Consequences of disease and treatment in ANCA-associated vasculitis

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Chapter one

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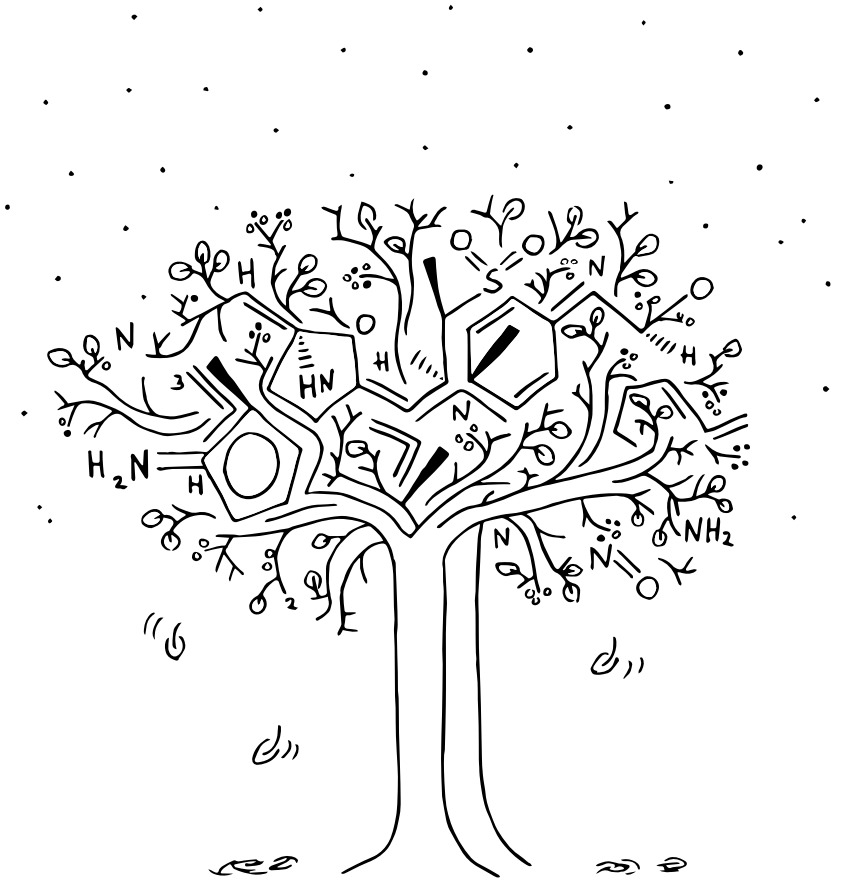

INTRODUCTION

Consequences of disease and treatment in ANCA-associated vasculitis

ANCA-associated vasculitides

ANCA-associated vasculitides (AAV) are a group of auto-immune diseases characterized by pauci-immune necrotizing inflammation of the small to medium-sized blood vessels [1]. In the majority of patients antineutrophil cytoplasmic antibodies (ANCA) are present. These antibodies are thought to play a role in the pathogenesis of these diseases [2]. AAV encompasses three clinical defined syndromes: granulomatosis with polyangiitis (GPA) (formerly known as Wegener's granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) [1]. Renal-limited vasculitis is often regarded a variant of MPA.

ANCA directed against proteinase-3 (PR3) or myeloperoxidase (MPO) can be found in over 90% of GPA and MPA patients [3]. PR3-ANCA are present in the majority of patients with GPA, however in some patients MPO-ANCA can be found as well. Conversely, half of the MPA patients present with PR3-ANCA and half with MPO-ANCA [4]. EGPA patients are often ANCA negative, but if positive mostly directed against MPO[5]. Considering the distinct clinic presentation, including the association with asthma, EGPA is not further discussed in this thesis.

