Chapter 8

Summary and future perspectives
Introduction

ANCA-associated vasculitis constitutes a group of primary vasculitides associated with antineutrophil cytoplasmic antibodies (ANCA), which includes Wegener’s granulomatosis, microscopic polyangiitis, renal limited vasculitis and the Churg-Strauss syndrome. Small and medium sized vessels are affected, which, together with granulomatous inflammation, results in clinical manifestations in the ear, nose and throat area, and in lungs and kidneys. AAV is a rare disease, with an incidence of 9.5-16 per million in northern Germany (1).

The etiology of this disease is currently unknown. It is believed that various environmental factors induce AAV in persons who are susceptible due to a certain genetic background (2-4), as described in chapter 1. These environmental factors include exposure to silica, solvents and livestock. Furthermore, infections seem to play a role. Infections in particular can lead to priming of neutrophils, resulting in expression of PR3 and MPO on their surface. Binding of ANCA to these antigens leads to further activation of neutrophils, eventually resulting in an amplified inflammation and damage to the endothelium. T cells are also involved in this inflammatory process. Genetic factors predisposing to the development of AAV include polymorphisms in genes encoding for cytokines, Fcγ receptors and α1-antitrypsin, and membrane expression of PR3 in neutrophils (5;6). Which additional environmental and genetic factors are of importance and how these factors interact, is, however, still unclear.

Treatment of AAV has improved over the years. As most patients can nowadays be brought into remission, concerns about mortality have been largely replaced by concerns about morbidity caused by treatment and recurrent episodes of disease activity. Refining current therapy by either risk-adapted strategies or individualized (pharmacogenetic-based) drug dosing is one option. Using new drugs is the other option.

Below, the findings of this thesis are summarized.

HLA

As the HLA system plays a central role in the distinction between self and non-self, an association between HLA-polymorphisms with the autoimmune disease AAV could be present. A strong association pointing to a specific antigenic factor would offer an opportunity to elucidate the etiology of AAV and to tailor the potentially toxic treatment to the individual patient. In chapter 2, we describe the associations found between HLA-antigens and AAV in 304 patients. Compared to healthy controls, we found a low frequency of DR13(6) and a higher frequency of DR4 and the ancestral haplotype A1B8DR3 in patients with AAV, particularly in patients with WG. In addition, we found an association between DR1 and WG, and between DR8 and CSS. How these associations lead to the development of AAV is still unclear. We found no association with HLA antigens and disease characteristics or course of AAV. Our findings are in line with other reports of a decrease of DR13(6) (7-12), an increase of DR4 (7-9;12-14) and an increase of DR8 (15) as well, though the increase of DR8 was reported in patients with WG and MPA and not in CSS patients, as observed in our study. As far as we know, we are the first to describe the increased presence of
the ancestral haplotype A1B8DR3, which is associated with an immune system that is more likely to develop autoreactive responses (16), in AAV patients.

Currently, it is unclear which role HLA plays in the development of AAV. Given the diversity and the relative weakness of most associations, found in various studies, it is unlikely that HLA is the only genetic factor leading to AAV. Moreover, associations do not prove causality. In addition, it is possible that not the HLA antigens, but linked immunomodulating genes are responsible for the development of AAV. Using more sophisticated methods to map the genes of AAV patients in homogeneous and large cohorts will be needed to clarify which (combination of) genes predispose to AAV and what mechanisms are involved.

We, like others (7;17;18), found no clear associations between HLA antigens and either disease characteristics at presentation or the course of AAV, except for a decreased frequency of renal involvement at diagnosis in patients with A9. Assessing HLA class I and II antigens before start of treatment will, therefore, not provide tools to tailor the treatment of these patients.

**Influenza vaccination**

Inducing autoimmunity or relapses of autoimmune diseases, like AAV, after vaccination against influenza, is feared by both physicians and doctors. In chapter 3 however, we found no increase of relapses after vaccination against influenza in a retrospective study on 230 patients with AAV. On the contrary, the relapse rate per 100 patients at risk was lower in patients who had been vaccinated within the previous year (3.4) than in patients who had not been vaccinated against influenza (6.3). Therefore, with regard to the occurrence of relapses, we conclude that it is safe to vaccinate AAV patients against influenza.

