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ANCA-associated renal vasculitis - epidemiology, diagnostics and treatment

This thesis was written as part of post-graduate studies in biomedicine under the scientific guidance of prof. Vladimír Tesař, M.D., PhD at the Nephrology Clinic, 1st Medical Faculty, Charles University, Prague.

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1. INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV), that is Wegener's Granulomatosis (WG), Churg-Strauss Syndrome (CSS), microscopic polyangiitis (MPA), and its renal limited form belong to the group of small-vessel vasculitides. Their annual incidence in Europe is 10 to 20 per million per year and they account for at least 5% of the causes of end stage renal failure. There is an increased incidence with age, with a median age of 56 years in studies over the past 10 years. Males slightly predominate (1,2).

As the title suggests, the AAV are strongly associated with ANCA. ANCA are most often directed to either proteinase 3 (PR3-ANCA) or to myeloperoxidase (MPO-ANCA). Both PR3 and MPO are located in the azurophilic granules of neutrophils and the peroxidase-positive lysosomes of monocytes. Two types of ANCA assays are currently widely used – a more sensitive indirect immunofluorescence assay (IIA) and a more specific enzyme-linked immunosorbent assay (ELISA). With IIA, two major patterns are observed. With the C-ANCA pattern, the staining is diffuse throughout the cytoplasm (C-cytoplasmic type of immunofluorescence). In most cases, antibodies directed against PR3 (detected by ELISA) cause this pattern. The perinuclear or P-ANCA pattern results from a staining pattern around the nucleus. The antibody responsible for this pattern (detected by ELISA) is usually directed against MPO. To maximize diagnostic utility, current guidelines recommend dual testing, to screen with IIA and to confirm all positive results with ELISAs directed against the vasculitis-specific target antigens (mainly PR3 and MPO) (2).

The clinical manifestation of WG, MPA, and CSS are extremely varied because they are influenced by the sites of involvement, and the activity versus the chronicity of the involvement. All three categories of vasculitis share features caused by the small vessel vasculitis, and patients with WG and CSS have additional features that define each of these syndromes. Generalized nonspecific manifestations of systemic inflammatory disease, such as fever, malaise, anorexia, weight loss, myalgias, and arthralgias, are often present in all the entities.

The diagnosis of WG is suggested from the clinical and laboratory findings and from the presence of ANCA that are more often directed against PR3 then to MPO (3). Renal disease is common (80%), manifested by acute renal failure and/or active urinary sediment with red cells, red cell and other casts, and proteinuria. Lung involvement is found in up to 90% of patients with WG, while E.N.T involvement also occurs in about 90%. Other organ systems that may become involved include: musculoskeletal system, skin, nervous system, eyes, heart and others. Basically, any organ may be affected (4,5).

Necrotizing glomerulonephritis is also very common in MPA. Pulmonary capillaritis often occurs, but, by definition, patients with MPA do not have granulomatous respiratory tract lesions. Similarly, E.N.T. lesions may occur in MPA, but they are caused by angiitis alone, without granulomatous inflammation. Neurologic, musculoskeletal and other organ involvement is similar to those with WG, eye involvement is less frequent than in WG. Patients with MPA have MPO-ANCA in 50%, PR3-ANCA in 40%, and are ANCA negative in 10% (5).

CSS is a necrotizing vasculitis with eosinophil-rich and

granulomatous inflammation affecting small to medium-sized vessels, involving the respiratory tract, and is associated with asthma and eosinophilia (6) Patients with CSS have MPO-ANCA in 60%, PR3-ANCA in 10%, and they are ANCA negative in 30%. The vasculitis typically involves the arteries of the lung (70%) and skin (60%), but may be generalized. Renal involvement is less frequent in CSS (45%), E.N.T. involvement occurs in about 50% of patients. On the other hand, neurologic manifestation is most frequent in CSS compared to other AVV.

RLV, or isolated (idiopathic) pauci-immune necrotizing/crescentic glomerulonephritis is distinguished from MPA and WG by the absence of extrarenal symptoms of vasculitis. It is more often MPO-ANCA positive and therefore considered a renal limited form of MPA.

AAV is a complex, immune-mediated disorder in which tissue injury results from the interplay between an initiating inflammatory event and a highly specific pathogenic immune response to previously shielded epitopes of neutrophil granule proteins. This generates high titer ANCA directed against antigens within the primary granules of neutrophils and monocytes. These antibodies produce tissue damage via interactions with primed neutrophils and endothelial cells. Inflammatory cytokine production and adhesion molecule activation or upregulation are important determinants of the pathogenic inflammatory responses noted in vasculitis. The exact mechanisms by which ANCA arise and their role in the etiology of AAV remain unclear. The exact events leading to the initiation of the disease are obscure. Infectious (chronic

nasal carriage of *Staphylococcus aureus)*, genetic, and environmental (exposure to silica-containing dust) risk factors and combinations of all three have been entertained (7).