Accumulating evidence suggests that patients with PR3-ANCA and MPO-ANCA differ with respect to genetic background, clinical presentation and outcome [4, 6, 7]. ANCA specificity has shown to correlate better with outcome compared to the clinical defined diagnoses. Therefore, PR3-ANCA-associated vasculitis and MPO-ANCA-associated vasculitis are increasingly regarded separate and defining entities [6-8].

Epidemiology and risk factors

ANCA-associated vasculitis can present at any age, but is mainly diagnosed after the fourth decade of life [9]. A slight male predominance is seen, especially in older and PR3-ANCA positive patients [10, 11]. AAV have an incidence of approximately 13-20 per million in Europe [12]. Geographical differences between the incidence of PR3 and MPO-AAV exist. While in Northwestern Europe the majority of patients are PR3-ANCA positive, in Southern Europe and Asia MPO-ANCA is more prevalent [13, 14]. For example, MPO-ANCA are detected in over 80% of Japanese patients [13].

These geographical differences might be explained by both genetic as well as environmental factors. Genome Wide Association Studies (GWAS) have shown genetic associations with the clinical subtypes of AAV, but even stronger associations with ANCA specificity. PR3-ANCA and MPO-ANCA were associated with different loci of the major-histocompatibility complex (MHC) and non-MHC loci [15]. Evidence also supports an environmental influence in the development of AAV. Several observations have fuelled the hypothesis that infections play a role in immune-system dysregulation and the development of AAV [16]. This is indirectly supported by studies showing a higher relapse rate in chronic S. aureus nasal carriers and a reduced incidence of relapses with trimethoprim/sulfamethoxazole therapy [17-19]. In addition, seasonal and geographical variations in the prevalence of disease activity are observed [12, 20].

Clinical presentation

Every small and medium-sized blood vessel can be involved in these diseases, giving rise to a multiplicity of potential clinical signs, symptoms and disease phenotypes, as seen in Figure 1. A phase of limited or very localised disease activity with non-specific complaints might proceed

overwhelming disease activity with life-threatening manifestations.
The upper and lower respiratory tract and kidneys are frequently involved in PR3-AAV. Ear, nose and throat involvement often presents as nasal crusting, nasal bleeding, hearing loss or hoarseness due to a subglottic stenosis. Lung involvement in MPO-ANCA is more often of fibrotic nature, whereas patients with PR3-ANCA more often present with nodular lesions and cavities. Alveolar bleeding, sometimes fulminant and life-threatening, can occur in both serotypes. This fibrotic propensity of MPO-ANCA-associated disease is also seen in kidney biopsies, and may explain the higher risk of

