Disease-activity in ANCA-associated vasculitis

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Risk factors for relapse in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis: Tools for treatment decisions?

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ABSTRACT

Current treatment based on the use of cyclophosphamide and corticosteroids has changed anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides from highly fatal into more chronic relapsing diseases. Relapses are a major problem in these diseases and cause increased morbidity and mortality. Current clinical research mainly focuses on achieving control of active disease while minimizing treatment related toxicity. Risks for long-term relapse and their sequelae are less clearly studied. It is noteworthy that, besides treatment, several other factors have been associated with the occurrence of relapses. Thus, compared to MPO-ANCA positive patients, patients with PR3-ANCA associated vasculitis have a significantly increased risk to experience relapse. In addition, ANCA-status during follow-up, levels of T cell activation, genetic background, and infectious and other exogenous factors have been linked to relapse as well. With a few exceptions, these associations are merely descriptive and not pathophysiologically proven. Furthermore, data on adapting treatment in accordance with risk factors for relapse are scarce. We review risk factors for relapse in ANCA-associated vasculitis, their potential pathogenic implications, and their possible role in preventive strategies and adaptations of current treatment policies.

INTRODUCTION

The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are a group of primary vasculitides affecting small to medium-sized vessels and associated with autoantibodies against neutrophilic proteases, in particular proteinase 3 (PR3) and myeloperoxidase (MPO). In Wegener's granulomatosis (WG) ANCA are mostly directed against PR3, whereas a large number of patients with microscopic polyangiitis (MPA), idiopathic necrotizing crescentic glomerulonephritis (iNCGN) and Churg-Strauss syndrome (CSS) are MPO-ANCA positive [1]. The introduction of cyclophosphamide and prednisolone has dramatically improved patient-outcome, and, currently, most patients with AAV can be brought into remission [2-4]. However, when immunosuppression is tapered and eventually stopped, relapses frequently occur [3]. Long-term follow-up of patients with WG showed a relapse rate of 50% or more within 5 years after diagnosis, and active disease during relapses was the main cause of mortality beyond the first year [3, 5-7]. Additionally, the number of relapses that had occurred was a strong predictor of damage during follow-up, as measured by the Vasculitis Damage Index [7]. Also for long-term renal survival in PR3-ANCA-associated vasculitis, the number of renal relapses is the primary determinant [8].

Relapses in ANCA-associated vasculitis constitute a clinically relevant problem. Therefore, a major challenge in current long-term treatment is the identification of patients at increased risk for relapse and the prevention of these relapses with minimal treatment related toxicity. Additionally, risk factors associated with the occurrence of relapses can provide insight into the pathogenic mechanisms underlying disease reactivation, and might lead to new, more individualized, risk-adapted treatment strategies. We will discuss the risk factors for relapse of disease and the potential preventive strategies identified thus far.

Treatment

Standard treatment for AAV consists of a combination of cyclophosphamide and prednisolone and is effective in inducing remission in the majority of patients. However, treatment related toxicity, both short- and long-term, occurs frequently, and relapses are encountered in many patients. In a meta-analysis of three randomized controlled trials continuous cyclophosphamide appeared to result in a lower relapse rate as compared to pulse cyclophosphamide, although the difference was not statistically significant (RR 1.79; 95% CI 0.85-3.75) [9]. In these trials patients on continuous treatment received significantly higher cumulative dosages of cyclophosphamide than patients on pulse treatment. Recently, Koldingsnes et al showed that a cumulative dosage of less than 10 g cyclophosphamide

during the first 6 months was associated with more relapses in patients with Wegener's granulomatosis (WG) (RR 2.83, 95% CI 1.33-6.02) [7]. However, higher cumulative doses of cyclophosphamide have also been associated with more damage during follow-up, as measured by VDI [7].