The pathogenesis of AAV is complex and is likely to involve many mechanisms. Although ANCA antigens are normally in the cytoplasm of neutrophils and monocytes, preactivation (priming) of these cells, as occurs following exposure to low amount of pro-inflammatory cytokines results in the release of small amounts of ANCA antigens at the cell surface. During priming the target antigens of ANCA, i.e. PR3 and MPO, are expressed at the cell surface and become accessible for interaction with ANCA. The interaction of ANCA and target antigens is followed by activation of neutrophils. The neutrophils undergo a respiratory burst and degranulation (8). The degranulation of neutrophils and release of chemoattractants and cytotoxic oxygen free-radicals causes tissue damage (9). In addition, primed neutrophils not only damage endothelial cells, but attract additional neutrophils to the site of damage, thereby creating an auto-amplifying loop. The release of MPO, PR3, elastase and other proteases from activated neutrophils also contributes directly to the local inflammatory process. Endothelial cells are the target of the initial injury resulting in swelling, necrosis and deadherence of endothelial cells. Lysed neutrophil granulocytes are found within affected vessels. In the lung, capillaries, venules and arterioles are infiltrated by polymorphonuclear leukocytes. Pulmonary microvascular necrotizing vasculitis (capillaritis) is the cause of pulmonary hemorrhage. In the kidney, rupture of the basement membrane subsequent to neutrophil degranulation gives rise to glomerular capillary thrombosis followed by a

cascade of events leading to focal segmental crescentic glomerulonephritis.

There is growing evidence that T cells may contribute to the pathogenesis of AAV, but their specific role is still uncertain. In active AAV, the cellular infiltrates in kidney, lung, and nasal tissue mainly consist of macrophages, T and B cells (10-14). The immunopathological process is T-cell-driven and ANCA production appears to be T-cell dependent. Peripheral blood T-cell responses to PR3-ANCA are seen in patients and to a lesser extent in controls. Understanding the mechanisms resulting in loss of tolerance in patients with systemic vasculitis may be of importance for prognosis and the development of new immunotherapies (15).

Prior to the introduction of immunosuppressive therapy, the outcome of the patients with AAV was fatal, with most patients dying in less than a year due to a vital organ failure (5). The current concept of therapy is an aggressive treatment of active disease and a lower-intensity therapy for the maintenance of remission. Most physicians favor a cyclophosphamide-corticosteroids combination regimen in the initial treatment of most patients with AAV. This is particular indicated in those with life-threatening disease, including patients with a serum creatinine concentration above 177 μmol/l, pulmonary involvement, CNS disease, and/or bowel perforation/infarction. Plasmapheresis should be added in patients with dialysis-dependent renal failure and life-threatening pulmonary hemorrhage at presentation, especially in those with high titer ANCA. Once complete remission is achieved,

cyclophosphamide is discontinued and either methotrexate (which is an option only in those with a serum creatinine < 177 μmol/L) or azathioprin is initiated. Maintenance therapy is usually continued for 12 to 24 months. Slow tapering of CS is initiated once there is a significant response, which usually occurs after one month. A low-dose (5-10 mg per day of prednisone), possibly in an alternate day regimen, is maintained for as long as immunosuppressive therapy is continued. In patients with chronic grumbling vasculitis despite conventional treatment, refractory or with contraindications to standard immunosuppressants several other immunosuppressants are tried with sometimes very promising results.

It has been noted that PR3-ANCA are predominantly found in patients with WG and MPO-ANCA in patients with MPA, its renal limited form, or CSS. Irrespective of the clinical diagnosis, PR3-ANCA and MPO-ANCA are markers of different disease entities within the spectrum of AAV. Patients with MPO-ANCA have a higher median age at presentation than patients with PR3-ANCA. The prevalence of renal involvement does not significantly differ between PR3-ANCA and MPO-ANCA positive patients, but, prior to treatment, renal function deteriorates significantly faster in PR3-ANCA. Moreover, kidney biopsies show a higher activity index and a lower chronicity index than biopsies from patients with MPO-ANCA. However, although PR3-ANCA positive patients show a more active renal disease, kidney survival does not differ between PR3-ANCA positive patients compared to MPO-ANCA positive patients. It is believed that the more acute clinical presentation of patients with PR3-ANCA results in the

earlier institution of immunosuppressive treatment explaining the comparable or even better renal outcome (16-17).

