Consequences of disease and treatment in ANCA-associated vasculitis

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Chapter nine

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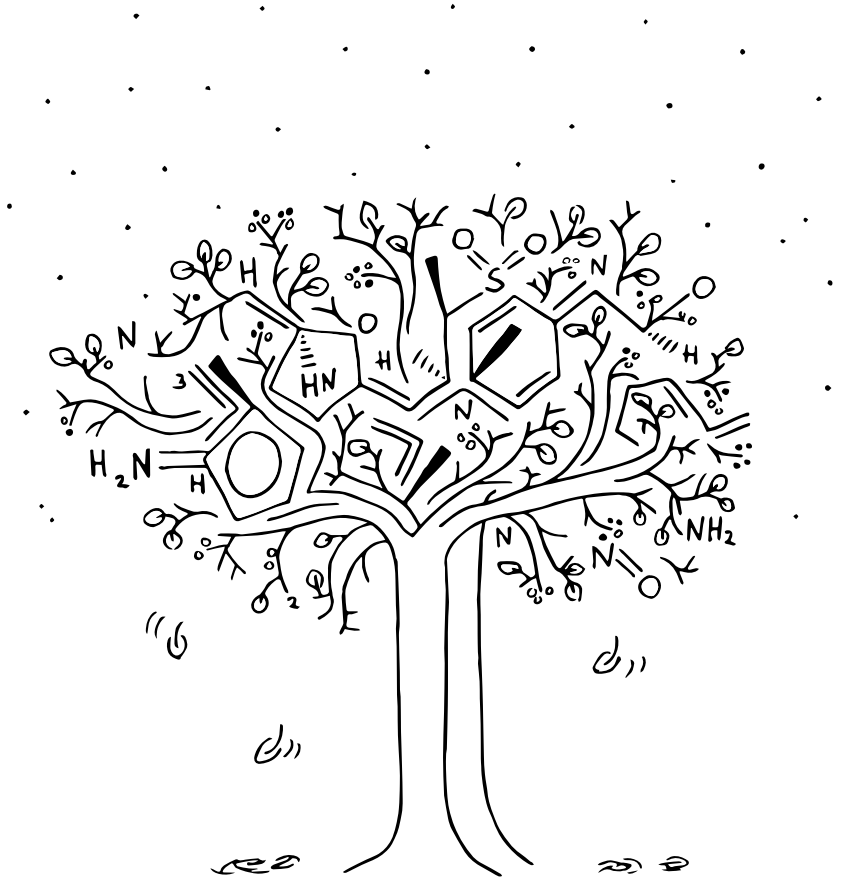

Summary, general discussion, clinical implications and future perspectives

Summary

The outlook of ANCA-associated vasculitis patients has substantially improved over the last decades. Whereas AAV were first fatal and patients were faced with a severely reduced life span, the 5-year survival rate is currently around 80% [1]. This is mainly the result of the introduction of cyclophosphamide in addition to glucocorticoids. Despite their fundamental contribution to increased survival, both drugs are associated with severe short-term and long-term side-effects. Only very few patients will not experience any consequences of their disease and its treatment [2, 3]. Optimizing treatment to reduce short and long-term damage associated with both disease and treatment is essential.

In **chapter 1** ANCA-associated vasculitis is introduced and the important issues in the care of AAV patients is discussed. The work in this thesis is focused on two issues regarding ANCA-associated vasculitis. In **Part 1**, the impact of AAV on reproduction and gonadal function are investigated. In order to prevent damage, several treatment options to individualize and thereby optimize treatment are studied in **Part 2**.

Part 1: Consequences of AAV

AAV is primarily diagnosed at older age (mainly beyond the 4th decade) and therefore only a limited number of pregnancies in AAV are reported. Many of them were associated with complications and poor outcome. In **Chapter 2** we report successful outcome for both the mother and the child in 22 pregnancies in 14 women diagnosed with AAV, to our knowledge the largest cohort reported in literature [4]. All pregnancies resulted in liveborn infants of which 90% in good health. Only 2 pregnancies ended in preterm delivery. Few complications during pregnancies were observed. We conclude that in female patients with AAV, favourable pregnancy outcome might be observed after counselling to conceive after at least 1 year of stable remission and no cyclophosphamide exposure within 1 year prior to conception.

