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ANCA-associated vasculitis : towards patient-tailored therapy

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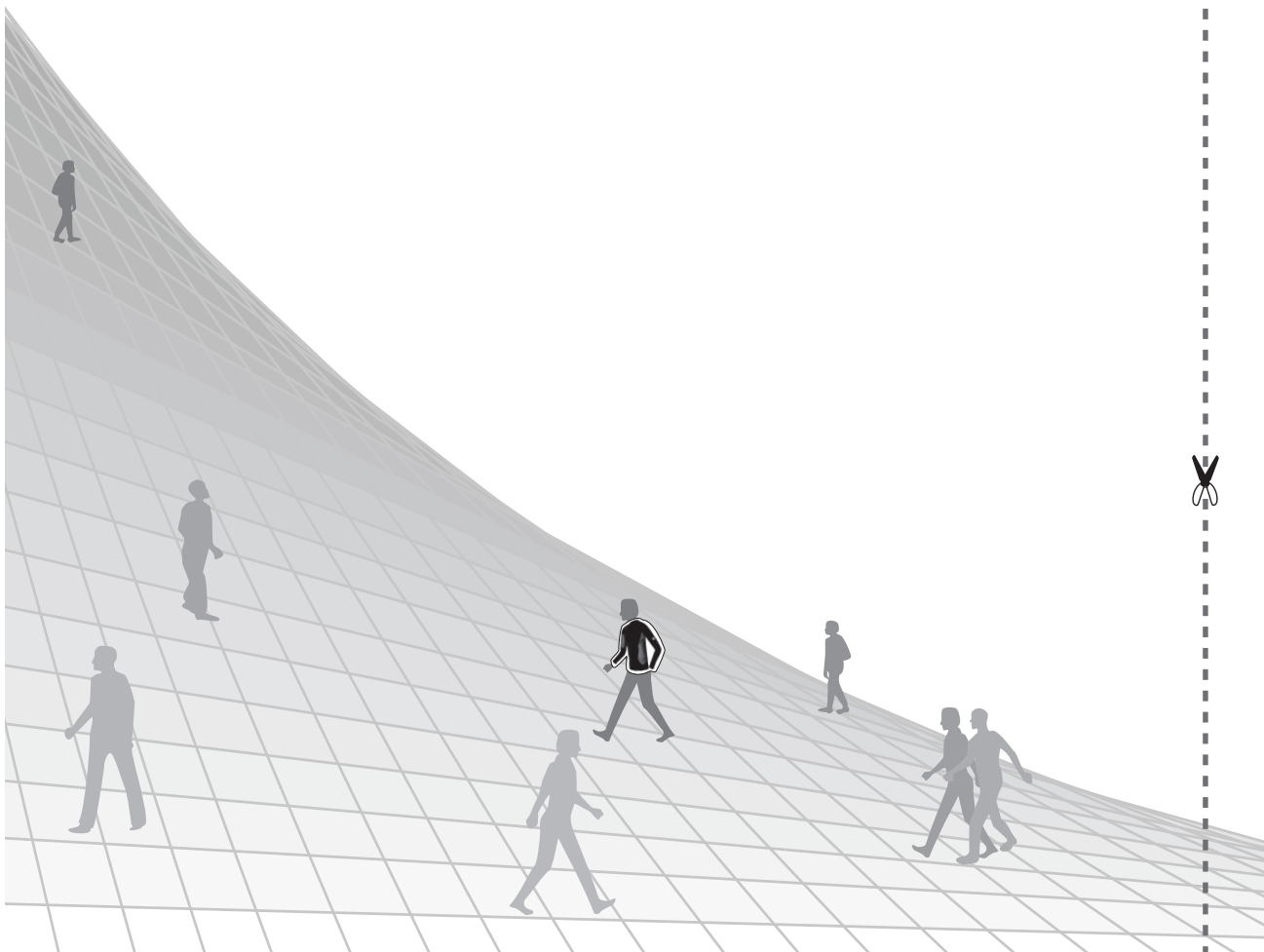
8