AAV is a relapsing disease. The reported relapse rate differs from 16% in 18 months of follow-up (18) to up to 50% in long-term observations (19). It has been noted by several groups that PR3-ANCA relapse more frequently than patients with MPO-ANCA associated vasculitis (16, 20-22). The reason for this difference is not clear. In patients with PR3-ANCA persistence of ANCA after induction of remission is a risk factor for relapse. Longitudinal observations made by several groups (20, 23, 24) showed that relapses of WG were preceded by rises in ANCA titres. It has been even suggested that rising titers of ANCA may be used as a guideline for the institution of immunosuppressive therapy, but this has not been proven beneficial to the patient as the amount of cyclophosphamide required to prevent relapses may be harmful to the patient in the long term due to its toxicity (25). Besides rising titers of ANCA, persistence of ANCA after induction of remission in WG has also been identified as a risk factor for an ensuing relapse (24, 26, 27), which suggests that long-term maintenance treatment should be instituted in patients who are persistently positive for ANCA after induction of remission. On the other hand, a persistently ANCA-negative status is not an absolute proof of remission. A second factor relevant for relapse in WG is chronic nasal carriage of *Staphylococcus aureus*. Stegeman et al. observed that 63% of patients with WG were chronic nasal carriers of *S. aureus* and that relapses occurred almost exclusively in these patients. In agreement with these data, maintenance treatment with co-trimoxazole resulted in a

reduction of relapses in WG (26).

AAV is a life-threatening disease that requires prompt recognition and therapy. Prognosis is an especially important tissue as the disease process is aggressive and the therapeutic options are inherently dangerous. Since the introduction of immunosuppressive therapy in the treatment of AAV, mortality has significantly decreased from 82% in 1 year to 59-95% in various patients groups during various follow-up periods, and remission rates have increased to up to 93% (20). At the time of disease presentation, clinicians are faced with several factors that may influence the outcome of the patients. The treatment used in AAV is toxic and carries the potential risk of life-threatening infection. Additionally, cytotoxic agents are associated with cancerogenesis, mutagenesis, infertility, and interstitial cystitis. Significant prognostic factors for mortality were found to be the entry age (28-31), the serum creatinine level and dialysis-dependence at presentation (28-31), the developing dialysis-dependence during follow-up (30), the multi-system manifestation and the presence of pulmonary hemorrhage (32). Overall, the morbidity and mortality results from several factors. In the early phase of the disease it is associated with irreversible organ dysfunction because of inflammatory injury (within days), further on with aggressive immunosuppressive therapy and its short-term adverse effects, namely infections (within months), and long-term sequellae, such as secondary tumors, myelodysplastic syndrome, accelerated atherosclerosis etc. (28, 30).

The reported renal survival in patients with AAV with renal involvement differs according to the severity of renal disease at presentation, from 65% at 5 years and 51% at 10 years (30), to

44% at 48 months (32). The strongest predictors of long-term renal outcome are the entry serum creatinine level and dialysis-dependence and the occurrence of renal relapses (28, 30-32).

OWN OBSERVATIONS - AIMS AND RESULTS

1. The diagnostics of AAV was described in "Multiple Extrarenal Complications of Wegener´s Granulomatosis" published in Case Report and Clinical Practice Revue. It is a case report of a patient with WG with an unusually high number of extrarenal complications. The patient had a two-year history of intermittent bloody nasal discharge; subsequently she developed a 'saddle' nose. After a febrile episode and arthralgias, the disease directly presented with a corneal perforation. The conjunctival, ENT and renal involvement were verified histologically. The peculiarities of this case included a double cardiac involvement - an acute myocardial infarction due to coronal arteritis and a vasculitis of the aortic valve - and gastrointestinal bleeding. Another relatively infrequent complication was the development of a very tight subglottic stenosis during the remission of the disease. Since all markers of pathologic activity were normal, we considered the development of stenosis as a result of reparative changes.

2. The etiologic factors of AAV were discussed in two papers - "Silica and Asbestos Exposure in ANCA-Associated Vasculitis with Pulmonary Involvement" published in Renal Failure and "Two Familial Cases of antineutrophil cytoplasmic antibody (ANCA)- associated vasculitis" published in Rheumatology.
First we carried out a study to find out whether patients with pulmonary involvement attributable to AAV have been exposed to silicon-containing materials. We included a total of 31 patients with AAV with pulmonary and renal involvement diagnosed in our center between the years 1993 and 2002. The