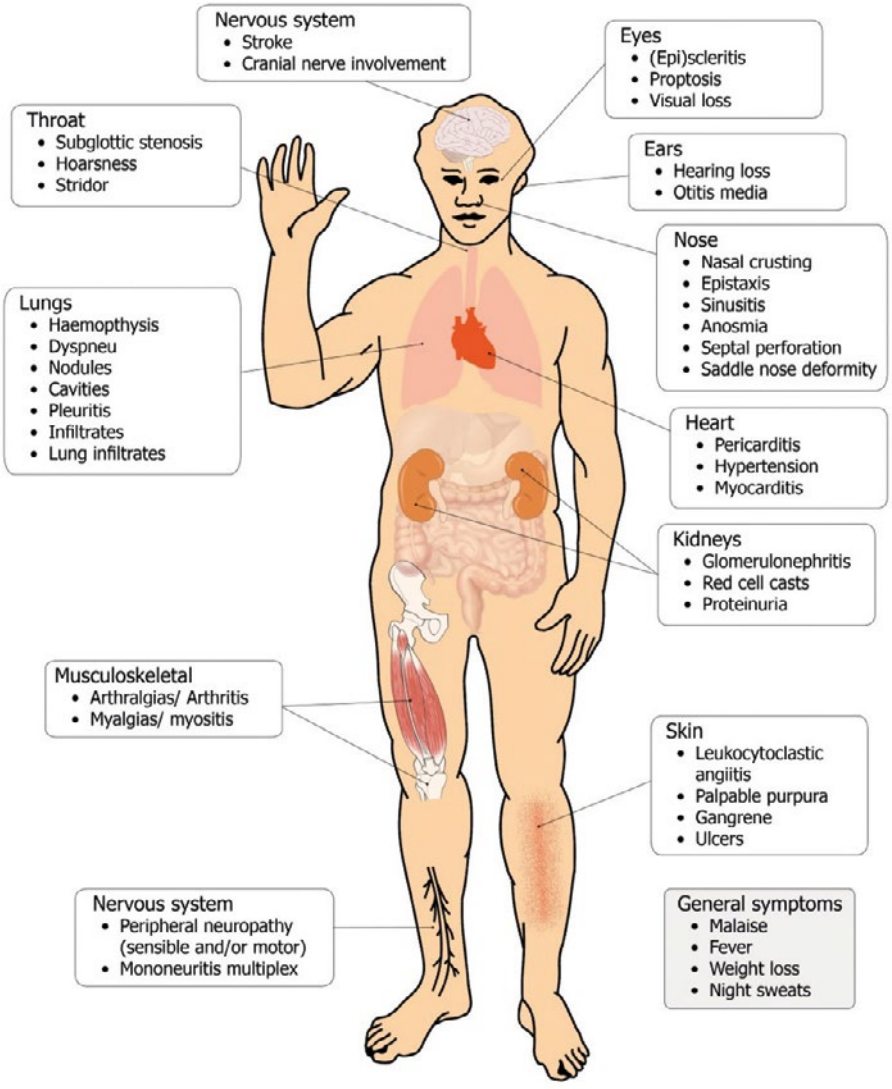

Figure 1. Possible symptoms of ANCA-associated vasculitis.

developing end-stage renal disease in MPO-ANCA positive patients. In addition, MPO-ANCA patients have worse renal function at presentation and this might also contribute to worse renal outcome [21]. Isolated necrotizing crescentic glomerulonephritis is more common in MPO-AAV compared to PR3-AAV, whereas more organ systems outside the kidneys are involved in PR3-AAV. These differences were already observed by Franssen et al. a decade ago and more recently discussed by Hilhorst et al. [4, 6].

Treatment

An effective treatment for granulomatosis with polyangiitis and microscopic polyangiits was not available until the 1960's, making these syndromes almost invariably fatal diseases up to then. Patients with untreated AAV have an average survival of only several months, and less than 20% survive the first year of disease. Glucocorticoid treatment improved survival, but still only an estimated 34% survived beyond the first year [22]. Reports showed beneficial effects of azathioprine during the 1960's, but the introduction of oral cyclophosphamide combined with high-dose glucocorticoids led to a substantial improvement in patient survival, nowadays up to 75% to 83% at five years [23-25]. Unfortunately, increases in survival came at the expense of serious acute and long-term side-effects of treatment. Cyclophosphamide can cause serious side-effects, such as malignancies, opportunistic infections, gonadal failure and bone marrow depression [3, 26]. These severe side-effects were the motivation for international scientific collaboration. Multiple large studies and trials were initiated in which alternatives for long-term oral cyclophosphamide have been studied and more recently alternatives and dosing protocols for glucocorticoids [27, 28].

Induction treatment

A major change in treatment was introduced after publication of the CYCAZAREM study in 2003 [29]. This study showed that after induction of remission, early switching to azathioprine maintenance therapy instead of continuation of cyclophosphamide, was not associated with an increased risk of relapse and adverse events. The long-term extension study observed a trend towards a higher relapse risk within the short-course cyclophosphamide group [30]. This is in concordance with previous observations [31, 32]. Reduction of cumulative cyclophosphamide exposure was also achieved by the use of pulse intravenous instead of oral cyclophosphamide. Unfortunately, the CYCLOPS study showed that lower cyclophosphamide exposure was associated with an increased risk of relapse during follow-up [33, 34].