In **Chapter 3** we studied menopause and primary ovarian insufficiency in women diagnosed with AAV [5]. We show that cyclophosphamide is related to earlier menopause and primary ovarian insufficiency in a dose- and age-dependent manner. Current treatment protocols reduce, but by no means eliminate, ovarian toxicity. When cyclophosphamide is inevitable, cyclophosphamide might be used to a cumulative dose of 16 gm., since our data show that this dose might still give women a 'window of opportunity' if a child is desired. Interestingly, both women treated with and without cyclophosphamide experienced involuntary childlessness. In women without cyclophosphamide exposure, their burden of disease was the main reason not to conceive. This study emphasizes the need for non-gonadal toxic treatment in women of childbearing age, but does also clearly underlines the need for better disease control, since involuntary childlessness occurred irrespective of treatment.

In male patients with AAV, a concerning lack of knowledge exist with respect to gonadal function. In **Chapter 4** we show that almost half of the male patients with AAV are to be considered androgen deficient. Data suggests that a higher disease burden, for example higher cumulative glucocorticoid dose, is inversely associated with the level of testosterone. Physical function was related to testosterone, especially in older patients. Fatigue was associated with testosterone, age, C-reactive protein (CRP) and the Vasculitis Damage Index. Treating deficient patients with testosterone might

improve fatigue and health-related quality of life in some patients with symptomatic androgen deficiency. Not screening for androgen deficiency, is a missed opportunity to treat a reversible contributing factor to impaired health-related quality of life and fatigue.

Part 2: Individualized treatment for AAV

In **Chapter 5** we discuss the challenges with respect to glucocorticoid withdrawal. Clinical experience shows that a considerable number of patients do not tolerate tapering well and a considerable part of the patients included in trials with a zero glucocorticoid target fail to reach this end-point, and therefore remain glucocorticoid-dependent. We hypothesize that in failure to withdraw glucocorticoids, the hypothalamic-pituitary-adrenal (HPA) axis plays a role. The high inter-patient variability with respect to tapering and its unpredictable nature might be explained by glucocorticoid sensitivity, which embodies a complex interaction between glucocorticoid regulation on many cellular and tissue levels. It is conceivable that both the development of side-effects as well as therapy efficacy are influenced by glucocorticoid sensitivity. This might result in over- and undertreatment of sensitive and resistant patients, respectively. Clearly, in glucocorticoid therapy one size does not fit all. We therefore advocate that future glucocorticoid therapy should be tailored to individual glucocorticoid sensitivity.

To further investigate HPA-axis function during glucocorticoid therapy, we present our study design and rationale for the CURVE study in **Chapter 6**: 'Recovery of the hypothalamic-pituitary-adrenal axis during glucocorticoid treatment after the induction of remission in ANCA-associated vasculitis. Through saliva collection and cortisol measurement we will study HPA-axis recovery. We hypothesize that impaired recovery is associated with commonly expressed complaints by patients. Patient recruitment is currently ongoing.

In **Chapter 7** we demonstrate the effectiveness and safety of trimethoprim/sulfamethoxazole therapy for the induction of remission in localised disease. We show that trimethoprim/sulfamethoxazole can induce remission in about two-third of patients and that CRP was a prognostic factor for therapy success. Eighty-nine per cent of patients with CRP <12.5 mg/L at start of therapy attained remission. We therefore concluded that trimethoprim/sulfamethoxazole can be considered as first-line therapy in localised disease if at presentation CRP is low.

