Anca associated vasculitis
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2001

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Summary and general discussion
Introduction
Primary systemic vasculitis can affect vessels of any type in any organ, and may therefore result in a wide variety of signs and symptoms. Autoantibodies directed against a variety of neutrophil constituents can often be detected in primary systemic vasculitis. Autoantibodies directed against proteinase 3 (PR3), a constituent of the azurophilic granules of the neutrophil granulocytes, and myeloperoxidase (MPO), a myeloid lysosomal enzyme, have been described in patients with Wegener's granulomatosis (van der Woude et al., 1985), microscopic polyangiitis (Savage et al., 1987), Churg-Strauss syndrome (Cohen Tervaert et al., 1991), and renal limited vasculitis (= idiopathic necrotizing crescentic glomerulonephritis) (Falk et al., 1988). The diagnostic potential of ANCA antibodies is now fairly well established.

These ANCA related syndromes form a distinct group with overlapping features within the spectrum of primary vasculitic syndromes and affect people of all ages (Falk et al., 1990a). These diseases are potentially life-threatening and cause substantial morbidity. Until today, the etiology and pathogenesis of ANCA associated vasculitis is poorly understood. As a result patients are treated with nonspecific immunosuppressives. This therapy is effective in inducing disease remission and preventing early relapses in most vasculitic disorders (Balow et al., 1993). Once remission is achieved, medication is tapered in order to prevent side effects but relapses of disease activity often occur (Hoffman et al., 1992). As relapses are frequently observed, immunosuppressive treatment frequently has to be restarted or intensified. Interestingly, changes in ANCA generally reflect changes in disease activity (Cohen Tervaert et al., 1989).

As an introduction to the specific items explored in the subsequent chapters, Chapter 1 gives an outline of several reported aspects of ANCA associated vasculitis. The general objective brought together in this thesis was to further elucidate relapses of disease activity in ANCA associated vasculitis. Its items are the occurrence, the prediction, the prevention, and the morbidity of clinical relapses in ANCA associated vasculitis.

Chapter 2 summarizes the literature on the occurrence of relapses and possibilities to prevent relapses. Immunosuppressive therapy nearly always induces disease remission in ANCA associated vasculitis. Once remission is achieved, medication is tapered in order to prevent side effects. Relapses occur in more than 50% of patients with ANCA associated vasculitis during tapering or after treatment is stopped. Within the spectrum of these vasculitides, relapses seem to occur more frequently in patients with Wegener's granulomatosis than in other forms of vasculitis. Nasal carriage of *Staphylococcus aureus* has been found to be an important risk factor for relapses in Wegener's granulomatosis. Prolonged prophylactic treatment with trimethoprim-sulfamethoxazole results in a reduced incidence of relapses in these patients. The second risk factor for a relapse that has been identified is ANCA. Persistence of ANCA or the reappearance of ANCA are important risk factors for the
development of a relapse. In addition, rising ANCA levels are associated with recurrence of the disease in many cases. Theoretically, quantification of ANCA can be used as a therapeutic guideline in patients with ANCA associated vasculitis. ANCA levels may either be used to institute 'pre-emptive' therapy, or to adopt an approach in which reductions of immunosuppressive treatment are postponed in those patients who remain ANCA positive. At present, too few studies have been performed to draw any conclusion whether one of these approaches should be used.

Damage of endothelial cells plays an important role in the pathogenesis of Wegener's granulomatosis. The soluble form of endothelial protein C receptor (EPCR) and the thrombin receptor, thrombomodulin (TM) have been described as markers of endothelial cell injury. We hypothesized that these markers of endothelial damage could be predictors of disease activity. Chapter 3 describes the relevance of sEPCR and sTM in Wegener's granulomatosis. In a minority of patients with active Wegener's granulomatosis we found significantly elevated levels of these soluble markers of endothelial cell damage sEPCR (22%) and sTM (46%). Moreover, the levels of sEPCR and sTM correlate with disease activity. Serial measurement of levels of these markers may have some potential as a marker of disease activity.

In Chapter 4 we present a novel approach for the quantitative analysis of ANCA. We have compared quantitative image analysis, which is based on a quantitative approach to the indirect immunofluorescence technique, with the currently used techniques for measuring ANCA levels, i.e. the semi-quantitative indirect immunofluorescence using serial dilutions and the antigen specific ELISA technique. We serially measured ANCA prior to a relapse in a cohort of patients with Wegener's granulomatosis using the different assays. The predictive value of a rise in ANCA as detected by image analysis is higher compared to the indirect immunofluorescence technique on which it is based. However, the ELISA technique performs marginally better in predicting relapses.