SUMMARY AND GENERAL DISCUSSION



SUMMARY AND GENERAL DISCUSSION

Research into the vasculitides has been going on for over a 100 years. The pioneers in the field, among them Kussmaul, Maier and Wegener,[1;2] set out to carefully describe the pathology and clinical course of the vasculitic diseases. For long, these were mostly fatal illnesses, one year survival without treatment being approximately 20%.[3] Fortunately, one year survival increased to over 90% with the introduction of steroids and, particularly, cyclophosphamide as the standard treatment regimen.[4] However, the serious adverse events associated with these immunosuppressants were soon recognized and relapse rates appeared to be high. With the advent of international multicenter randomized therapeutic trials,[5-8] effective and safer therapy regimens were established. The NORAM trial demonstrated that methotrexate was as effective as cyclophosphamide in remission induction in limited Wegener's granulomatosis. However, relapse rates were reported to be higher with methotrexate.[5] The CYCAZAREM trial demonstrated that azathioprine is a good substitute for cyclophosphamide as remission maintenance therapy: azathioprine was as effective in maintaining remission but was associated with less serious adverse events than cyclophosphamide.[7] In severe renal ANCA-associated vasculitis (AAV), using plasma exchange as adjunctive therapy was shown to be associated with an increased chance of renal function recovery, as demonstrated in the MEPEX trial.[8] In the fourth trial, named CYCLOPS, pulse cyclophosphamide was shown to be as effective as daily oral cyclophosphamide in inducing disease remission, and was associated with a reduced cumulative cyclophosphamide exposure.[6] While these four trials greatly increased insights into the efficacy of different therapeutic modalities in ANCA-associated vasculitis, as well as introduced ways to safely reduce the occurrence of serious adverse events, all trials thus far only had limited follow-up.

Long-term patient follow-up

To investigate the long-term follow-up of patients treated with current standard therapies, a long-term follow-up study was instigated by the European Vasculitis Study Group, wherein data from the four trials previously described were pooled. This long-term follow-up study aimed to describe long-term patient outcomes, such as patient and renal survival, and possible prognostic factors at presentation. The results of this long-term follow-up study regarding patient and renal survival are reported in the **second and third chapter** of this thesis. **Chapter 2** describes that the mortality

rate of patients treated with current therapies is still 2.6 times higher than that of an age-matched background control population. The increased mortality risk is especially apparent in the first year after diagnosis, in which infections and active vasculitis account for the majority of early deaths. Especially older patients with severe renal impairment have a high risk of dying in the first few months after presentation; this reflects the severity of their disease as well as the increased susceptibility of these patients to the toxicity of current therapies. While an increased mortality risk early after diagnosis is apparent in patients with ANCA-associated vasculitis, the mortality rate among patients who survive the first year after diagnosis is still 1.3 times higher than that of age-matched population controls. Causes of death after the first year mainly comprise infections, cardiovascular disease, and malignancies.

Chapter 3 of this thesis describes that 20% of AAV patients developed end stage renal failure (ESRF) over a median time of follow-up of 5.2 years. This percentage is comparable to those in previous studies, which reported development of ESRF in 20-40% of patients (median time of follow-up range 3.4 years to ≥ 5 years).[9-14] **Chapter 3** describes that patients with reduced renal function at baseline are likely to still have an impaired renal function after 5 years of follow-up. Consistent with this finding, patients who presented with a reduced renal function, as well as patients who harbored MPO-ANCA, were at risk of developing ESRF. These findings are in agreement with those reported in the literature, since many studies have described baseline serum creatinine or glomerular filtration rate as (one of) the most important predictor(s) for renal outcome.[11-17] In the cohort under study, those patients who experienced one or more renal relapses during follow-up had a significantly higher risk of developing ESRF than those patients who did not have renal flares. Concluding, on a group level, renal function at baseline is the most robust determinant of renal outcome in terms of dialysis dependency. On an individual basis, however, even patients who present with initial dialysis dependency may recover with respect to renal function, at least for a while.[18] In any case, the clear correlation between baseline renal function and long-term renal survival underlines the importance of a timely diagnosis and prompt initiation of therapy to try to prevent irreversible loss of renal function. However, a timely diagnosis is often hampered by the often silent nature of renal manifestations and the resulting patient and doctor's delay.