Various alternative treatment strategies have been tested, with treatment related toxicity and relapse rate as primary outcome measures. Maintenance therapy with low dose methotrexate following remission induction with cyclophosphamide in an open-label prospective trial resulted in a high rate of, predominantly renal, relapses after a median follow-up of 25 months (26 relapses in 71 patients) [10]. In addition, a randomised study of the European vasculitis study group (EUVAS) demonstrated that induction and maintenance therapy with cyclophosphamide therapy or methotrexate, both limited to an unusual short period of 12 months, resulted in a high number of relapses during 18 months follow-up [11]. However, the relapse rate was even greater in the methotrexate group as compared to the cyclophospamide group (70% vs. 45%, p=0.016).

Additional attempts have been made to reduce cyclophosphamide exposure by limiting the period of cyclophosphamide treatment to 3 months following remission. The European multicenter trial comparing cyclophosphamide to azathioprine during remission in AAV (CYCAZAREM) showed that after induction of remission with cyclophosphamide, patients who switched to azathioprine did not experience more relapses after 18 months follow-up in comparison to cyclophosphamide maintenance (disease-free survival 86.3% in the cyclophosphamide group and 84.5% in the azathioprine group) [4]. Whether these results persist on longer follow-up is, however, questionable. We analysed outcome after 5 years in newly treated patients at our center who were diagnosed between 1990 and 2000 and had been treated with cyclophosphamide only (1990-1996) or were switched to azathioprine following 3 months of stable remission (1997-2000). We found that, despite a relapse rate at 18 months that was not different (disease-free survival 88.6% in the cyclophosphamide group and 89.6% in the azathioprine group), patients who switched to azathioprine (n=48) tended to have more relapses than those on cyclophosphamide maintenance (n=88) during long-term follow-up (RR 1.51; 95% CI 0.92-2.68, p=0.099), although this difference did not reach statistical significance [12]. Relapses tended to occur especially in the third and fourth year of follow-up after immunosuppressive therapy was stopped. This suggests ongoing smouldering disease, which, upon stopping of immunosuppressive maintenance therapy, results in a clinically apparent relapse. Possible prevention by continued immunosuppressive maintenance therapy would be rational when subgroups at increased risk for relapse can be identified during the initial period of follow-up.

Immune activation

ANCA specificity and levels

In WG about 85% of patients are PR3-ANCA positive, whereas patients with MPA and idiopathic NCGN are more frequently MPO-ANCA positive [1]. Apart from their specific clinical hallmarks, these diseases differ in relapse rate; reported relapse rates in cohorts of patients with WG are 50% during a mean 8 years follow-up 3 and 44% within 3 years 6 compared to 25-35% within 2.5 to 5 years in other ANCA-associated vasculitides [6, 1, 14]. Consequently, relapses are associated with ANCA specificity and occur more frequently in PR3-ANCA-associated vasculitis compared to MPO-ANCA-associated vasculitis [15-20]. Also in our own cohort diagnosed between 1990 and 2002 PR3-ANCA positive patients were more likely to experience relapse than MPO-ANCA positive patients (with a relative relapse risk of around 3.7; 95%CI 1.6-4.1; p=0.002) (Figure 1). This difference in relapse rate based on ANCA specificity seems to occur also within one disease entity. In our center patients with WG who were PR3-ANCA positive (n=137) experienced significantly more relapses than the small group of WG patients with MPO-ANCA (n=13) (RR 4.4; 95% CI 1.1-5.0) (Figure 2A) [21]. Likewise, within the group of patients with MPA and iNCGN, those with PR3-ANCA (n=12) show a higher relapse rate as compared to those with MPO-ANCA (n=36). The relative risk for relapse for PR3-ANCA positive patients as compared to MPO-ANCA positive patients within this group was comparable to that found in our group of WG patients (Figure 2B).