In Chapter 5, we investigated the prevalence of ANCA with specificity for myeloperoxidase (MPO-ANCA) in a large cohort of 121 patients with biopsy-proven pauci-immune (= few or no immune deposits) crescentic glomerulonephritis using a variety of assays applying different (recombinant) antigens for the detection of MPO-ANCA. In almost all patients with pauci-immune necrotizing crescentic glomerulonephritis MPO-ANCA or ANCA with specificity for proteinase 3 (PR3-ANCA) can be detected. Serum samples from patients with MPO-ANCA associated pauci-immune necrotizing crescentic glomerulonephritis nearly always recognize both recombinant MPO and native MPO in both direct and capture ELISA. False positive results by disease controls without pauci-immune necrotizing crescentic glomerulonephritis are obtained less frequently using a capture ELISA compared to a direct
ELISA. Thus, in patients with pauci-immune necrotizing crescentic glomerulonephritis a capture ELISA is the preferred method to detect MPO-ANCA, and recombinant MPO is an alternative antigen source for native MPO for the detection of MPO-ANCA in these patients.

Prediction of relapses in Wegener’s granulomatosis by measuring levels of PR3-ANCA or MPO-ANCA remains a controversial issue. The results of a prospective study in 100 patients with Wegener's granulomatosis in order to assess the value of serial quantification of ANCA by indirect immunofluorescence and ELISA for the prediction of disease activity are presented in Chapter 6. We demonstrated that serial measurement of ANCA levels is valuable for the early prediction of relapses in patients with Wegener's granulomatosis since rises in ANCA are found in the majority of patients prior to a relapse. Furthermore, we showed that quantitation of ANCA by ELISA is superior to quantitation of ANCA by titration of serial dilutions in indirect immunofluorescence for the prediction of an ensuing relapse. The additional measurement of rises in the IgG3 subclass of PR3-ANCA increases the positive predictive value of a rise in ANCA but, given its low sensitivity, is of little clinical utility.

It has been demonstrated in a pilot study that relapses of Wegener’s granulomatosis can be prevented by early treatment with cyclophosphamide and steroids based on levels of ANCA as detected by indirect immunofluorescence. Since ANCA quantitation by ELISA was superior to indirect immunofluorescence (Chapter 6), a randomized multi-center study was conducted in 100 PR3-ANCA positive patients with vasculitis to investigate the value of early treatment with azathioprine and steroids based on serial quantification of ANCA by ELISA for the prevention of relapses (Chapter 7). Based on our preliminary data, we conclude that pre-emptive treatment based on rises in ANCA levels might be valuable for the prevention of imminent relapses in patients with PR3-ANCA positive vasculitis. However, despite pre-emptive treatment no appreciable reduction in late relapses was observed and after an initial decline of ANCA levels, these eventually rose above baseline values after prednisolone treatment was stopped.

Since the initial anecdotal reports on the successful treatment of Wegener's granulomatosis with trimethoprim-sulfamethoxazole, its place as monotherapy or additional therapy in the therapeutic armamentum for this disease has been unclear. In Chapter 8, ANCA levels were measured in a cohort study of 31 consecutive patients with Wegener's granulomatosis presenting with disease activity limited to the upper and lower airways who were treated with trimethoprim-sulfamethoxazole only. We evaluated whether ANCA levels in these patients were helpful to predict a treatment failure. The disease was controlled in 60% for at least 24 months analyzed on an intent to treat basis. In patients obtaining a complete remission on trimethoprim-sulfamethoxazole the disease controlled survival was a high as 87% at 24
months. Treatment failure was more likely in patients with disease activity outside the ENT region and in patients with a *Staphylococcus aureus* negative nasal culture at initiation of therapy. Patients with decreasing ANCA titers after therapy was started were not less prone to relapses compared to patients with constant or increasing ANCA titers.

ANCA associated vasculitis is a relapsing-remitting disease. One of the major side-effects of long-term treatment with steroids in combination with cyclophosphamide is osteoporosis which may result in the increased occurrence of fractures. In Chapter 9, we describe the result of a study in which we measured the prevalence of reduced bone mineral density in a cross-sectional cohort of 99 patients with ANCA associated vasculitis and correlated bone mineral density findings with disease episodes and cumulative doses of steroids and cyclophosphamide. Osteoporosis and osteopenia are frequently observed in patients with ANCA associated vasculitis. Bone mineral density values as compared to age and sex matched controls, however, are only significantly reduced in males. We found that the cumulative dose of steroid therapy is a significant contributor to bone loss of the lumbar spine and proximal femur. We were not able to identify a role for cyclophosphamide therapy.

