shown to have a significantly different disease course than patients with MPO-ANCA positive GPA.[4,6] Unizony *et al.* investigated treatment response in patients enrolled in the Rituximab in ANCA-associated Vasculitis (RAVE) trial according to both clinical diagnosis (GPA versus MPA) and ANCA-serotype (PR3-ANCA versus MPO-ANCA).[7] Treatment response did not differ between GPA and MPA patients. In contrast, treatment response did differ between PR3-ANCA positive and MPO-ANCA positive patients: PR3-ANCA positive patients were shown to respond better to rituximab than to the combination of cyclophosphamide and azathioprine. These findings have led to the following question: is it the name of the disease or is it the serotype that is more important with respect to disease classification and individual patient prognosis?

Our genetic findings taken together with the clinical findings discussed previously underscore the need to account for ANCA-serotype in the classification of ANCA-associated vasculitis. Arguments to incorporate ANCA-serotype in the classification of ANCA-associated vasculitis include:

- Distinct genetic subsets correlate more strongly with ANCA-serotype than with clinical classification
- ANCA-serotype correlates better with clinical features than clinical classification
- ANCA-serotype is more reproducible and reliable than clinical classification
- ANCA-serotype better reflects the disease mechanism

However, there are problems with using only ANCA-serotype to classify patients. For example, using current assays, 5-10% of ANCA-associated vasculitis patients are ANCA-negative.[8-13] Moreover, better understanding of the granuloma formation in ANCA-associated vasculitis may reveal therapies that specifically target this component of the disease, which requires acknowledging this phenotype. Therefore, for now, a classification system based primarily on ANCA-serotype with subsequent subdivision based on clinical diagnosis – e.g. PR3-ANCA positive GPA – may be the best way to characterize patients until we truly understand the similarities and differences in the pathogenesis of the ANCA-associated vasculitides. This will provide practical classification criteria better aligned to patient phenotype, treatment responses, and outcomes, and will allow logical design and testing of future therapies.

In **Chapter 3**, we focused on ear, nose, and throat (ENT) involvement and ANCA-serotype with respect to renal histology at diagnosis and renal outcome. Previously, Hauer *et al.* demonstrated that MPO-ANCA positive vasculitis patients have more chronic lesions in their renal biopsies than PR3-ANCA positive vasculitis patients. The authors hypothesized that this finding may reflect an association of MPO-ANCA with a clinical phenotype of smouldering disease.[14] This could be indicative of different disease mechanisms in PR3-ANCA vasculitis and MPO-ANCA vasculitis, in line with the results presented in **Chapter 2**. However, PR3-ANCA positivity is also closely correlated with ENT disease.[15, 16] Thus, another hypothesis for the fewer chronic lesions in the renal biopsies of PR3-ANCA positive patients is that the clinically overt ENT manifestations in these patients lead to earlier diagnosis of ANCA-associated vasculitis by reducing patient's and/or doctor's delay.

In our study, patients with ENT involvement had fewer chronic lesions in their renal biopsies and better renal function at diagnosis and 5-year follow-up than patients without ENT involvement. Our results suggest that this is not caused by earlier diagnosis due to the clinically overt ENT symptoms: in the same patients, other manifestations that would also have reduced patient and/or doctor's delay, such as lung involvement, cutaneous manifestations, and

arthralgia/arthritis, were not associated with renal histology or renal outcome. To exemplify this point further; a study by Bligny *et al.* reported higher survival in ANCA-associated vasculitis patients with ENT involvement than patients without ENT involvement.[17] The authors argued that this is not likely caused by earlier diagnosis and treatment, as 87% of patients without ENT involvement had lung involvement, which, theoretically, also would have led to earlier diagnosis. Interestingly, a study by Poulton *et al.* even demonstrated an increased diagnostic delay in ANCA-associated vasculitis patients with ENT involvement.[18] The authors argued that both physicians and patients focused on the more common causes of the ENT symptoms instead of regarding them as signs of vasculitis. As could be expected, patients with skin manifestations often had a prompt diagnosis of ANCA-associated vasculitis.[18]