Alternatives to cyclophosphamide were assessed with varying degrees of success. Studies reports conflicting outcomes of trimethoprim/sulfamethoxazole monotherapy for the induction of remission in localised AAV confined to the upper and lower respiratory tract. Whereas some studies show high remission rates ranging from 58-93%, others report high failure rates up to 73% [35-38]. The place of trimethoprim/sulfamethoxazole in the treatment of this group of patients has yet to be determined. Methotrexate compared to cyclophosphamide for induction of remission in early systemic disease showed similar remission rates at 6 months. Again, this was accompanied by a higher relapse rate during follow-up, necessitating subsequent treatments and longer duration to obtain remission [39]. Mycophenolate mofetil showed efficacy in several small case and cohort studies and in patients who could not be treated with cyclophosphamide [40-42]. Larger studies are needed to determine the role of mycophenolate mofetil in the induction treatment of AAV. In contrast, rituximab for the

induction of remission has shown to be equally successful as cyclophosphamide. In addition, no difference with respect to adverse events and relapse were reported in two large trials comparing rituximab and cyclophosphamide [43, 44]. These results are promising, although much has to be learned about this drug. The effect on B-cell depletion on the long-term, the relation with B-cell restitution and relapse and long-term safety are still unknown.
Overall, it has been difficult to replace cyclophosphamide for other at least as effective drugs with less side-effects. It also proved to be difficult to further lower the cumulative dose to less than the treatment of 4-6 months as described in the CYCAZAREM study. Treatment options besides cyclophosphamide would be a valuable addition to standard induction treatment of AAV.

Maintenance treatment

After the introduction of an induction and maintenance phase in the treatment of AAV several options for maintenance therapy have been studied and will be discussed briefly here. Early start of azathioprine was not associated with and increased risk of relapses and is now often used as the comparative drug [29]. Mycophenolate mofetil compared to azathioprine maintenance was associated with a higher relapse rate during a 42-month study and is not routinely used [45]. Methotrexate as another maintenance treatment option, was shown similarly effective compared to azathioprine, however, a trend towards more severe adverse events was observed in the methotrexate group. This suggest that methotrexate could be considered for maintenance of remission, although it cannot be used patients with severely impaired renal function. Although, trimethoprim/sulfamethoxazole has shown to reduce the incidence of relapses [18], it was shown inferior to methotrexate in the maintenance of remission and is therefore not recommended as a maintenance agent. Rituximab maintenance therapy is promising, but long-term safety, efficacy and cost-effectiveness have still to be addressed [46].
Currently, azathioprine is still the mainstay in maintenance therapy and rituximab, mycophenolate mofetil and methotrexate should be considered alternatives [47]. In the future, rituximab might become first-line, however stronger evidence has yet to come from larger trials.

Glucocorticoids

Glucocorticoids have been the cornerstone in treatment even before the introduction of cyclophosphamide[22]. For the induction of remission, high-dose glucocorticoids is started besides cyclophosphamide. After remission is achieved, glucocorticoids are tapered and withdrawn. There is a paucity of evidence to support a particular glucocorticoid regimen and the protocols used vary widely. Duration of therapy may be as long as 6 months up to 27 months [48]. A high inter-patient variation is observed with respect to tapering and many patients experience complaints during tapering or withdrawal. In addition, a substantial part of patients is not able to discontinue glucocorticoids [49]. Unclear is whether this failure to taper or withdraw the low-dose prednisolone is caused by insufficient control of the vasculitic disease or is more related to insufficient recovery of the hypothalamic-pituitary-adrenal (HPA) axis. Strikingly, despite the central role of glucocorticoids in the treatment of AAV, no longitudinal studies have investigated the effect of tapering on the recovery of the HPA axis.