While the long-term follow-up studies described in **chapters 2 and 3** demonstrate the need to rapidly gain control of potentially life-threatening disease manifestations, it is also emphasized that it is important not to expose patients to undue risks of heavy immunosuppression. While cyclophosphamide and steroids are still the key players in induction regimens for ANCA-associated vasculitis, it is important to search for other modalities that could maintain or even improve efficacy regarding remission induction, but with more favorable safety profiles.

Rituximab

Over the years the cumulative dosage of cyclophosphamide in the treatment of vasculitis has been successfully reduced in a number of ways, particularly by administering cyclophosphamide in intravenous pulses and substituting it with azathioprine for remission maintenance purposes. However, because of its still unfavorable safety profile, there is an ongoing search for newer, safer, at least equally effective therapies, aiming to replace cyclophosphamide use entirely.

Two recent international, randomized, controlled trials under the names RITUXVAS and RAVE investigated a potentially promising biological, namely the anti-CD20 agent rituximab. The RITUXVAS trial compared a rituximab-based regimen with a standard cyclophosphamide/azathioprine regimen for the treatment of active, generalized vasculitis. Sustained remission at 12 months was one of the primary endpoints. This was obtained in 76% of patients in the rituximab group compared to 82% in the control group, these percentages being not significantly different. The rituximab-based regimen was therefore not inferior to standard intravenous cyclophosphamide therapy. However, the other primary endpoint was the occurrence of severe adverse events, and contrary to what was hypothesized, rituximab therapy appeared not to be associated with a reduction in early severe adverse events when compared to standard therapy.[19] In the other trial, RAVE, patients were randomly assigned to receive either rituximab or standard therapy with oral cyclophosphamide. The patients in the experimental group in RAVE did not receive a single dose of cyclophosphamide, which is different from the RITUXVAS trial, in which two intravenous pulses of cyclophosphamide were given to the patients in the experimental group, to aid rapid disease control. The primary endpoint of the RAVE study was disease remission without the use of prednisone at 6 months. This endpoint was reached by 64% of patients in the rituximab group, compared to 53% of patients in the control group.

Concluding, at 6 months of follow-up, the RAVE trial demonstrated that rituximab therapy was not inferior to cyclophosphamide, in agreement with the 1 year results from RITUXVAS.[20]

Chapter 4 of this thesis describes a clinicopathologic study involving patients who were recruited from the experimental limb of the RITUXVAS trial. Renal biopsy specimens were available of 30 patients who received experimental treatment with a rituximab-based regimen. Investigation of these biopsies demonstrated that predominantly $CD3^+$ T cell tubulitis and tubular atrophy correlated with impaired renal function during follow-up. This may be an indication that in these patients who received mainly rituximab and much less cyclophosphamide as opposed to standard treatment, T cell tubulitis was not effectively treated, affecting renal outcome. Although B cell-depleting regimens are a promising modality in the treatment of ANCA-associated vasculitis, caution is required when administering specific anti-CD20 therapy. The extent of T cell infiltration detected on renal biopsy examination might eventually be a factor of consideration when devising actual patient-tailored therapy. A patient with extensive $CD3^+$ T cell tubulitis might turn out not to be the best candidate for rituximab.