Advances in medical care have transformed diseases like Wegener's granulomatosis and systemic lupus erythematosus from diseases with a high mortality to conditions with serious chronic morbidity. In Chapter 10 we describe the patients' perceptions of the effects of Wegener's granulomatosis and systemic lupus erythematosus on health, function, income, and interpersonal relationships. Seventy-nine patients with Wegener's granulomatosis and 114 patients with systemic lupus erythematosus completed a self-administered questionnaire. All patients experienced substantial functional morbidity. Furthermore, Wegener's granulomatosis as well as systemic lupus erythematosus had a considerable impact upon the psychological and social life. Therefore, our study demonstrates that Wegener's granulomatosis and systemic lupus erythematosus are associated with substantial medical morbidity resulting in physical and occupational disability. Both diseases have a profound impact on patients' lives.

**General Conclusions**

The work described in this thesis is focussed on relapses of disease activity in ANCA associated vasculitis during follow-up. Its subjects are the occurrence (Chapter 6), the prediction (Chapter 3, 4, 6), the prevention (Chapters 7, 8), and the morbidity (Chapter 9, 10) of clinical relapses in ANCA associated vasculitis. Although we are still a long way from curing this disease, some things have become clear. First of all, since relapses are associated with morbidity and even mortality, early detection and prediction of relapses is of great importance. We studied several markers of endothelial damage, inflammation, and autoantibodies to endothelial cells/neutrophil constituents in order to predict a relapse of disease activity. An association between markers for endothelial
damage, inflammation and disease activity was established (Chapter 3), and endothelial
damage can be detected prior to clinical disease activity. Furthermore, increases in ANCA
antibodies precede clinical disease activity in the great majority of patients (Chapter 3,4,6)
indicating that serial measurement of ANCA is valuable for monitoring patients with ANCA
associated vasculitis.
Secondly, we wondered whether we could prevent an ensuing relapse by pre-emptive
treatment based on an increase in ANCA to avoid renewed immunosuppressive therapy
(Chapter 7). Based on our preliminary data, we conclude that combined courses of
azathioprine in combination with prednisolone are capable of postponing relapses of disease
activity. Pre-emptive treatment based on a rise in ANCA was only instituted once. Since
subsequent rise(s) in ANCA prior to relapse were observed in patients randomized for pre-
emptive treatment, it remains unclear whether these relapses could have been prevented by
repeated courses of pre-emptive treatment, or whether pre-emptive treatment should have
been prolonged. Further studies are needed to identify which patients might benefit most of
pre-emptive treatment based a rise in ANCA, and which treatment protocol combines low
toxicity with effective protection against late relapses.
Alternatively, trimethoprim-sulfamethoxazole may be advocated as the initial treatment in
patients with Wegener's granulomatosis limited to the ENT region. Even in patients with ENT
involvement in combination with mild episcleritis, pulmonary lesions, and arthralgias,
prolonged treatment with trimethoprim-sulfamethoxazole may prevent relapses for prolonged
periods and therefore obviate the need for the more toxic conventional treatment with
cyclophosphamide and corticosteroids (Chapter 8).
Last but not least, auto-immune diseases such as Wegener's granulomatosis and systemic
lupus erythematosus are associated with substantial disease and treatment related medical
morbidity (e.g. osteoporosis) resulting in physical and occupational disability (Chapter 9,10).

Future perspectives
Pathophysiology of ANCA associated vasculitis
Since ANCA and disease activity are clearly related, many groups have examined the possible
pathophysiologic role of ANCA. At present, there is no definitive proof that ANCA play a
causative role in the development of systemic vasculitis and/or necrotizing crescentic
glomerulonephritis. However, experimental data from in vitro studies suggest that ANCA
and/or ANCA-antigen-related autoimmune responses are implicated in the pathophysiology
of these diseases (Falk et al., 1990b; Savage et al., 1992; Buttrum et al., 1994; Franssen et al.,
1999; Muller Kobold et al., 1999a; Radford et al., 2000). It is, however, questionable whether
ANCA levels are of direct pathophysiological relevance. From studies in experimental models
it is clear that ANCA in itself are not sufficient for disease induction. In a rat model it was
demonstrated that immunization with MPO results in the induction of MPO-ANCA without
the development of lesions and that, subsequently, immune-complex deposition is needed
before vasculitis and glomerulonephritis are induced (Heeringa et al., 1998). It has been suggested that during infections PR3 and MPO is released from activated neutrophils and/or monocytes, and subsequently bound to endothelial cells serving as 'planted' antigens and new targets for ANCA resulting in \textit{in situ} immune-complexes, which in turn attract other neutrophils. This cascade may lead to endothelial cell apoptosis, detachment and lysis (Müller Kobold et al., 1999b; Kallenberg et al., 1994). Infections may also be the origin of pro-inflammatory cytokines that prime circulating neutrophils (Pinching et al., 1980). Primed neutrophils express PR3 and MPO enabling ANCA to bind to those cells and further activate them, thus reactivating quiescent disease.