In our study, ENT involvement overruled ANCA-serotype in the multivariable analysis and was still associated with better renal outcome in a sub-analysis including only PR3-ANCA positive patients. Thus, independent of ANCA-serotype, ENT involvement itself seems to be associated with better renal histology and outcome. The findings of this study can be viewed in the context of the two distinct pathological features in GPA, namely: vasculitis, which is predominantly localized in the kidneys, and granulomatosis, which predominantly involves the airways.[19] The clinical heterogeneity of GPA reflects a spectrum of manifestations ranging from predominantly vasculitic disease at one end to predominantly granulomatous disease at the other. Thus, in our study, the patients with ENT involvement could have had a predominantly granulomatous disease, while the patients without ENT involvement had a predominantly vasculitic disease. In this light, the better renal prognosis in the patients with ENT involvement could be representative of the more benign renal course of 'granulomatous GPA' compared to 'vasculitic GPA'.

The pathological pathways leading to vasculitis or granuloma formation are hypothesized to be different. Immunological studies indicate that these two distinct pathological features correspond to different T cell immune responses; i.e. a T helper cell 1 (Th1)-type response in granulomatous disease and a Th2-type response in vasculitic disease.[20-22] Further studies are needed to adequately assess the potential prognostic value of ENT involvement, which, in turn, might provide further insight into the pathogenesis of GPA.

Histopathological classification of ANCA-associated glomerulonephritis

The histopathological classification of ANCA-associated glomerulonephritis was devised by an international working group of renal pathologists and nephrologists with the aim of further adding to the prognostication of patients with ANCA-

associated glomerulonephritis.[23] Thus far, 21 validation studies, consisting of cohorts from Asia, North-America, Australia, and Europe, have investigated the prognostic value of the classification system.[24-44] **Chapter 4** reviews the findings of these validation studies and puts them into a broader perspective. In general, the studies confirmed the predictive value of the classification system for renal outcome in the focal and sclerotic classes. However, several studies showed conflicting results with respect to the crescentic and mixed classes.[24-37]

A number of factors may have contributed to the inconsistent results between the validation studies. As indicated by their denominator, biopsies in the mixed class may indeed show mixed lesions in which either an acute or chronic phenotype can predominate. Therefore, differences in the compositions of the mixed classes in the various validation studies may have contributed to the different outcomes reported for this class. It could be argued that, in its current form, this class is too heterogeneous to have a straightforward predictive value in the classification system. A second explanation for the inconsistent results between the validation studies might be a problem of definitions; the crescentic class was originally defined as the predominance of cellular crescents, however, this definition may have lacked important details leading to inter-observer discrepancies among pathologists.[23, 29] Differences in patient population and treatment most probably also contributed to the variable results between the validation studies. Unfortunately, due to their retrospective design, it was not possible to fully account for treatment in most of the validation studies. A number of validation studies suggested that inclusion of tubulointerstitial parameters could increase the predictive value of the classification system. In contrast, Berden *et al.* argued that inclusion of tubulointerstitial parameters does not add meaningfully to the predictive value of the classification system and only increases its complexity.[23]

Currently ongoing studies that aim at the optimization of the histopathological classification of ANCA-associated glomerulonephritis are discussed in the 'Future perspectives' section of this chapter.

Treatment and outcome

Over the last decades, the introduction of immunosuppressive treatment has improved the prognosis of patients with ANCA-associated vasculitis dramatically. Maintenance of remission, on the other hand, still represents a challenge, necessitating prolonged immunosuppressive treatment.[45] Despite the clear success of conventional immunosuppressive therapies, their long-term side effects jeopardize outcomes. One of the long-term side effects of immunosuppressive treatment is the occurrence of malignancies.[46]

Chapter 5 reports the results of the first 10-year follow-up study investigating malignancy risk in patients with ANCA-associated vasculitis treated with current

treatment regimens. In these patients, overall malignancy risk was 2.21 times increased compared to the general population. This increased malignancy risk was attributable solely to the increased risk of non-melanoma skin cancer (NMSC). Importantly, in contrast to previous studies,[47] in our study the risks of bladder cancer, lymphomas, and leukaemia were not increased. These findings most likely reflect the decrease in cyclophosphamide exposure in the treatment of ANCA-associated vasculitis in the last decade.[8-10, 13]

The close relationship between disease activity and immunosuppressive treatment exposure makes it difficult to distinguish disease-related from treatment-related effects on malignancy risk. Nonetheless, a subgroup analysis – based on year of diagnosis and subsequent treatment – demonstrated that malignancy risk was no longer significantly increased after the major reduction in cyclophosphamide exposure that resulted from the publication of the CYCAZAREM trial.[10] Moreover, malignancy risk was directly correlated to the duration of cyclophosphamide treatment.