Cellular immunity in ANCA-associated vasculitis: T cell characteristics

Although rituximab is a promising drug, not only for refractory vasculitis but also as a substitute for cyclophosphomide in remission induction, it might not effectively treat T cell-mediated disease manifestations. Since the discovery of the relationship of circulating ANCA and the systemic small vessel vasculitides in the eighties, many investigators have focused on the role of humoral immunity in these diseases. However, over the years, marked abnormalities of the effector cells of cellular immunity have been described as well.

Chapter 5 describes that the relative lymphopenia encountered in patients with Wegeners granulomatosis seems to be compensated by an increase in activated and memory T cells. Markers of activation are highly expressed on peripheral blood T cells, generally irrespective of disease activity or therapy. The relative increase in memory T cells might underly the relapsing and remitting nature of the disease and potentially these cells might be targeted with therapy. In autoimmune diseases, the role of regulatory T cells is a popular subject of study, but in vasculitis the role of these cells

is not yet clear. Besides regulatory T cells, also T helper cell responses are thoroughly investigated, and of particular interest to autoimmune diseases is the subset of Th17 cells. These cells are thought to form a bridge between innate and adaptive immune responses, but their role in vasculitis remains to be elucidated. While there is substantial evidence of activated T cells in peripheral blood, it is unclear whether this is representative of T cells in the tissues. In pauci-immune glomerulonephritis effector cells of cell-mediated immunity are also prominent, but some of the abnormalities encountered in peripheral bood, such as high expression of activation markers such as CD25, do not seem to be reflected on T cells encountered in renal biopsy material. It is apparent that the abnormalities encountered on peripheral blood T cells often do not correspond well to clinical parameters. Abnormalities of T cells present in tissues that are directly affected by the disease process could potentially relate better to clinical disease. **Chapter 5** describes that while patients' T cells evidently differ from control cells in several aspects, the origin of these differences remains to be elucidated.

Humoral immunity in ANCA-associated vasculitis: anti-plasminogen antibodies

Chapters 4 and 5 highlight the role of cellular immunity in the pathogenesis of ANCA-associated vasculitis. In contrast, in **chapter 6** the focus is on humoral immunity, specifically on antibodies directed against plasminogen, a key component of the fibrinolytic system. Antibodies recognizing different serine proteases of the coagulation/fibrinolysis cascade were previously detected in various autoimmune diseases.[21-24] **Chapter 6** describes the identification of anti-plasminogen antibodies in ~25% of patients with ANCA-associated vasculitis in two independent patient cohorts, one from the United Kingdom (UK) and one from the Netherlands. Furthermore, in 17.6% of patients from the UK cohort, antibodies recognizing tissue plasminogen activator (tPA) were detected.

Antibodies reactive with plasminogen, but not with tPA, were previously described in a North American cohort of patients with proteinase 3-ANCA-associated vasculitis and were related to the occurrence of thromboembolic events in these patients.[25] Notably, the antibodies recognizing plasminogen in the North American patients also recognized complementary proteinase 3, the peptide translated from the antisense DNA strand of the proteinase 3 gene. Various microbial proteins demonstrate homology to complementary proteinase 3. The immune response following infection with

one of these agents might account for the development of antibodies against complementary proteinase 3, which in their turn can cross-react with plasminogen. We did not examine antibodies directed against complementary proteinase 3, but the occurrence of anti-plasminogen antibodies in both proteinase 3- and myeloperoxidase-ANCA-positive patients in the two European cohorts indicates differences from the North American cohort.

Chapter 6 underlines that anti-plasminogen antibodies are likely to have pathogenic significance. *In vitro* fibrin clot lysis experiments demonstrated that the majority of patient IgG samples that inhibited fibrinolysis harbored either anti-plasminogen and/or anti-tPA antibodies. Although unfortunately no comprehensive data on venous thromboembolic events were available, it was apparent that one of the Dutch patients with a particularly high titer of anti-plasminogen antibodies had a history of deep venous thrombosis. Investigating renal biopsy material of the patients under study showed that seropositivity for anti-plasminogen antibodies correlated with a higher proportion of glomeruli exhibiting fibrinoid necrosis and cellular crescents, hallmark histologic renal lesions that are closely associated with disturbances in coagulation. The data presented in **Chapter 6** indicate that therapies aiming at enhancing or replacing fibrinolytic activity may be of benefit in the treatment of vasculitis patients who harbor antibodies against components of the coagulation/fibrinolysis cascade, particularly plasminogen.