In **Chapter 8** the efficacy and safety of mycophonlate mofetil compared to cyclophosphamide is investigated in ANCA-positive patients presenting with a nonlife-threatening relapse. In the mycophenolate mofetil group a lower remission rate at six months was observed, though this difference did not reach statistical significance. There was a significant interaction between treatment and disease severity at study entry on successful remission induction. Patients treated with mycophonlate mofetil and high disease severity were less likely to attain remission compared to patients who received cyclophosphamide. Efficacy of cyclophosphamide appeared to be unrelated of disease severity at study entry. After remission was attained, a similar number of relapses occurred and at the end of 4 years of follow-up a similar percentage of patients was in remission. With this study, we showed that mycophonlate mofetil is a viable alternative for cyclophosphamide in relapses with mild to moderate disease severity.

General discussion, clinical implications and future perspectives

Consequences of AAV

AAV have changed from fatal diseases into chronic and relapsing diseases. Unfortunately, survival came at the expense of short- and long-term damage caused by both the treatment and the disease itself. Damage can manifest in every organ and present in the form of impaired quality of life or psychological distress. Not frequently occurring, but of major impact on patients' lives, is the effect of disease and treatment on reproductive health. Firstly, the treatment can affect fertility and subsequently cause primary ovarian insufficiency. Secondly, both active disease as well as its treatment are highly undesirable during pregnancy.

Cyclophosphamide treatment is associated with early onset of menopause and the occurrence of primary ovarian insufficiency (**Chapter 3**). The results of our cohort study confirmed previous studies by showing that cyclophosphamide influenced the onset of menopause in an age- and dose-dependent manner. No increased risk of primary ovarian insufficiency was observed in cyclophosphamide naïve patients. Contrary to what is expected, also women without previous cyclophosphamide exposure were reported to be involuntary childless. The burden of disease was the main reason not to conceive. This clearly highlights the need for better disease control and the prevention of damage. Gonadal preservation, like oocytes cryopreservation, might be considered in young woman who desire a child in the future [6]. However, when prompt therapy is warranted in severe disease, these techniques might take too much time or are not possible due to a poor clinical condition. As mentioned before, women can be involuntary childless irrespective of treatment. Therefore, improving reproductive potential should preferably be attempted by improving disease control with non-gonadotoxic therapy.

In addition to mycophenolate mofetil, which may form a viable alternative to cyclophosphamide (see **Chapter 8**), rituximab could be considered as well. This monoclonal antibody directed against B-cell surface antigen CD20, is an attractive alternative to cyclophosphamide, since it does not affect ovarian reserve and has therefore not been associated with earlier menopause. Importantly, we must keep in mind that on the short-term rituximab was not inferior to cyclophosphamide, but long-term experience is scarce [7-9]. Still, rituximab might be considered first-choice in women of childbearing age due to the potential benefits in this patient population.

Pregnancies in women diagnosed with AAV can have favourable outcome for both the mother and the child (**Chapter 2**). With careful management, few complications were observed during and after pregnancy and the vast majority of newborns were in excellent health at birth and follow-up. Our findings are supported by a recent study, that showed excellent outcome of pregnancy in women with stable remission as well [10]. In contrast to our study, they found a lower relapse rate after delivery. This might be attributable to the higher number of women on maintenance therapy in their cohort compared to the low number of women on any medication during pregnancy in our cohort. It remains uncertain whether the postpartum period should be regarded a high-risk period for relapse. Considering the rarity of events, larger studies conducted within international collaboration are needed. Based on the current evidence, initiating therapy for relapse reduction post-partum is not indicated, but careful follow-up after delivery is desirable.