Outcome of AAV

Survival

Survival after diagnosis of AAV significantly improved over the past decades [25]. Nowadays 5-year survival rates range from approximately 75% to 83% mostly depending on severity of renal involvement and age [24, 25, 50]. Deaths within the first year after diagnosis are primarily from infections (48%) and not the result of active vasculitis (19%). After the first year, cardiovascular disease (26%), malignancies (22%) and infections (20%) are the main causes of death [50].

Relapse

Cyclophosphamide in combination with glucocorticoids have changed AAV from fatal diseases into chronic and relapsing diseases. In contrast to better survival, the relapse rate does not seem to decrease over time [25]. Renewed disease activity exposes patients to more immunosuppressive therapy, accumulating damage both due to disease and treatment and uncertainty about the future. Relapse occurs within 5 year in 30- 50% of patients [51, 52]. Especially PR3 positive patients are at risk of relapse [51]. This has questioned the need for maintenance therapy in MPO-AAV, and also indicates that indefinite maintenance therapy would result in overtreatment of 50% of PR3 positive patients as well [47].

Damage

Increased survival comes at a price. Initial and relapsing disease leads to accumulating damage caused by both the disease and its treatment. In 1997 the Vasculitis Damage Index (VDI) was developed to score the amount of damage accrued after the diagnosis of vasculitis, irrespective of the causative factor [53]. It was shown that severe disease, defined as a Vasculitis Damage Index (VDI) of ≥5 was associated with a six-fold increase in mortality [54]. Analysis of several EUVAS trials showed that about a third of patients experienced severe damage within seven years after diagnosis, while only 8% of patients had no items of damage after long-term follow-up. Damage was primarily renal: eGFR<50 mL/min, proteinuria and hypertension. Nasal crusting, hearing loss and peripheral neuropathy were also frequently recorded. The most observed treatment-related items were hypertension (42%), osteoporosis (14%), malignancies (13%) and diabetes mellitus(10%) [55]. These findings are in line with other studies demonstrating development of ear- nose and throat damage and renal damage most frequent [3, 56]. The whole spectrum of damage shows great variety and this reflects the character of these systemic multi-organ diseases.

Treatment-related damage has mostly been linked to cyclophosphamide and this has been the motivation for various trials to replace or reduce the use of cyclophosphamide, as discussed previously. The first reports of bladder cancer after the use of cyclophosphamide go back as early as the 1960's. In the following years, it was reported that AAV patients were at increased risk of several malignancies. An overall 2.4-fold risk of malignancies and a 33-fold increased risk of bladder cancer was reported by Hoffman et al. [3]. More recently, long-term follow-up showed an increased risk of malignancies in a dose-dependent manner. A cumulative dose of less than 36 grams was not associated with a measurable increased risk of malignancies, except for non-melanoma skin cancer [57]. After the introduction of a short induction phase followed by a maintenance phase with less