As the retrospective design of our study allows the possibility of bias by indication, our results should be confirmed in prospective randomized controlled studies. In such a study from our center, vaccination against influenza did not result in an increase in relapses in 72 patients with quiescent AAV (19).

Apart from the safety of vaccination against influenza, the efficacy of this vaccination should be investigated, especially because it is possible that influenza infections lead to an increase in disease activity in patients with autoimmune diseases (20;21). Preliminary data from our center show that vaccination results in protective antibody titers in patients with quiescent AAV, even though these titers were lower than in healthy controls (19). Furthermore, the influence of (the dose of) standard and new immunosuppressive drugs on the efficacy of vaccination has not yet been studied in patients with AAV. In patients with RA, protective antibody titers were found in patients using prednisolone and methotrexate (22). However, azathio- prine might decrease the response to vaccination in patients with SLE (23). In addition, mycophenolate mofetil in combination with prednisolone and cyclosporin leads to less protective antibody titers in transplant patients (24), although controversy on this subject exists (25). Vaccination is also less effective in patients with RA using Rituximab, a monoclonal antibody against CD20, which is also used in patients with AAV (26).

In our cohort, 40% of the patients were not vaccinated consistently against influenza. In particular, younger patients were not vaccinated. This is also seen in patients with RA (27), mostly due to the fact that vaccination was never offered (28). When the secondary care informs about and advises in favor of vaccination, the
vaccination grade will raise substantially (29). Considering that vaccination is safe and effective, care must be taken, perhaps by the secondary line, to ensure that all AAV patients receive their vaccination.

**TPMT genotype and TPMT activity**

The metabolism of 6-mercaptopurine, the active metabolite of azathioprine, is dependent on the enzyme thiopurine methyltransferase (TPMT). TPMT inactivates 6-mercaptopurine and catalyses the conversion of 6-mercaptopurine into hepatotoxic metabolites. The gene encoding TPMT is polymorphic, which leads to differences in activity of the enzyme. In chapter 4, we found that 7 out of 108 patients (6%) with AAV were heterozygous for one variant allele, all with correspondingly lower TPMT activity than the patients with homozygous normal alleles. None of the patients were homozygous for variant alleles. We found no relation between TPMT variant alleles or TPMT activity and the occurrence of both relapses and adverse effects, in particular hepatotoxicity and leukocytopenia. However, as measured by the [leukocyte]×[azathioprine] product, it seemed that sensitivity for leukocytopenia was higher in the group with the lowest TPMT activity.

Our results must be viewed in the light of the retrospective design of our study. Especially, minor adverse effects may not have been registered correctly. Further, with an expected low prevalence of TPMT genotype or TPMT activity variants, a larger sample size is probably needed to find significant correlations. Another factor that may have influenced our results is that all our patients had been treated with cyclophosphamide before starting azathioprine, which is not the case in other diseases like inflammatory bowel disease.

It is possible that other gene polymorphisms associated with TPMT activity than the four we tested for, are responsible for the lack of efficacy or occurrence of adverse effects in patients with AAV. In addition, it is possible that variants in other enzymes than TPMT, involved in the metabolism of azathioprine, like inosine triphosphate pyrophosphatase (30;31) or glutathione-S-transferase (32), can explain the observed lack of efficacy and toxicity of azathioprine. Finding alternative explanations for the fact that azathioprine is not effective or causes adverse effects in some patients with AAV will be valuable, considering the fact that the role of the TPMT polymorphisms and abnormal TPMT activity, we tested in the present study, was (at most) of minor significance.

**Mycophenolic acid in non-transplant renal diseases**

In chapter 5, we reviewed the increasing experience with mycophenolic acid, the active metabolite of mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS), in the treatment of non transplant renal diseases. Most experience with MMF exists in the induction treatment of proliferative lupus nephritis. Several small sized controlled studies with short follow-up showed that MMF induced remissions at least as often as cyclophosphamide. Several recent meta-analyses of these studies concluded that MMF was even better and less toxic than cyclophosphamide (33-35). In addition, use of MMF as first-line therapy was more cost-effective and resulted in higher quality of life than cyclophosphamide (36). For maintenance therapy in proliferative lupus nephritis, MMF seemed more effective than cyclophosphamide, in one small study (37). However, as large randomized
controlled studies with sufficient follow-up are still lacking, more trials are clearly needed and are underway.