In contrast to these findings, two recent studies reported higher rates of preterm delivery [11, 12]. In one study this was driven by medically indicated preterm delivery and in the other study it was self-

reported. Unfortunately, both studies lack further specification and the exact medical indication is unknown. Chen and colleagues also reported a significant higher neonatal and maternal morbidity. Both cohorts included patients diagnosed with several forms of systemic vasculitis. With regard to pregnancy, systemic vasculitides carry individual risks related to the disease and are therefore not fully interchangeable [13]. For example, in Takayasu's arteritis hypertension, pre-eclampsia and intra-uterine growth restriction are frequently observed, in which the nature of disease is thought to play a role. Both Behçet's disease as well as AAV are primarily associated with venous thrombosis, whereas large vessel vasculitis is associated with arterial thrombosis and the subsequent occurrence of transient ischaemic attacks and stroke [14, 15]. Preferably, analysis of pregnancies should be disease specific.

Rituximab is not associated with gonadal toxicity. In addition, it appears not to be teratogenic or embryotoxic in contrast to cyclophosphamide and mycophenolate mofetil [16, 17]. This makes rituximab an attractive alternative in women of childbearing age. Rituximab use prior to conception did not seem to be related to adverse pregnancy outcome or B-cell depletion in the newborn in a small series of women with AAV [18]. Data from the rituximab global safety database showed that pregnancy after rituximab treatment resulted in live births in the majority of cases and the rate of pre-term delivery was only moderately increased (19%) [16]. Rituximab was indicated for severe diseases including haematological malignancies and results might thereby be confounded by treatment indication and concomitant medication.

IgG antibodies, like rituximab, can pass the placental barrier[19]. Rituximab use during established pregnancy resulted in higher rituximab levels in the newborn compared to the mother in several studies [16]. This can result in fetal B-cell depletion and cytopenias were present in 7 out of 11 newborns documented in the global safety database. None of the cytopenias was associated with infection, but immunosuppression and subsequent infection in the newborn remain a serious concern. Complete B-cell reconstitution can take up to 6 months after delivery [20]. Pregnancy is therefore still discouraged until 12 months after the last administration of rituximab. The effect of rituximab exposure preconception and antepartum on pregnancy and neonatal outcome needs further evaluation.

When a relapse develops azathioprine and glucocorticoids can be used and even cyclophosphamide could be used in the second to third trimester as last resort. Current literature is limited on rituximab during established pregnancy, but shows good neonatal outcome even in the presence of cytopenias. However, the risk of immunosuppression and infection in the newborn should be weighed against the benefits of treatment for the mother. Individualized treatment is necessary in these rare cases.

Cyclophosphamide affects male fertility in a dose-dependent manner as well [21, 22]. Azoospermia, as a result of damage to the germinal epithelium, is reported in the majority, if not all, men during oral cyclophosphamide treatment. This can persist after discontinuation of therapy, resulting in infertility [23, 24]. Cryostorage of semen is the easiest approach in men who wish to preserve fertility an should always be offered. Other methods, like gonadal tissue cryopreservation are being developed [24]. Leydig cell impairment can occur after treatment with cyclophosphamide, but these cells appear more resistant than the germinal epithelium. Testosterone levels are normal in many patients undergoing cyclophosphamide treatment, and histological evidence does not show Leydig

cell toxicity [21]. Higher doses can result in increased levels of luteinizing hormone, suggesting a compensation for (partial) cell dysfunction [24].
In AAV, the occurrence of male hypogonadism or infertility is rarely reported. One small series reported a high prevalence of hypogonadism, but associations with complaints were not assessed [25]. We confirmed this finding, by showing that almost half of the male patients in our study is to be considered androgen deficient (**Chapter 4**). Testosterone levels were associated with, amongst others, the cumulative glucocorticoid dose prior to this study. High- dose glucocorticoids are known to suppress the hypothalamic-pituitary-gonadal axis and our results could be suggestive for a more persistent down regulation in part of the patients in remission [26].
We further showed that testosterone levels were associated with fatigue and health-related quality of life. Rationally, impaired health-related quality of life and fatigue cannot be fully explained by reduced testosterone levels. Over the last years, studies have clearly shown that fatigue and quality of life are determined by multiple bio-psychosocial factors [27, 28]. Interestingly within quality of life, fatigue appears one of the most important contributing factors [29]. Androgen deficiency could therefore be one of the main contributing factors to impaired health-related quality of life and fatigue. Subsequently, testosterone replacement therapy might be indicated in some AAV male patients. Although recent concerns about an increased risk of cardiovascular events during testosterone replacement therapy might warrant cautious, testosterone itself has been inversely associated with cardiovascular risks in large prospective cohort studies [30-32]. The study of Basaria, that showed an increased cardiovascular risk during testosterone replacement therapy, had some methodological concerns and the severity of androgen deficiency was questionable. Subsequent treatment resulted in high-normal to supraphysiological testosterone levels. This is not in line with the recommendations of the Endocrine Society Guideline [33].
A multi-factorial problem demands a multi-factorial approach. Psycho-social interventions and physical therapy could be considered besides optimal control of disease. However, our study showed that not screening for androgen deficiency could be a missed opportunity to treat a contributing factor. Hence, prospective intervention studies investigating the effect and safety of testosterone replacement therapy are warranted.

