Introduction
Chapter 1

Vasculitis

Vasculitis is characterized by inflammation of vessel walls. Vessels of any type in any organ can be affected resulting in a wide variety of signs and symptoms. The disease may be systemic or limited to one or just a few organ systems. Vasculitis may be present as a primary disease of the blood vessels (primary or idiopathic vasculitis, see Table 1 (Jennette et al., 1994)) or as part of another underlying disease (secondary vasculitis, e.g. vasculitis associated with serum sickness, viral hepatitis, systemic lupus erythematosus, rheumatoid arthritis). These secondary vasculitides are, in contrast to primary vasculitis, frequently characterized by immune complex deposition within the vessel wall suggesting that immune complex deposition from the circulation or in situ complex formation is pathophysiologically related to the vasculitic process.

Table 1 Classification of systemic primary / idiopathic vasculitides

<table>
<thead>
<tr>
<th>I.</th>
<th>Predominantly affecting large- and medium-sized blood vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Takayasu's arteritis</td>
</tr>
<tr>
<td>2</td>
<td>Giant cell arteritis / temporal arteritis</td>
</tr>
<tr>
<td>II.</td>
<td>Predominantly affecting medium-sized blood vessels</td>
</tr>
<tr>
<td>1</td>
<td>Polyarteritis nodosa</td>
</tr>
<tr>
<td>2</td>
<td>Kawasaki's disease</td>
</tr>
<tr>
<td>III.</td>
<td>Predominantly affecting medium- and small-sized blood vessels</td>
</tr>
<tr>
<td>1</td>
<td>Churg-Strauss syndrome</td>
</tr>
<tr>
<td>2</td>
<td>Wegener's granulomatosis</td>
</tr>
<tr>
<td>3</td>
<td>Microscopic polyangiitis</td>
</tr>
<tr>
<td>IV.</td>
<td>Predominantly affecting small-sized blood vessels</td>
</tr>
<tr>
<td>1</td>
<td>Henoch-Schönlein purpura</td>
</tr>
<tr>
<td>2</td>
<td>Essential cryoglobulinemic vasculitis</td>
</tr>
<tr>
<td>3</td>
<td>Cutaneous leukocytoclastic angiitis</td>
</tr>
</tbody>
</table>

(adapted from Jennette et al., 1994)

Antineutrophil cytoplasmic antibodies (ANCA)

Autoantibodies directed against constituents of polymorphonuclear leukocytes and/or monocytes were first described in 1982 by Davies et al. in a few patients with renal vasculitis (Davies et al. 1982). In 1985 it became apparent that ANCA are a sensitive and specific marker for Wegener's granulomatosis (van der Woude et al., 1985). Since then, ANCA have also been described in patients with other forms of vasculitis, i.e. microscopic polyangiitis (Savage et al., 1987), Churg-Strauss syndrome (Cohen Tervaert et al., 1991a), and renal limited vasculitis (= idiopathic necrotizing crescentic glomerulonephritis) (Falk et al., 1988). ANCA were originally detected by indirect immunofluorescence on ethanol fixed neutrophils (Wiik et al., 1989). At least three different patterns of fluorescence have been distinguished: a
cytoplasmic/classic pattern (cANCA) with accentuation of the fluorescence intensity in the area with the nuclear lobes, a perinuclear pattern (pANCA), and a more diffuse cytoplasmic staining pattern (atypical ANCA). Approximately 90% of the sera that produce a cANCA pattern react with proteinase 3 (PR3), a serine protease from the azurophilic granules of myeloid cells (Falk et al., 1997). In patients with primary systemic vasculitis predominantly affecting medium- and small-sized blood vessels, approximately 75% of the sera producing a perinuclear pattern (pANCA) react with myeloperoxidase (MPO), a myeloid lysosomal enzyme (Cohen Tervaert et al., 1991b). In ANCA-positive patients with other nonvasculitic diseases (e.g. inflammatory bowel disease) often antigenic specificities are recognized, such as lactoferrin and elastase, may occur (Kallenberg et al., 1992). The diagnostic potential of PR3-ANCA and MPO-ANCA are now fairly well established (Table 2). In a patient with signs and symptoms of vasculitis, ANCA with specificity for PR3 (PR3-ANCA) suggests a diagnosis of Wegener's granulomatosis, whereas ANCA with specificity for MPO (MPO-ANCA) is highly sensitive for either microscopic polyangiitis, idiopathic necrotizing crescentic glomerulonephritis, or active Churg-Strauss syndrome (Cohen Tervaert et al., 1996). A number of in vitro and animal studies, in addition, have suggested that ANCA are involved in the pathophysiology of ANCA related syndromes.