For other immunologically mediated renal diseases, less data exist. In general, mycophenolic acid seems to offer an alternative in case current standard therapy fails (38;39). Whether mycophenolic acid can replace current standard therapy is presently not clear.

Mycophenolate mofetil for remission induction in ANCA-associated vasculitis

In chapter 6, our experience is described with MMF as induction treatment in 32 patients with AAV, who could not be treated with cyclophosphamide. All but one patient responded within 5 months of therapy with MMF and prednisolone. However, the relapse rate was high (59%) and overall median relapse free survival was only 16 months in these negatively selected patients, with predominantly longstanding and frequently relapsing AAV. Most patients were diagnosed as WG, experienced many relapses before, and were PR3-ANCA positive, all known risk factors for relapse (40). Despite these characteristics, median relapse free survival was 41 months in the 78% of patients in whom a complete remission could be obtained.

Our results are in line with findings of other studies showing MMF is able to induce remissions (41-45). One recent small (n=35) open randomized study with 6 months of follow-up, included mostly MPO-ANCA positive AAV patients with moderate renal involvement (serum creatinine <500 µmol/l; mean: 315 µmol/l) and treated these with either MMF or intravenous cyclophosphamide (46). MMF was superior to cyclophosphamide, with remission induced in 78% of patients, compared with 47% when using cyclophosphamide. In addition, MMF led to recovery of renal function more frequently than cyclophosphamide (44 vs 15%, respectively).

The results of our and other studies are promising enough to recommend large randomized controlled studies with sufficient follow-up to evaluate whether MMF offers an equally (or more) effective and less toxic alternative for cyclophosphamide based regimens. At this moment, we coordinate a multi-center study in the Netherlands that compares the effect of MMF as induction therapy with that of cyclophosphamide in patients with a relapse of AAV.

Venous thromboembolism

In chapter 7, we investigated the incidence of VTE in a large homogeneous cohort of AAV patients (n=198) and the possible influence of disease activity and classic risk factors for VTE on the occurrence of VTE. We found an increased incidence of VTE just prior to and after the diagnosis of AAV of 1.8/100 person years, compared to 0.3 in a healthy population of the same age (47). The incidence increased to 6.7/100 person years in periods with active AAV. VTEs occurred less frequently in patients with WG and in those with PR3-ANCA. We found no significant differences in the prevalence of classic risk factors for VTE between patients who developed an AAV-associated VTE and those who did not. These results must be seen in the context of the retrospective design of this study. We may have missed (asymptomatic) VTEs, which could have been diagnosed in a prospective study.
The cause of the increased risk of VTE, that we and others (48;49) found in AAV patients, especially when the disease is active, is unknown. We observed that the presence of classic risk factors could not explain this increased risk for VTE, as did others (49;50). Possibly, changes in endothelial function and hypercoagulability, especially during active disease, can explain this increased risk for VTE, and these options should be explored. Treatment with cyclophosphamide and high doses of corticosteroids may also explain the increased incidence of VTE in AAV patients. Combination chemotherapy containing cyclophosphamide is known to increase the risk of VTE (51). This alleged adverse effect of current therapy of AAV justifies the search for new treatments of AAV even more.

It seems reasonable to assume that the increased incidence of VTE in patients with (active) AAV justifies prophylaxis against VTE, but this should be evaluated in randomized studies.

Final conclusions

Several HLA-antigens, or linked immunomodulating genes, may predispose to the development of AAV, as a decreased prevalence of DR13(6) and an increased prevalence of DR4 and the haplotype A1B8DR3 in patients with AAV was found. Vaccination against influenza does not increase disease activity of AAV, unlike other environmental factors, and infections in particular. Assessing TPMT genotype and TPMT activity does not predict efficacy or toxicity of maintenance therapy with azathioprine and will therefore not offer a tool to individualize and improve treatment of ANCA-associated vasculitis. Mycophenolic acid, the active metabolite of mycophenolate mofetil, is a promising new drug, which can be used to treat immunologically mediated renal diseases and to induce remissions of ANCA-associated vasculitis. The risk of venous thromboembolism is increased in patients with ANCA-associated vasculitis and, when vasculitis is active, equals that of patients with a history of venous thromboembolisms.


SUMMARY AND FUTURE PERSPECTIVES