Individualized treatment of AAV

Key to (future) management in vasculitis patients is improving disease control with agents exerting limited toxicity and minimum side-effects. This will minimize the development of damage, comorbidities and may reduce the impact of disease and treatment on patients' lives.
As already discussed in the introduction, many attempts to replace cyclophosphamide or reduce the cumulative dose have been undertaken [7, 8, 34-38]. However, cyclophosphamide has shown to be one of the most potent drugs in the treatment of AAV. The main motivation for replacing cyclophosphamide was its association with serious long-term side-effects as malignancies, gonadal failure and bone marrow depression [39-42]. However, several reports including our own study on the onset of menopause showed that the majority of side-effects are (cumulative) dose-dependent. The shortening of standard induction regimes have led to a reduction in the incidence of primary ovarian insufficiency (**Chapter 3**). In addition, cumulative cyclophosphamide dosages up to 36 grams were not associated with an increased risk of malignancies, except for non-melanoma skin

cancer [5, 39, 40]. We might wonder whether replacement of cyclophosphamide should be the ultimate goal or finding additional treatment options and tailoring therapy?

Two potential treatment options were studied in this thesis. Trimethoprim/sulfamethoxazole was investigated as induction treatment in patients with localised disease (**Chapter 7**). Mycophenolate mofetil was studied as induction treatment in patients with a non-life-threatening relapse of AAV (**Chapter 8**). Both treatment options have shown efficacy in small studies and case series, but larger studies are needed to explore their role in the treatment of AAV [38, 43-50].

Trimethoprim/sulfamethoxazole, a double-drug folate antagonist, was shown to be effective in a substantial part of patients diagnosed with localised disease (**Chapter 7**). Therapy effectiveness was highest among patients with a low CRP, reflecting low systemic inflammation. Trimethoprim/sulfamethoxazole could therefore be considered first-line therapy in these patients with very limited disease.

The observations that concomitant treatment with trimethoprim/sulfamethoxazole reduces the incidence of relapses, also supports an effect of this agent on the course of disease [51]. A reduction of the incidence of infections might be one of the mechanisms of action of this antibiotic agent. Several studies have linked infections to autoimmunity, including studies in AAV [52-54]. In addition, it has been shown that *Staphylococcus aureus* carriers have an increased risk of relapse [55]. Assuming that infections play a role in triggering the onset of disease or disease relapses, it would be interesting to study whether other antibiotics, for example nasal mupirocin, would show similar results. Besides the infection hypothesis, studies have shown that trimethoprim/sulfamethoxazole exerts anti-inflammatory effects, which might attenuate or dampen inflammation [56-58]. The exact mechanism of action of this drug in AAV remains to be elucidated and larger comparative, prospective trials should be conducted to establish the role of trimethoprim/sulfamethoxazole induction therapy in localised AAV.

