Chapter 1

General introduction and outline of the thesis
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Introduction

ANCA-associated vasculitis (AAV) constitutes a group of primary vasculitides associated with antineutrophil cytoplasmic antibodies (ANCA), which includes Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA), renal limited vasculitis (RLV) and the Churg-Strauss syndrome (CSS). Small and medium sized vessels are affected in AAV. AAV is a rare disease, with approximately 2000 patients living in the Netherlands.

The etiology of AAV is unknown. However, both genetic and environmental factors seem to play a role. Supporting the role of genetic factors in the development of AAV are the findings of familial cases (1) and an elevated relative risk (1.56 (95% CI: 0.35-6.90)) of having WG in first-degree relatives of WG patients in a large Swedish study (2). Furthermore, several associations between AAV and polymorphisms of genes that encode for immunologically important proteins, like α1-antitrypsin, Fcγ receptors and cytokines have been described (reviewed in (3-5)). One of the other genetic factors leading to increased susceptibility to the development of AAV may be the presence of polymorphisms in the human leukocyte antigen (HLA) genes. Environmental factors, like exposure to silica, livestock and solvents (6), use of drugs, like PTU (7) and infections (both viral and bacterial) (8) may lead to the development of AAV in individuals with a genetic predisposition (reviewed in (3-4)).

ANCA are antibodies directed against proteinase 3 (PR3) and myeloperoxidase (MPO), both constituents of azurophilic granules in neutrophils and monocytes. These antibodies are highly specific and sensitive for AAV and can be used both as a diagnostic tool and for monitoring of therapy and follow-up (9). Rises of titers of ANCA often precede clinical relapses (10). Except for their use in clinical practice, these antibodies have a pathogenetic role in the development of AAV. ANCA stimulate neutrophils, which are primed by cytokines, like TNFα, to adhere to endothelium and to degranulate their granules and induce a respiratory burst with the release of toxic oxygen radicals and lysosomal enzymes onto the vessel wall, leading to damage. Neutrophils that have been activated by ANCA also release factors that activate the alternate complement pathway, thereby amplifying the inflammation (11;12).

Today, cyclophosphamide, in combination with corticosteroids, forms the cornerstone of the treatment of AAV. The prognosis of AAV patients has drastically improved since this combination therapy was introduced in the 1970s, as most patients (90%) can be brought into remission. Before the introduction of this combination therapy, AAV was usually fatal, with an average survival of 5 months (13). However, treatment with cyclophosphamide leaves us with three problems. First, many patients (up to 50%) experience a relapse within 5 years (14), leading to substantial morbidity caused by both the recurrent vasculitis and repeat exposure to this drug (14-16). Second, current treatment is toxic, in particular when cumulative dose is high. About 42% of patients experience serious side effects (14) and treatment accounts for about 28% of damage seen in AAV patients after a follow-up of 25 months (16). Third, with longer patient survival and increasing number of relapses, leading to high cumulative exposure to these drugs, some patients can no longer be treated with current therapy at the moment of renewed disease activity. Therefore,
new therapies are needed, either by refining current therapy or introduction of new drugs. Reducing exposure to cyclophosphamide is one of the methods to refine current treatment. After a stable remission has been induced, azathioprine replaces cyclophosphamide to maintain this remission. This strategy has proven effective and less toxic than long-term treatment with cyclophosphamide (17). Individualizing therapy may be another method to refine current therapy. As 50% of patients experience a relapse within 5 years, part of the patients are overtreated and part are probably undertreated. Identifying those at risk for a relapse, for instance those who are PR3-ANCA positive (reviewed in (18)), would probably reduce damage from recurrent disease activity and exposure to toxic drugs. Last, assessing the ability of the patient to metabolize the drugs used may be a different method to individualize and refine therapy. Introduction of new and less toxic drugs is another way to avoid exposure to drugs that lead to substantial morbidity.

The outline of this thesis will be discussed below, in more detail.

**ANCA-associated vasculitis and HLA antigens**

The development of AAV seems to be controlled by both genetic and environmental factors. One of the genetic factors may be the human leukocyte antigen (HLA) system. The HLA system plays a key role in the distinction between self and non-self and is involved in antigen presentation. It is also possible that other immune-modulating genes, like those encoding for TNF-α and C4A, located near the HLA genes on chromosome 6, are involved in the etiology of AAV.

The relationship between HLA antigens and AAV has been investigated in several small studies, which report conflicting data (19-42). The associations found are not consistent, which can mainly be explained by small sample size and differences in patient selection. Whether a relation between HLA antigens and the course of AAV exists, is currently not known. In other autoimmune diseases, like systemic lupus erythematosus (SLE), carriers of DR3 and DR4 are at lower risk of developing nephritis, in contrast to carriers of DR2 antigens (43). Identifying patients at risk for relapses or more serious disease before start of treatment will provide tools to tailor the treatment of these patients.

In chapter 2, we investigated the association of HLA-antigens with AAV, comparing HLA antigens of a large cohort of patients with AAV with healthy blood donors. We also investigated the possible relationship between HLA-antigens and the presentation and course of AAV.
Influenza vaccination

Environmental factors, like vaccinations and occurrence of infections, are believed to play a role in the development of AAV. Several mechanisms have been proposed to explain the induction of autoimmunity following vaccinations, such as molecular mimicry, polyclonal activation and bystander activation (44-46).