Many studies have been published on the usefulness of ANCA titration for prediction of relapses. During induction treatment, ANCA levels fall or become negative in 30% to more than 80% of the patients within the first few months. Patients who subsequently remain ANCA negative during follow-up have a very low risk to develop a relapse. In contrast, persistence or reappearance of ANCA after induction of remission has been associated with the occurrence of relapses. A significant association was found between C-ANCA positivity in the first year after diagnosis and the subsequent occurrence of relapses [15-18, 22, 23], whereas this relation is less clear for patients positive for P-ANCA [14, 17, 18, 24]. Recently, we showed that in patients with PR3-ANCA associated vasculitis, a positive C-ANCA-titer at switch to azathioprine, after induction of stable remission by cyclophosphamide, was associated with an increased risk to experience relapse (RR 2.6, 95% CI 1.1-8.0), with a relapse-free survival of only 18% at 5 years [19].

With respect to the predictive value of ANCA rises for relapses, as measured by indirect immunofluorescence (IIF) or ELISA, divergent results have been reported. Rises in C-ANCA titer were followed by a relapse in 57 to 82% of the patients; rises in PR3-

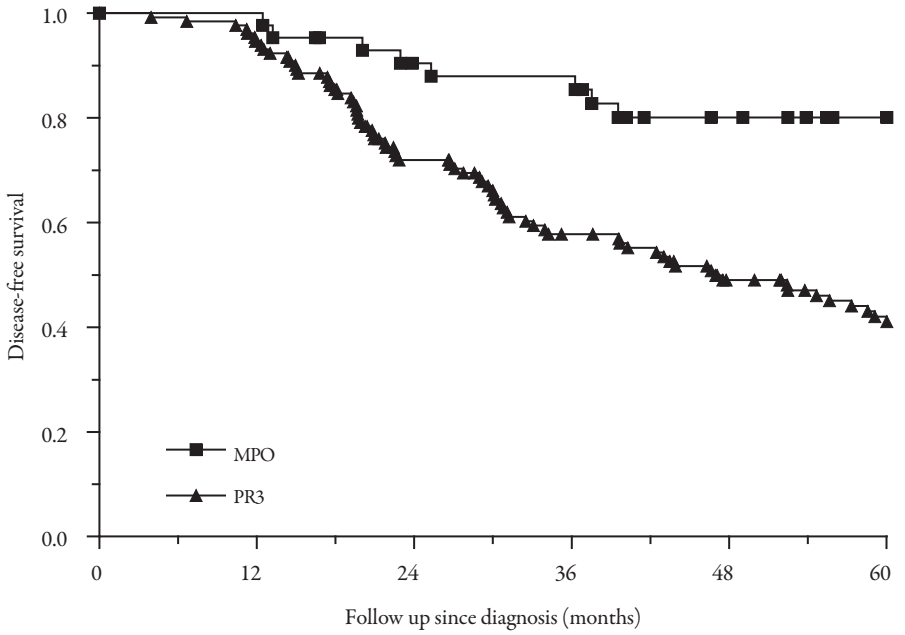

Figure 1. Disease-free survival in 174 patients diagnosed with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis and treated with cyclophosphamide and prednisolone at the University Hospital Groningen from 1990 to 2002 according to ANCA specificity. There is a significant difference in disease-free-survival between patients with MPO- (■) (n = 43) and PR3-ANCA-associated vasculitis (▲) (n=131); RR 3.7 (95% CI 1.6-4.1); p=0.002.

ANCA and MPO-ANCA by ELISA were followed by relapses in 59 to 100%, and 79 to 100% of cases, respectively (reviewed in [21]). Several difficulties come up when interpreting studies on serial ANCA measurements. Many studies are retrospective and concern few patients, they do not standardise the interval between ANCA measurements, and do not define a relapse. Additionally, the methodology of ANCA measurement differs widely. Using predefined criteria for a relapse and a standardised interval for sequential ANCA measurement, and eliminating inter-assay variation by prospectively measuring sequential samples within the same assay, we found that 4-fold rises in C-ANCA titers by IIF or >75% increase in PR3-ANCA levels as determined by direct ELISA predicted the occurrence of a relapse within a period of 12 months in 50% and 58% of cases, respectively [25]. This study showed rises in PR3-ANCA levels to be a slightly better predictor than rises in C-ANCA titers (Figure 3) [25]. Attempts have been made to improve the association between changes