Since the majority of patients with Wegener's granulomatosis are chronic nasal carriers of \textit{Staphylococcus aureus} and since chronic nasal carriage of \textit{Staphylococcus aureus} is a substantial risk factor for the development of relapses of this disease, \textit{Staphylococcus aureus} may play a role in inducing vasculitis (Stegeman et al., 1994). Supporting evidence comes from elevated levels of antibodies directed to staphylococcal antigens that are found in WG patients. One of these antigens is staphylococcal cationic phosphatase. This antigen, which is a naturally occurring staphylococcal antigen, causes an immune-complex-mediated glomerulonephritis in an animal model, which may be aggravated by the presence of autoantibodies against myeloperoxidase (Brons et al., 2000). We postulate that nasal carriage of \textit{Staphylococcus aureus} in patients with Wegener's granulomatosis induces a chronic inflammation of the upper respiratory tract, which could lead to the initiation of vasculitis/glomerulonephritis.

Finally, superantigens produced by \textit{Staphylococcus aureus} could also play a role in the induction of Wegener's granulomatosis. Superantigens are capable of activating autoreactive T-cells that mediate auto-immune vessel wall destruction and inducing autoreactive B-cells to produce autoantibodies (Cohen Tervaert et al., 1999).

\textit{Treatment of ANCA-associated vasculitis}

As long as the pathophysiology of ANCA associated vasculitis is unclear, nonspecific immunosuppression will be the initial approach of treatment. Because cytotoxic drugs like cyclophosphamide have severe short- and long-term side-effects, several attempts have been made to tailor treatment in order to minimize side-effects. Recent studies with either azathioprine (Jayne, 2000a), methotrexate (de Groot et al., 1996), or cyclosporin (Haubitz et al., 1998) indicate that the long-term use of cyclophosphamide can possibly be avoided by using these drugs after remission is induced.

Newer immunosuppressive concepts were tested during the past few years. As we have demonstrated in this thesis, pre-emptive treatment based on quantification of PR3-ANCA by ELISA can be used as a therapeutic guideline in patients with ANCA associated vasculitis. Further studies are needed to identify which patients might benefit most of pre-emptive treatment, and which treatment protocol combines low toxicity with effective protection.
against late relapses. New immunosuppressive drugs like mycophenolate mofetil (Nowack et al., 1999; Stegeman et al., 2000), 15-desoxyyspergualin (Birck et al., 2000) and leflunomide (Metzler et al., 1998) have been tested in small groups of patients with promising results.

Other attractive approaches include therapy directed against cytokines, adhesion molecules and/or lymphocytes. Cytokines might play a role both in the initiation of vasculitis by priming of polymorphonuclear neutrophils and in the effector phase by upregulation of adhesion molecules and the activation/attraction of inflammatory cells. Cytokines can be modulated either by blocking monoclonal antibodies or by receptor antagonists. The use of antibodies directed against tumor necrosis factor is currently being investigated in a randomized placebo-controlled multi-center trial in Wegener's granulomatosis (Stone, 2000). Adhesion molecules play an important *in vivo* role in polymorphonuclear neutrophil migration and in ANCA mediated polymorphonuclear neutrophil cytotoxicity to endothelial cells. Drugs that inhibit the adhesion of leukocytes to endothelial cells form an attractive alternative to prevent activation of polymorphonuclear neutrophils and cell migration in the effector phase of ANCA associated vasculitis (Lockwood et al., 1999). For induction of remission in progressive disease resistant to standard therapy or when standard therapy is not tolerated, lymphocyte depletion using antithymocyte globulin or monoclonal anti-T cell antibodies has been used in small series of patients (Lockwood et al., 1993; van der Woude et al., 2000).

Finally, immunoablation using high-dose cytotoxic medication with or without stem cell rescue has led to the prolonged remission in a few patients with refractory vasculitis (Tyndall et al., 1999).

The first step towards a specific treatment may be the inactivation/removal of ANCA. The administration of intravenous immunoglobulin has been used successfully in resistant cases of ANCA associated vasculitis (Jayne et al., 2000b). This therapy may inhibit and/or down regulate ANCA, besides saturating Fc-receptors, by the presence of anti-idiotypic antibodies in the preparation which interact with the idiotypic network. Given the suspected role of ANCA in the pathophysiology of vasculitis, other approaches include plasma exchange (Pusey et al., 1991) and the selective removal of ANCA using specific immunoabsorption columns (Alexandre et al., 2000).

Given the suspected role of *Staphylococcus aureus* in the pathophysiology of Wegener's granulomatosis, other approaches include eradication of this microorganism by antimicrobial treatment. Prolonged treatment with trimethoprim-sulfamethoxazole treatment may lead to partial or complete remission in patients presenting with active WG limited to the upper and lower airways (this thesis) and is known to prevent relapses (Stegeman et al., 1996). Whether this is due to some as yet unknown immunosuppressive or anti-inflammatory effect or due to its antimicrobial activity is unknown.

**Literature**