### Table 2 Characteristics of ANCA-associated vasculitides

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical</th>
<th>Anti-PR3 Antibody</th>
<th>Anti-MPO Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Churg-Strauss syndrome</td>
<td>Asthma, eosinophilia, neuropathy</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Wegener's granulomatosis</td>
<td>Nose bleeds, nephritis, lung infiltrates</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>Microscopic polyangiitis</td>
<td>Nephritis, purpura, hemoptysis</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Idiopathic necrotizing and / or</td>
<td>Nephritis, malaise</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>crescentic glomerulonephritis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PR3 = proteinase 3; MPO = myeloperoxidase; ++++ = > 70% of patients; +++ = 30-70% of patients; ++ = 10-30% of patients; + = 3-10% of patients (adapted from Cohen Tervaert et al., 1996)

### ANCA-associated vasculitis

Within the spectrum of primary vasculitic syndromes, the ANCA related syndromes form a distinct group with overlapping features. Most patients have a prodromal flu-like onset consisting of malaise, myalgias, arthralgias, fever and weight loss. This flu-like onset appears within days to weeks before the onset of overt vasculitic or nephritic disease. ANCA associated vasculitis includes four major syndromes: Wegener's granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, and idiopathic necrotizing and/or crescentic glomerulonephritis (Table 2). Wegener's granulomatosis is differentiated from the others by the presence of necrotizing granulomatous inflammation of the upper and lower respiratory tract, which is usually accompanied by systemic necrotizing small vessel vasculitis and
glomerulonephritis. Churg-Strauss syndrome is differentiated by the presence of (a history of) asthma, allergic rhinitis, systemic eosinophilia, in addition to systemic vasculitis with or without glomerulonephritis. Microscopic polyangiitis is characterized by necrotizing and/or crescentic glomerulonephritis and a multisystem vasculitis involving small vessels. Microscopic polyangiitis shares many features with Wegener's granulomatosis and Churg-Strauss syndrome, but lacks necrotizing granulomatous inflammation of the respiratory tract and asthma (Jennette et al., 1994). In idiopathic necrotizing and/or crescentic glomerulonephritis the vasculitic process is limited to the kidneys. The diseases mentioned above affect people of all ages but are most common in older adults in their 50s and 60s, and they affect men and women equally (Pettersson et al., 1995; Falk et al., 1990).