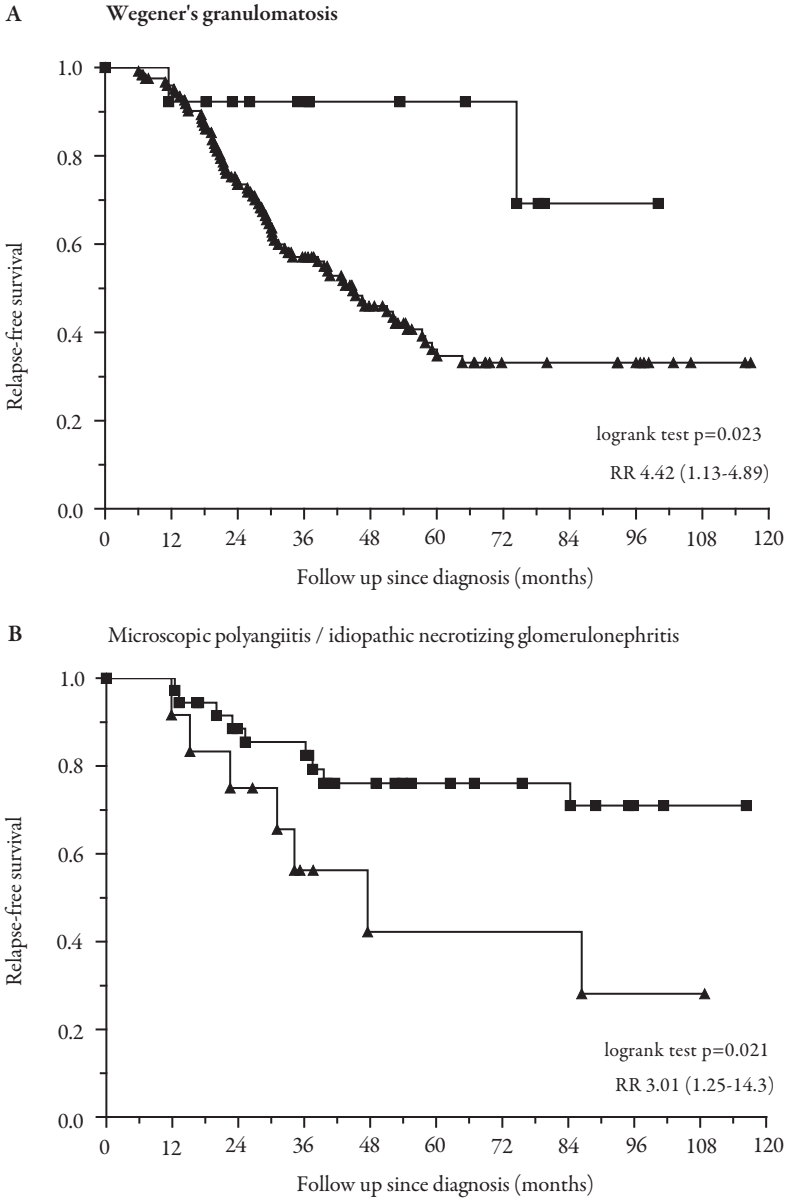

Figure 2. A Disease-free survival during follow-up in patients diagnosed with Wegener's granulomatosis between 1990 and 2000 at the University Hospital Groningen according to ANCA specificity (PR3-ANCA; n=124; ▲, and MPO-ANCA; n=13; ■). (Adapted with permission from reference 21). **B** Disease-free survival during follow-up in patients diagnosed with microscopic polyangiitis and idiopathic necrotizing crescentic glomerulonephritis between 1990 and 2000 at the University Hospital Groningen according to ANCA specificity (PR3-ANCA; n=12; ▲, and MPO-ANCA; n=36; ■).

in ANCA-levels and vasculitic disease activity by analyzing IgG subclasses of ANCA or specific functional aspects of the interaction of ANCA with their antigens, such as neutrophil activating capacity and interference with PR3 and MPO inhibition by α-1-antitrypsin and ceruloplasmin, respectively. So far, the results of these studies have been negative and analysis of these functional aspects of ANCA *in vitro* are too cumbersome to have a place in the surveillance of patients.