Histopathologic classification of ANCA-associated glomerulonephritis

Chapter 7 of this thesis, describes a proposal for a histopathologic classification of ANCA-associated glomerulonephritis. While the diagnostic and prognostic value of the renal biopsy in ANCA-associated vasculitis is widely known, until now there was no histopathologic classification system. The classification system described in **chapter 7** has been developed by an international working group of renal pathologists and nephrologists. The report contains a first validation exercise on a set of 100 renal biopsies that were previously collected and scored in a standardized manner. Briefly, the system contains four categories: the focal category, comprised of biopsies wherein ≥ 50% of glomeruli are not yet affected by the disease; the crescentic category, wherein at least half of the glomeruli have cellular crescents; the mixed category wherein biopsies show a combination of normal, crescentic, and sclerotic glomeruli and the sclerotic category, wherein biopsies are characterized by ≥ 50%

globally sclerotic glomeruli. The validation study demonstrated that the phenotypical and numerical orders of the classes correspond to the order of severity of renal function impairment during follow-up. Our proposed classification schema proved practical during a first validation exercise, and will hopefully be of aid in the prognostication of patients at the time of diagnosis, as well as facilitate uniform reporting between centers. Amendments to this classification system to make it more useful are welcomed and will hopefully come from additional validation studies.

Future perspectives

Since the first descriptions, over a 100 years ago, of the clinical manifestations of what we now know as ANCA-associated vasculitis, insights into etiology, therapeutic modalities and prognosis have dramatically improved, and are still improving. However, many questions are still unanswered and new questions arise every day. Despite continuing advances in therapy, patients with vasculitis continue to have excess mortality compared to the general population. This increased risk of death persists after the first acute presentation of the disease and is in part related to toxicity of current therapies. While the unfavorable safety profile of cyclophosphamide, the cornerstone of vasculitis treatment today, has long been recognized, it becomes more and more apparent that the burden of corticosteroids is responsible for a substantial part of therapy-related adverse events as well. Future trials will investigate different dosing regimens of steroids regarding efficacy and safety.

Newer therapies, such as rituximab, first showed promising results in patients with refractory disease, unresponsive to standard therapy regimens, but were recently demonstrated in two independent randomized controlled trials to be potential substitutes for cyclophosphamide as remission induction therapy. Although promising, the long-term effects of rituximab regarding efficacy and safety are unknown. Regarding renal histology and rituximab, it might be the case that rituximab does not treat T cell tubulitis well enough, which potentially might encourage tubular atrophy, a negative prognostic factor when it comes to renal outcome. Future studies might answer the question whether these findings in the renal biopsy will be directly useful to guide therapeutic decision making.

Ongoing clinical trials aim to improve therapy by means of investigating large groups of patients with ANCA-associated vasculitis, but in fact patients within these groups

demonstrate very heterogeneous disease manifestations. It is well-known that disease manifestations are highly variable amongst patients with ANCA-associated vasculitis, but evidence accumulates that the same might be true regarding etiologic factors. A relationship between disease flares and nasal carriage of *Staphylococcus aureus* might be present in some patients, infection with *Escherichia coli* strains might cause vasculitis in others, and yet other patients might stand a higher risk of thromboembolic events because of anti-plasminogen antibodies in their circulation. Combine all these factors with different genetic backgrounds, and it is directly evident that, no matter how ideal the concept of actual patient-tailored therapy is, the realization thereof is not straightforward and can be called challenging at the least.

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