ANCA related small-vessel vasculitides are potentially life-threatening diseases with high mortality. Prolonged immunosuppressive therapy (>1 year) with cyclophosphamide and steroids is effective in inducing disease remission and preventing early relapses in most vasculitic disorders (Balow et al., 1993; Gaskin et al., 1992; Fauci et al., 1978; Fauci et al., 1983; Hoffman et al., Ann Int Med 1992; Andrassy et al., Clin Nephrol 1991). Continuous use of cyclophosphamide to sustain remission can not be recommended, however, since this treatment regimen is associated with severe and potentially lethal adverse effects such as the occurrence of opportunistic infections and the development of malignancies (Stillwell et al., 1988; Radis et al., 1995). Therefore, cyclophosphamide is tapered or stopped and replaced by azathioprine once remission is achieved to prevent adverse effects, a policy recently tested in a rigorous multicenter trial and proven to be equally effective in the follow-up for 18 months (Gaskin et al., 1992; Jayne, 2000). Azathioprine is considered less effective in inducing remission than cyclophosphamide, but its long-term toxicity is much lower (Bouroncle et al., 1967; Norton et al.,1968). Other alternative maintenance therapy regimens, such as methotrexate (de Groot et al., 1996), cyclosporin A (Haubitz et al., 1998), mycophenolate (Nowack et al., 1999), or trimethoprim-sulfamethoxazole (Stegeman et al., 1996) have been described. Relapses, however, are frequently observed in these forms of vasculitis and treatment in such cases has to be intensified or reinstituted (Hoffman et al., 1992; Gordon et al., 1993; Nachman et al., 1996; Guillevin et al., 1999; Reinhold-Keller et al., 2000).

Interestingly, changes in ANCA during followup generally reflect disease activity. It has been demonstrated that the persistence or reappearance of ANCA is a risk factor for the development of a relapse of disease activity (Stegeman et al., 1994; De'Oliviera et al., 1995; Jayne et al., 1995). In addition, relapses of Wegener's granulomatosis are frequently preceded by rises in the titer of cytoplasmic/classic ANCA (cANCA) as detected by indirect immunofluorescence (Cohen Tervaert et al., 1989) and relapses can be prevented by treatment with immunosuppressives based on rises in cANCA (Cohen Tervaert et al., 1990). These data suggest a pathophysiological role in vivo for these autoantibodies.
Aim of this thesis

During follow-up, relapses of disease activity occur in the majority of patients with ANCA associated vasculitis. The general objective brought together in this thesis was to further elucidate the characteristics and consequences of these relapses. Investigated items are the occurrence, the prediction, the prevention, and the morbidity of clinical relapses in ANCA associated vasculitis.

First, we reviewed the literature on the occurrence of relapses and possibilities to prevent relapses (Chapter 2). Secondly, as the relation between changes in ANCA and disease activity are variable, we investigated whether markers of endothelial damage, inflammation, and/or autoantibodies to endothelial cells could be better predictors of disease activity in Wegener's granulomatosis than ANCA (Chapter 3). To elucidate the value of quantitative image analysis for monitoring ANCA levels, we compared this technique with the currently available semi quantitative (i.e. indirect immunofluorescence) and quantitative (i.e. ELISA) techniques using samples from a cohort of PR3-ANCA positive patients with Wegener's granulomatosis. The results are described in Chapter 4. Next, we investigated the prevalence of MPO-ANCA in a large cohort of patients with biopsy-proven pauci-immune (= few or no immune deposits) crescentic glomerulonephritis using different assays and (recombinant) antigens for the detection of MPO-ANCA (Chapter 5). The results of a prospective study in 100 patients with Wegener's granulomatosis designed to assess the value of serial quantification of ANCA by indirect immunofluorescence and antigen-specific enzyme-linked immunosorbent assay (ELISA) for the prediction of relapses are presented in Chapter 6. Since PR3-ANCA detection by ELISA proved superior to indirect immunofluorescence, a randomized multi-center study was conducted in PR3-ANCA positive patients with vasculitis to investigate the value of early treatment based on serial quantification of ANCA by ELISA for the prevention of relapses (Chapter 7). In Chapter 8, ANCA levels were measured in a cohort study of 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 treatment failure. It is generally accepted that relapses are associated with disease and treatment related morbidity. We describe in Chapter 9 the result of a study on the prevalence of reduced bone mineral density in a cross-sectional cohort of patients and correlated bone mineral density findings with disease episodes and cumulative doses of steroids and cyclophosphamide. Finally, the patients' perceptions of the effects of Wegener's granulomatosis and systemic lupus erythematosus on health, function, income, and interpersonal relationships are the topics of Chapter 10. Finally the data are summarized and discussed in Chapter 11.
Chapter 1

Literature