In summary, the data reported in **Chapter 5** demonstrate the effectiveness of international efforts to reduce toxicity in the treatment of ANCA-associated vasculitis. Despite the advances achieved, the road towards treatment modalities with less adverse effects in terms of, amongst others, infection risk, infertility, and malignancy occurrence in patients with prolonged cyclophosphamide exposure, is still long. Recently, rituximab was successfully introduced in the treatment of ANCA-associated vasculitis with the aim of, amongst others, further reducing these adverse treatment effects.[13, 48, 49]

Chapter 6 reports the first study that compares the long-term malignancy risks between rituximab-based and cyclophosphamide-based treatment in ANCA-associated vasculitis. Strikingly, malignancy risk was 4.61 fold increased in patients treated with cyclophosphamide compared to patients treated with rituximab. Malignancy risk was 3.10 fold increased in patients treated with cyclophosphamide compared to the general population. This increased malignancy risk was again solely attributable to the occurrence of NMSC. In contrast, malignancy risk was not increased in patients treated with rituximab. Interestingly, patients treated with both rituximab and cyclophosphamide had a lower malignancy risk than patients treated with cyclophosphamide alone, despite the mean cumulative cyclophosphamide dose being lower in the latter group. In addition, there was a trend towards an inverse dose-response relationship between the cumulative rituximab dose and malignancy risk, i.e., the more rituximab a patient received, the lower the malignancy risk. Notably, patients who cumulatively received more than 6.0g rituximab had a lower malignancy risk than the general population. This effect was observed for both haematological and non-haematological malignancies.

These findings hint towards the possibility that rituximab has an inhibitory effect on (haematological and non-haematological) malignancy occurrence. B cell

depletion – the mechanism of action of rituximab – was shown to increase anti-tumour immunity in murine models.[50, 51] This enhanced anti-tumour immune response is hypothesized to be caused by decreased IL-10 production by B regulatory cells, leading to enhancement of the anti-tumour effects of cytotoxic T cells.[50] In humans, the hypothesis that rituximab enhances the anti-tumour immune response is supported by the trend towards a lower risk of developing a second primary (non-haematological) malignancy in patients with non-Hodgkin lymphoma treated with rituximab-containing chemotherapy, compared to patients treated with conventional chemotherapy not including rituximab.[52, 53] Clearly, elucidation of the effects of B-cell depletion on anti-tumour immunity requires further investigation.

In conclusion, the results of the study reported in **Chapter 6** demonstrate that patients with ANCA-associated vasculitis treated with rituximab have a decreased burden of malignancy, surpassing expectations from clinical trial data.[54, 55] Moreover, our results suggest that rituximab may have an inhibitory effect on malignancy occurrence. Therefore, regarding the occurrence of malignancies, rituximab seems a superior alternative to cyclophosphamide in the treatment of ANCA-associated vasculitis.

Future perspectives

Despite the major advances made in the understanding of the pathogenesis, the diagnostic approach, and the treatment of ANCA-associated vasculitis, many questions are still unanswered and new questions arise every day. The results of the meta-analysis presented in this thesis confirm that genetic factors contribute to the pathogenesis of ANCA-associated vasculitis. However, functional studies remain to be performed to establish the precise role of these genetic variants and their effects on the disease process. Future studies in genetics and epigenetics will likely not only lead to a deeper understanding of the pathogenesis of ANCA-associated vasculitis, but perhaps also to a personalised medicine approach to clinical management and therapeutic target selection. In systemic lupus erythematosus, researchers have developed a personalized transcriptional immunomonitoring approach that enables patient stratification based on molecular networks best correlating with disease progression.[56] The development of such a personalized transcriptional immunomonitoring approach in ANCA-associated vasculitis would create some order in this complex heterogeneous disease, bringing patient-tailored treatment one step closer.