toxic agents, dosages generally not exceed 36 grams.
Cyclophosphamide is an alkylating drug and causes cross-linking of DNA strands, which interferes with cell division. Not surprisingly, besides malignancies, cyclophosphamide in women is also associated with earlier menopause and its extreme form primary ovarian insufficiency [58, 59]. Limited data is available on the effect of orally administered cyclophosphamide on the onset of menopause or the incidence of primary ovarian insufficiency in women with AAV [60]. It is plausible that also here cyclophosphamide affects fertility in a dose-dependent manner. AAV are mainly diagnosed after the fourth decade of life, reports on pregnancy in AAV patients are therefore scarce. Reported outcome is often poor with a high rate of complications. In men, cyclophosphamide can also cause infertility and gonadal damage. However, the influence on testosterone production is far less pronounced [61]. In AAV, only one small study reported a high prevalence of androgen deficiency male patients, however, causality could not be inferred with this study [62].
Cyclophosphamide as a cause of damage has received much attention. Looking at the results of the EUVAS study, it becomes apparent that other agents, namely glucocorticoids, probably account for a high burden of induced damage and comorbidities. Hypertension, osteoporosis and diabetes mellitus are present in a high number of patients, as shown by the long-term follow-up of six EUVAS trials [55]. This is in line with the findings of the WGET study, in which 5-10% of patients had hypertension, diabetes mellitus, significant muscle atrophy or weakness, osteoporosis and cataract after a median follow-up of two years [56]. Considering the nature of the damage and comorbidities, it is likely that glucocorticoids played a role in their development. In addition, glucocorticoids contribute to the susceptibility to infections. This notion is supported by subgroup analysis of the EUVAS trials showing that increased cumulative glucocorticoid use was independently associated with higher levels of damage and having cataract and hypertension. Patients with longer duration of glucocorticoid treatment were more likely to have severe damage (VDI ≥5) which has been associated with a six-fold increase in mortality [49, 54]. Although causality could not be inferred, substantial evidence suggests the importance of reducing glucocorticoid treatment.
Finally, AAV patients are faced with an unpredictable course of disease with an uncertain prognosis. It is not surprising that AAV have been shown to impact emotional well-being and quality of life [63-65]. In addition, both physical and psychological impairments can influence role functioning and occupational and social participation [66, 67]. Recording damage will therefore not fully capture the burden of disease and the impact on patients' lives. Indeed, patients regard fatigue as the major burden of disease and severe organ damage was ranked substantially lower [68]. We might question whether fatigue and low levels of quality of life are not forms of damage as well.

AIMS OF THIS THESIS

Advances in treatment of AAV has led to significant improvements in survival of AAV patients. Nowadays, the outlook of patients is increasingly determined by the development of comorbidities, damage and relapses. It has been shown that AAV patients experience high levels of damage and health-related quality of life is substantially impaired. In this thesis the impact of disease and treatment are being investigated to guide future treatment strategies.
Part 1 focuses on the consequences of disease and treatment on the reproductive system and gonadal function in men and women. The effect of therapy, especially cyclophosphamide, on

fertility is a major concern for woman in their reproductive age, warranting further investigation. In **Chapter 2** we evaluate the outcome of pregnancies in women diagnosed with AAV. Earlier menopause and more severely, primary ovarian insufficiency does not only affect reproduction but also has considerable implications for bone mineral density, general and sexual well-being, and cardiovascular health. **Chapter 3** investigates the influence of AAV therapy on the onset of menopause. **Chapter 4** investigates the occurrence of androgen deficiency in male patients with AAV. In addition, the role of testosterone in fatigue and impaired quality of life is being examined.

Part 2 focuses on alternative treatment options for subgroups of patients to individualize and thereby optimize treatment. Tailoring treatment prevents over- and undertreatment and presumably may prevent damage. In **Chapter 5** we discuss the challenges of tapering of glucocorticoids and argue for an individualized treatment which takes glucocorticoid sensitivity into account. To expand on the hypothesis that a high inter-individual variation exists and the inability to withdraw glucocorticoids might be attributable to a delayed recovery of the hypothalamic-pituitary-adrenal axis, **Chapter 6** describes the study design and rationale of the CURVE study. This prospective, longitudinal observational study investigates the recovery of the hypothalamic-pituitary-adrenal axis and its relation with commonly expressed complaints. One treatment does not fit all, and alternatives or additional therapeutic options are needed. **Chapter 7** explores the possibility to use trimethoprim/ sulfamethoxazole for the induction of remission in localised and early systemic disease, thereby completely avoiding the toxicities if treatment with glucocorticoids, methotrexate or cyclophosphamide. **Chapter 8** investigates the efficacy and safety of mycophenolate mofetil compared to oral cyclophosphamide for the treatment of non-severe relapses in ANCA positive patients.

Chapter 9 summarizes and discusses the main findings of the previous chapters in the context of the current literature. Implications for future treatment and research are discussed.

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Part 1

Consequences of ANCA-associated vasculitis