Patients with autoimmune diseases like AAV are advised to be vaccinated against influenza because this viral infection is a major concern in patients with AAV. Infections in general, lead to hospitalization in 26-46% and mortality in 3% (14;15), though the exact contribution of influenza to these infections is unknown. Furthermore, influenza infections can possibly increase disease activity of autoimmune diseases, like multiple sclerosis and rheumatoid arthritis (47;48). On the other hand, vaccination against influenza may induce AAV, as described in case reports or small series (reviewed in (49;50)). In the Netherlands as well, vasculitis was reported after vaccination against influenza (51). Also, in patients already known to have AAV, relapse of disease following vaccination against influenza has been reported (52). However, most reported associations between autoimmunity and vaccination were not confirmed in larger studies (49;53;54).

No studies have so far been performed that evaluate the safety of vaccination against influenza, with respect to the occurrence of relapses, in patients with AAV. In chapter 3, we investigated the relation between vaccination against influenza and the occurrence of relapses in patients with AAV.

TPMT genotype and TPMT activity

One of the possible opportunities to refine the treatment of AAV may be to determine the genotype and/or activity of the enzyme thiopurine methyltransferase (TPMT). This TPMT enzyme influences the metabolism of azathioprine, a drug that is used as maintenance treatment after remission has been induced by cyclophosphamide. Azathioprine is a prodrug that is metabolized to the active immunosuppressant 6-mercaptopurine, which in turn is metabolized to active and inactive metabolites (55). 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. Approximately 89% of the individuals in Caucasians are homozygous for the normal or high TPMT activity allele, whereas 11% of Caucasians are heterozygous for one of four possible variant alleles, resulting in intermediate TPMT activity. Few individuals (0.3%) are homozygous for variant alleles, associated with absent TPMT activity. Use of azathioprine in patients who are homozygous for normal alleles may lead to reduced immunosuppressive action of 6-mercaptopurine, and to increased risk of hepatotoxicity. Intermediate TPMT activity, associated with heterozygous alleles, increases the risk of myelosuppression. In the rare patient with homozygous variant alleles severe myelosuppression develops (56).
Whether variant TPMT genotypes and activity are clinically relevant in patients with AAV has not yet been investigated. In other diseases, like SLE and inflammatory bowel disease (IBD) contradictory results with regard to the relation of TPMT activity and both efficacy (57-60) and toxicity (57,60-71) of azathioprine have been reported.

In chapter 4, the clinical relevance of TPMT genotype and activity in patients with AAV will be described. We expected relapses to occur less and leukocytopenia more frequently with lower TPMT activity and more hepatotoxicity in patients with high TPMT activity.

Mycophenolic acid

Mycophenolic acid (MPA) is a relatively new immunosuppressive drug that is used for the prevention of rejection in kidney transplantation since the 1990s. MPA has not only proved effective in preventing rejection, but also seems to cause less adverse effects than other immunosuppressive drugs (72-75). Given its’ favourable profile, MPA has also been used in autoimmune diseases like AAV.

MPA is the active metabolite of mycophenolate mofetil (MMF, CellCept) and the slow release formulation enteric-coated mycophenolate sodium (EC-MPS, Myfortic). MPA is a non-alkylating drug that suppresses the immune response by inhibiting proliferation of activated lymphocytes. MPA blocks the enzyme inosine monophosphate dehydrogenase (IMPDH), which is essential for the de novo synthesis of the purine guanine, and thereby inhibits lymphocytes to proliferate (76-81). While lymphocytes completely depend on the enzyme IMPDH for synthesis of guanine, most other human cells use other pathways for this synthesis, and are therefore, not or less affected by MPA. In addition, MPA has antifibrotic effects (82).

As many renal diseases are immunologically-mediated diseases, MPA might be beneficiary in these diseases. In chapter 5, the value of MPA for the treatment of non-transplant renal diseases is reviewed.

Currently, only little experience with MMF for the treatment of AAV is available. A total of 46 patients with active AAV receiving induction treatment with MMF have been reported in 2 uncontrolled studies and 2 small case series (83-86). The majority of patients (82%) responded well to MMF, but the relapse rate was high (56% after 2 years). Long-term experience regarding the toxicity of MMF, usually in combination with calcineurin inhibitors and steroids, is available in transplantation medicine, which suggests that treatment with MMF is safe with prolonged follow-up (87).

In chapter 6, our experience with MMF as remission induction therapy in patients with AAV who could not be treated with cyclophosphamide, is described.
Venous thromboembolism

Even with a short period of inflammation, like urinary tract or airway infection, the risk of venous thromboembolism (VTE) appears increased (88). In autoimmune diseases with chronic inflammation, like SLE (89) and IBD (90), an elevated risk of VTE has been found as well. Though deep venous thrombosis (DVT) and pulmonary embolism (PE) have been described in AAV patients (91;92), the incidence of VTE in these patients has not been studied until recently. In a prospective study, Merkel et al found an increased incidence of 7.0/100 person years of VTE in WG patients (93), which was confirmed in a retrospective study that found an incidence of 4.3/100 person years (94). For comparison, in healthy men the incidence is 0.3/100 person years (95). The cause of this increased incidence of VTE in AAV patients is unclear. However, in both studies, VTEs occurred mainly during active disease.

In chapter 7, we investigated the incidence of VTE in our cohort of patients with AAV. Furthermore, we evaluated the influence of disease activity and the presence of classic risk factors for VTE on the occurrence of VTE.

In chapter 8, the results of the thesis are summarized and discussed. Future perspectives will be outlined.
References