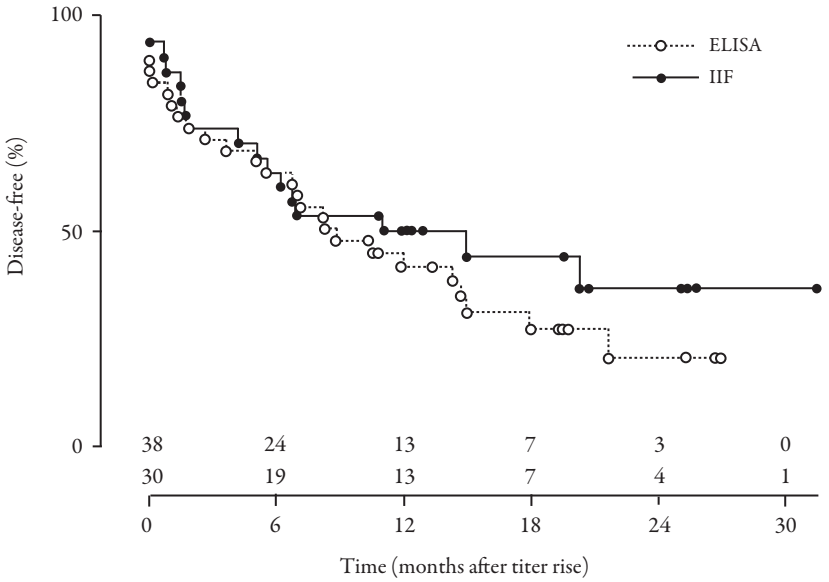

Figure 3. Percentage of patients with Wegener's granulomatosis who did not experience disease relapses in the indicated time period after a rise in antineutrophil cytoplasmic antibodies as measured by either indirect immunofluorescence (IIF; n=30) or antigen-specific enzyme-linked immunosorbent assay (ELISA; n=38). The numbers above the horizontal axis indicate the number of patients who were still at risk for a relapse at 6, 12, 18, 24, and 30 months after the rise in antibody levels as detected by ELISA (upper numbers) or IIF (lower numbers). (Reprinted with permission from Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc., and the authors [25]).

T cell activation and cytokines

At the moment of active disease, activation of T cells can be found in patients with AAV. Abundant presence of T cells in involved tissues and markers of T cell activation on

circulating lymphocytes can be demonstrated. In addition, serum markers of T cell activity, notably sIL-2R (sCD25) and sCD30, are elevated in active disease and correlate with disease activity [26-29]. Moreover, activation of circulating T cells seems to persist during remission and may point to an ongoing immune stimulating process, which may predispose to renewed disease activity [30, 31]. Likewise, Ohlsson et al reported that low plasma levels of the immunosuppressive Th2 cytokine IL-10 during remission were associated with an increased risk of subsequent relapse in AAV [32].

Disease activity has also been correlated with altered presence of specific T cell subsets. Both in $CD4^+$- and $CD8^+$ T cell subsets expansion of memory effector cells and reduction in naive T cell subpopulations have been described. Again, these alterations seem to persist during remission [33]. Whether individualized differences in these distributions may be related to clinical disease activity is currently unresolved.