Mycophenolate mofetil was studied for the induction of remission in non-severe relapses in ANCA-positive patients (**Chapter 8**). Mycophenolate mofetil induced remission in a substantial part of the patients, but was inferior to oral cyclophosphamide in case of severe disease. After remission was achieved, relapses developed in a similar proportion of patients of both groups. At 4 years of follow-up a similar percentages of patients was in remission. One could opt to only treat patients with mycophenolate mofetil, when initial disease is mild or moderate (i.e. BVAS<19). Based on these findings, it is tempting to speculate that this approach could improve treatment outcome.

At the same time as our study, mycophenolate mofetil was studied for the induction of remission in newly diagnosed patients. Results have only been presented in abstract form, but the remission rate with mycophenolate mofetil was similar compared to our study [59].

The rationale for tailoring glucocorticoid therapy has extensively been discussed in **chapter 5** of this thesis. In short, glucocorticoids can cause severe short- and long-term toxicity and contribute the high levels of damage observed in AAV patients. Despite this association, many patients remain glucocorticoid-dependant for years for unclear reasons [60].

During tapering a high inter-patient variability with respect to tapering is observed. Whereas some patients tolerate tapering well, others do not and experience various non-specific complaints. These tend to resolve quickly after increasing the glucocorticoid dose. Whether this could be attributed to disease activity or other causes should be investigated. We hypothesized that a delayed recovery

of the hypothalamic-pituitary-adrenal axis could be related to the occurrence of these complaints. We further hypothesized that the high inter-patient variability with respect to tapering and the occurrence of side-effects could be explained by differences in glucocorticoid sensitivity. Glucocorticoid availability is regulated on many cellular and tissue levels and small variations in the regulation might eventually result in larger effects in the end [61]. Several genetic polymorphisms have been demonstrated to predispose patients to a more sensitive or resistant glucocorticoid profile. Already, clinical outcomes have been associated with polymorphisms encoding for the glucocorticoid receptor or enzymes regulating glucocorticoid availability at tissue levels [62-65]. With increasing use of genetic analyses, a composite of individual sensitivity might be incorporated in clinical practice in future. Whether this approach would lead to less side-effects and higher treatment efficacy remains to be addressed. But is tempting to speculate that individualized treatment might improve both efficacy and safety.

Currently, the PEXIVAS trial aims to investigate whether a reduced dose glucocorticoid regimen could be safely used to prevent side-effects, including infections without undermining treatment efficacy [66]. This large randomized controlled trial will presumably add to the current discussion on glucocorticoids. Whether or not on population level outcomes may improve, a future fine-tuning based on sensitivity would be physiologically most sound.

We might wonder, are there any new agents on the horizon? Amongst others, the complement C5a inhibitor CCX168 is being studied as an agent to replace or reduce the dose of glucocorticoids as concomitant therapy in patients with renal disease (NCT02222155) [67]. CCX168 prevents the expression of adhesion molecules and migration of neutrophils and other immune competent cells by chemotaxis. Preliminary results of a small phase 2 trial showed that CCX168 appeared to be at least as effective, if not, more effective than glucocorticoids alone [68]. Larger trials are currently underway.

In line with our search for less toxic treatment in non-severe relapsing disease, others report promising results with the use of abatacept (CTLA4-Ig) in a small open-label study [69]. A high number of patients achieved remission and prednisolone could be withdrawn in a substantial part of the patients. Currently, a randomized trial is being conducted to compare abatacept with methotrexate in this patient population (NCT02108860).

Conclusion

ANCA-associated vasculitides have changed from fatal diseases into chronic, relapsing conditions. Nowadays, the outlook of patients is mainly determined by the development of relapses, comorbidities and damage. This thesis clearly demonstrated the consequences of both disease and treatment, and underlines the need for less toxic agents and better disease control. Expanding treatment options is of major importance, but will not suffice. Tailoring of therapy on individual sensitivity, disease and patient characteristics should ultimately result in improved efficacy, less disease and treatment-related damage, and improved quality of life. In ANCA-associated vasculitis, one size does not fit all.

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