The genetic differences between PR3-ANCA vasculitis and MPO-ANCA vasculitis indicate that future genetic and clinical studies should be sufficiently powered to allow for independent analysis of PR3-ANCA positive and MPO-ANCA positive patients. Currently, a GWAS including GPA and MPA patients

is being conducted that is sufficiently powered to investigate genetic associations in the PR3-ANCA and MPO-ANCA subgroups independently.[57] Moreover, an ongoing GWAS is investigating whether there are genetic differences between MPO-ANCA positive and MPO-ANCA negative patients with eosinophilic granulomatosis with polyangiitis (EGPA).[57] Preliminary results of this GWAS point towards profound differences in the pathogenesis of these two EGPA subtypes and suggest a number of therapeutic approaches that might be effective in EGPA.

Current treatment strategies in ANCA-associated vasculitis do not take into account specific patient and disease characteristics. However, genetic and clinical data suggest that treatment should be tailored to specific parameters. Possible parameters that, now or in the future, could be taken into consideration for refining treatment, in the form of patient-tailored therapy, are: genetic markers, ANCA-serotype, severity scores, and scores and/or markers predicting relapse. A step in this direction is set by the identification of altered gene expressions that can predict treatment response in MPA patients.[58] The MAINRITSAN 2 trial (ClinicalTrials.gov NCT01731561) is currently investigating treatment response in rituximab treatment according to a fixed administration protocol versus individually tailored rituximab treatment based on ANCA-status and peripheral blood CD19 lymphocyte reappearance. In refractory rheumatoid arthritis, similar parameters (rheumatoid factor, anti-CCP antibodies, and serum IgG concentration) were shown to predict treatment response to rituximab.[59]

Presently, we are stepping into a new therapeutic era with targeted therapies – e.g. monoclonal antibodies such as rituximab – that could further improve the treatment of ANCA-associated vasculitis. This is a field for ongoing and future trials that investigate the optimal dosing and duration of rituximab both as induction and maintenance treatment. The currently ongoing RITAZAREM trial (ClinicalTrials.gov NCT01697267) will provide the largest trial dataset for the use of rituximab as remission-induction treatment for ANCA-associated vasculitis. This trial compares rituximab and azathioprine maintenance treatment following rituximab induction-treatment, and explores whether prolonged B-cell depletion leads to sustained treatment-free remission after treatment discontinuation. Although the first studies, as demonstrated in this thesis, indicate that rituximab treatment has resulted in less morbidity in the long run, more long-term follow-up studies with longer follow-up are warranted.

Moreover, it becomes more and more apparent that the burden of corticosteroids is responsible for a substantial part of the treatment-related adverse events in ANCA-associated vasculitis. Several ongoing studies are investigating different dosing regimens of steroids regarding efficacy and safety. The currently ongoing PEXIVAS trial (ClinicalTrials.gov NCT00987389) is the largest trial conducted in ANCA-associated vasculitis thus far and has recruited more than 700 patients from over 100 centres over the world. This 2-by-2 trial addresses the

effect of a reduced cumulative dosing regimen of glucocorticoids and the effect of adjuvant plasma exchange in patients with severe ANCA-associated vasculitis.

Regarding histology, future studies might answer the question whether renal biopsy findings will be directly useful to guide therapeutic decision-making. A large international validation study of the histopathological classification of ANCA-associated glomerulonephritis in which histopathological, clinical, and therapeutic data are being incorporated is currently being conducted. Moreover, the biopsies included in the 21 previously performed validation studies are now being collected with the aim of re-evaluating them by a group of experienced pathologists. The aim of these studies is to evaluate the predictive value of the classification system, formulate clearer and more concise definitions, and possibly also revise the histopathological classification of ANCA-associated glomerulonephritis.

Overall, the vasculitis patient and research communities can be optimistic about the future of research in ANCA-associated vasculitis and the potential for this work to unravel the puzzle of this complex disease and, most importantly, to improve the lives of patients with ANCA-associated vasculitis.

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