Genetic factors

Many polymorphic genetic factors, especially involving genes coding for immunologically functioning products, have been associated with AAV. For some of these factors hypotheses have been formulated regarding how they may influence the pathophysiology of AAV. However, only very few genetic factors have been found to relate to disease course and relapse.

mPR3 epression and neutrophil activation

Neutrophils store proteinase 3 (PR3) in specific and secretory vesicles, but PR3 is also constitutively present on the membrane of resting neutrophils [34, 35]. Between individuals the percentage of neutrophils with PR3 membrane expression differs from 0 to 100%, and also the level of expression may differ. The percentage of neutrophils expressing membrane proteinase 3 (mPR3) within one person is stable, and probably genetically controlled [35], [36]. WG patients tend to have higher percentages of mPR3 expressing neutrophils compared to healthy individuals [34, 37]. Within patients with WG the level of mPR3 expression on neutrophils is associated with the incidence of relapse with a relative risk of around 0.5 for those with low mPR3 expression during a median 80 months follow-up [34]. *In vitro* data show that stimulation by PR3-ANCA of $mPR3^+$ primed neutrophils results in higher degrees of superoxide generation, and more degranulation than $mPR3^-$ primed neutrophils from the same individual [38]. We found that stimulation of unprimed mPR3+ neutrophils with anti-PR3 antibodies could induce early activation, as detected by actin polymerization [39]. Moreover, the degree of actin polymerization upon stimulation with anti-PR3 antibodies correlated with the level of constitutive mPR3 expression on neutrophils. These data suggest that the degree of mPR3 expression has functional and, possibly, pathogenic consequences.

Fcγ Receptor polymorphisms

Fcγ receptors display genetic polymorphism, like the R/H131 polymorphism for FcγRIIa, and the V/F158 polymorphism for FcγRIIIa. Functional consequences of these polymorphisms are differences between genotypes in ligand affinity and specificity, as well as cell distribution [40]. Dijstelbloem et al showed that patients with WG were more prone to experience relapses in the first 5 years after diagnosis when they homozygously expressed both the R131 form of FcγRIIa and the F158 form of FcγRIIIa (n=12), both considered low affinity phenotypes, compared to all other FcγR phenotypes (n=79) (RR 3.3; 95% CI 1.6-6.8) [41]. Differences in affinity may affect clearance of immunecomplexes or opsonized micro-organisms. Otherwise, Fcγ-receptors may be involved in ANCA induced neutophil acitvation.

HLA-DR

Only weak associations of AAV with HLA-antigens were observed, with a reduced frequency of HLA DR13 in patients with AAV [42, 43]. Spencer et al studied a cohort of 59 patients and reported an association between persistence of ANCA during follow-up and the presence of HLA DR2 [44]. Likewise, we recently found an increased relapse rate during follow-up in HLA DR3 positive patients [45]. Whether these associations are primarily with class II genes or are due to linkage with other immunologically important polymorphisms located close to class II genes on chromosome 6, such as the gene encoding for TNF-α, is at present unclear [43].

Myeloperoxidase

Genetic polymorphisms in the promoter region of the MPO gene, which influence MPO expression, have been described. The MPO-463GG genotype was associated with two- to three-fold higher levels of MPO mRNA and protein expression than were GA/AA genotypes in myeloid leukemia cells [46]. In patients with MPO-ANCA-associated vasculitis the MPO-463GG genotype was more frequently present in female, but not in male patients [20]. In addition, MPO-ANCA positive vasculitis patients with this GG genotype were older at diagnosis and had a significantly reduced risk to experience relapse of disease compared to patients with GA/AA genotypes (RR 0.31; 95%CI 0.05-0.70). These observations were not confirmed in a recent study by Fiebeler et al, who did not find differences in the frequencies of MPO G-463A promoter polymorphisms between MPO-ANCA- and PR3-ANCA-associated vasculitis patients and healthy controls [47]. The possible association of GA/AA genotypes with earlier onset of vasculitis and more frequent relapses suggests that higher MPO levels could somehow be protective.

Other genetic influences

Polymorphisms in other immune-response genes have been studied in AAV. So far most of these polymorphisms have not been related to patient-outcome or relapse risk.

In the CTLA-4 gene, which is involved in T cell activation, both an $(AT)_n$ microsatellite and a single nucleotide polymorphism in the promoter region have been linked to WG [48, 49]. Regarding the IL-10 (-1082) polymorphism, a shift towards the homozygous AA genotype was found in both WG and MPA [50, 51]. *In vitro* the IL-10 -1082AA homozygous genotype has been shown to correlate with low IL-10 secretion by ConA-stimulated peripheral blood mononuclear cells, which may be in line with the finding that low plasma levels of IL-10 during remission are associated with an increased risk of subsequent relapse [32, 52]. Patients with renal involvement of PR3-ANCA associated vasculitis had an increased risk of developing end-stage renal disease when carrying the proinflammatory IL-1β/IL-1Ra genotype characterized by high secretion of IL-1β and low secretion of its antagonist IL-1Ra [53].

Polymorphisms in the α-1-antitrypsin (a1-AT) gene, in particular the α1-AT PiZ allele which results in lower plasma concentrations of α1-AT, have been linked to AAV [54]. a1-AT is the natural inhibitor of the lytic activity of PR3. In a cohort of 99 patients with PR3-ANCA associated vasculitis, the 18 PiZ-positive patients did not have a higher relapse rate but did have more disseminated disease and a higher risk of fatal outcome [55]. Finally, the PR3 gene itself shows polymorphisms, and in WG an association was demonstrated with the A-564G polymorphism in the PR3 promoter which affects a putative transcription factor binding site [56]. So far no functional or clinical consequences of this polymorphism have been described.

Infection and microbiological factors

Given the fact that target antigens of ANCA are expressed and released by activated neutrophils and monocytes/macrophages, it is tempting to speculate on a relation between infection induced activation of these cells and subsequent development of disease activity in patients with AAV. Indeed, several cases of bacterial and other infections have been reported in which ANCA with specificity for PR3 and MPO have been detected [57]. Especially in cases of bacterial endocarditis, in which also vasculitic symptoms may occur, finding of PR3-ANCA may pose a diagnostic problem [58]. With respect to the occurrence of relapses, it has been suggested that infections may lead to disease activity and the site of infection might even determine the location of active vasculitis [59]. In addition, a seasonal influence in disease onset, with a higher incidence of WG in winter months, has been described, suggesting the

possible influence of (viral) airways infection on disease activation [60]. However, other studies, although documenting a high incidence of especially upper airways infection in WG, failed to provide a strong link between the occurrence of infections and relapse of active disease in ANCA associated vasculitis [22, 61].

Staphylococcus aureus has been shown a frequent cause of secondary infections of the paranasal sinuses in patients with WG [62]. It has been shown that 60 to 70% of patients with WG, far exceeding the percentage seen in healthy individuals, are chronic nasal carriers of *S. aureus*, which may explain the preponderance of this organism in causing infections. In addition, it was found that nasal carriage is a significant risk factor (RR 7.2; 95%CI 1.6-31.5) for the occurrence of relapses in WG and that nasal carriage was associated with persistently positive C-ANCA titers during follow-up [22]. These data suggest a role for *S. aureus* in disease activation in WG, although the pathophysiology of this association is currently unclear. *S. aureus* has the ability to produce several immune activating proteins like superantigens which polyclonally stimulate T cells expressing particular Vβ patterns. We found, however, no clear association between T lymphocyte Vβ repertoire in patients with WG and the presence of particular *S. aureus* derived superantigens in *S. aureus* isolates from their nasal cavities [63]. We did find, however, a relation between the occurrence of relapses in WG patients and the presence of TSST-1 superantigen in *S. aureus* isolates from the nasal cavity. For the other ANCA related vasculitic diseases associations between disease activity and infections or the presence of microbiological organisms are less clear. Cases of MPA (and WG) in association with parvovirus B19 infection have been described [57, 64]. Likewise, cases of Churg-Strauss syndrome have been described in association with infestation/infection with Ascaris, Aspergillus sp., and HIV [65]. In the majority of patients with active AAV, however, a clear precipitating event cannot be identified. Finally, many cases have been published in which a temporal relation of vasculitic disease activity with vaccinations have been described. As many individuals receive vaccinations and large cohort studies or randomized trials on the effects of vaccination on vasculitic activity in patients with AAV have not been performed, the role of vaccinations in precipitating disease activity is unclear [66, 67].

The potential role of infections in ANCA-associated vasculitis is supported by the finding described in case series that some patients with WG and limited disease activity can be successfully treated with trimethoprim-sulfamethoxazole [68-70]. In addition, a prospective, placebo-controlled randomized trial has shown that treatment with trimethoprim-sulfamethoxazole for 2 years in patients with WG who are in remission significantly reduces the risk for relapse by 60% [61]. However, in another prospective study 42% of patients with

generalized WG on co-trimoxazole maintenance relapsed after a median 13 months whereas 29% of patients without co-tromoxazole relapsed after a median 22.5 months [70].

Whether the effect of trimethoprim-sulfamethoxazole is caused by its anti-microbial effects and suppression of *S. aureus* or by its immune modulating effects on inflammatory cells as has been postulated based on *in vitro* data, is as yet unresolved [71]. Currently, data on the potential efficacy of other antibiotic agents in ANCA-associated vasculitis are not available.

Can treatment in ANCA-associated vasculitis be adapted to risk factors for relapse?

The morbidity and mortality associated with relapses combined with treatment related toxicity ask for prevention. Although their pathophysiological role is not always well understood, the identification of several risk factors has made tailored intervention strategies feasible. First and foremost, patients with PR3-ANCA associated vasculitis have a more than 3-fold higher risk for relapse within 5 years following diagnosis than patients with MPO-ANCA-associated vasculitis, an effect which seems to be independent from the specific diagnosis. The lower absolute risk for relapse in MPO-ANCA-associated vasculitis makes it probable that prolonged maintenance treatment in this group will not be effective, unless other specific risk factors with high predictive value can be identified. In PR3-ANCA-associated vasculitis, patients who remain or again become C-ANCA positive during induction of remission have an increased relapse risk, which could indicate that these patients may benefit from prolonged immunosuppressive maintenance therapy. However, the toxicity of long-term immunosuppression could outweigh the benefit of a potential reduction of relapses. We are currently evaluating in a randomized study whether long-term azathioprine maintenance treatment in PR3-ANCA positive patients who remain C-ANCA positive after induction of remission by cyclophosphamide for 3 months, indeed reduces the occurrence of relapse. This study will provide data that can demonstrate whether pre-emptive treatment with toxic agents is effective in this subgroup of patients with PR3-ANCA-associated vasculitis who have an increased risk to experience a relapse during long-term follow-up, and does not lead to increased toxicity.

Studies that evaluate soluble or cellular markers of immune activation at several points in time for their predictive value have to be performed in order to see whether they can improve the identification of high and low risk groups additive to the predictive role of ANCA. Finally, patients who have previously experienced a relapse apparently have a relapse prone phenotype and may be in need for adapted treatment when disease activity occurs. Solid data supporting this view are, however, lacking.

Several genetic factors, i.e. constitutive membrane PR3 expression, Fcγ Receptor-, HLA DR antigen- and MPO-polymorphisms, have been identified as risk factors associated with relapse. So far, the applicability of these factors in individual treatment strategies appears limited and data on treatment adaptations based on these factors are absent. Moreover, sufficiently large size studies evaluating multiple polymorphisms and their interaction with relapses have not been undertaken. Exogenous factors, particularly chronic nasal carriage of *S. aureus*, have been identified as a risk factor for relapses in WG. Since an association is by no means equivalent to a causative role, it still remains to be proven that eradication of *S. aureus* (if possible) will lead to a reduction in relapses. Whether or not caused by an effect on *S. aureus*, trimethoprim-sulfamethoxazole has been proven effective in preventing relapses in WG and maintenance therapy in patients with WG should be considered.

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