patients were asked to complete questionnaires designed by the occupational health physicians to evaluate their exposure to silica-containing chemicals and estimate their extent. Seven AAV patients (22.6%) had at one time been exposed to silicon-containing chemicals (12.9% to SiO2, 9.7% to asbestos). The mean length of exposure was almost 4 years. In all cases the exposure was considered as low. We explored the possibility that the exposure was more likely to be associated with sex, smoking, and specific ANCA pattern or disease category. None of these was proven significant, although there was a tendency towards higher exposure in the MPO group. The results were compared to those of 30 age, sex, and residence-matched controls, where no patients were found to have any previous occupational exposure to silicon-containing materials. The difference was statistically significant ($p<0.05$). Exposure to smoking was comparable in the AAV and control groups (41.9% vs. 43.3% of smokers). It is largely accepted that AAV is genetically based but environmentally triggered, and there is an increasing evidence of a pathophysiologic role of silica in AAV, although the mechanisms by which silica may induce AAV are not well known. We were able to show a significantly higher anamnestic exposure to silicon-containing compounds in our group of AAV patients compared to the control group.
Morover, the patterns of pulmonary involvement in AAV _ not yet satisfactorily described in literature _ were discussed in this paper. The majority of our patients had an obstructive disease on spirometry and either normal or reduced TLCO. However, as the tests were mostly performed in remission, these findings reflect the consequences of pulmonary AAV attributable to vasculitis damage, and not the vasculitis activity.

Subsequently, the role of a genetic predisposition to AAV was addressed in a description of two familial cases of AAV. In the first family, the father presented with an acute renal failure due to PR3-ANCA-associated disease three years before his daughter had exactly the same manifestation. However, at the time of her admission to our ward she had already had E.N.T. involvement, which was later attributed to Wegener's granulomatosis. The second family involved two sisters, who were diagnosed with microscopic polyangiitis with a more gradual decline of renal function within four months of each other. The younger sister actually suggested the diagnosis of her sibling based on her symptoms and probably saved her a significant portion of renal function.

To our best knowledge, these are the first two familial cases of AAV from the Czech Republic described so far. They illustrate several very important aspects of the AAV from both theoretical and practical points of view. They show that the clinical manifestation of the different AAV are extremely varied because they are influenced by the sites of involvement and the activity versus the chronicity of the involvement. Generalized nonspecific manifestations of systemic inflammatory disease are often present in all entities. These contitutional and frequent E.N.T. or respiratory symptoms make the diagnosis difficult and lead mostly to a treatment with a variety of antibiotics. The detection of blood in the urine very often prompts another course of antibiotics for a presumed urinary infection and subsequently, as the finding does not resolve, an extended urologic work-up is done. Another tricky feature of the disease is the possibility of temporary spontaneous withdrawal of the symptoms, which further

confuses and delays the diagnosis. As the disease reappears or persists, it may start to resemble a malignancy. We have had several patients in whom the diagnosis of WG was established from a kidney or lung lobe removed because of a mass in the organ, and morphological features of a necrotizing granulomatous inflammatory disease made the situation clear (unpublished data). Admittedly, there are patients with an isolated mass and no other symptoms in whom the invasive procedures seem to be justified, but they are rare. An erroneous clinical and histologic diagnosis of carcinoma with a subsequent oncologic treatment in the setting of a histological diagnosis of cutaneous vasculitis _ as happened to the female patient of the first family _ is a grave mistake.

However varied the AAV may be, their clinical presentation in the two families described was in some aspects remarkably similar. Both the father and daughter from the first case study had WG with ANCA directed against PR3. In both of them a history of E.N.T. involvement preceded the presentation with dialysis requiring renal failure. The renal biopsies in both had some corresponding features. The two sisters had both MPA with MPO-ANCA. Their clinical presentation with nonspecific constitutional symptoms and the histological and laboratory evidence of a rather slower decline in the renal function was the same. The disease in the older sister was detected earlier thanks to the younger one. Her morphological features were more active without chronic changes and her symptoms were therefore presumably lesser pronounced. We were unable to obtain additional objective data on their father's illness. Nevertheless, the daughters decribed a suggestive picture of AAV with pulmonary involvement that resembled that of WG.

The difference in the disease presentation in the two families shows that PR3-ANCA and MPO-ANCA are markers of different disease entities within the spectrum of AAV.

Last, but not least, the presented case reports raise the question of a familial predisposition in AAV. Our patients within the two families shared a similar genetic background with the HLA haplotype A 01, B 57, Cw 06, DRB1 07/11 in the affected members of the first family and A 02/30, B 8/62, Cw 03, DRB1 04 in the two sisters. The healthy members of both families were thoroughly checked, including for ANCA, and no signs of AAV were found. Mutations in the gene encoding a-1 antitrypsin (AAT), the natural in vivo inhibitor of PR3, are more frequently found in patients with AAV. All our patients had AAT levels within the normal range. The patients described in our study did not share the same environment. The fact that two members of the two families fell ill with the same disease and their similar HLA support the hypothesis that genetic predisposition plays an important, although not yet fully understood role.

3. Inflammatory cytokine production and adhesion molecule activation or upregulation are important determinants of the pathogenic inflammatory responses noted in vasculitis. The role of T cells was addresses in

"Regulatory Cytokines in ANCA-associated Vasculitis" published in proceedings from the 11th European Meeting on Cardionephrology. We examined 48 peripheral blood samples of patients with AAV and 21 peripheral blood samples of age- and sex-matched healthy controls. Using flow cytometry, the following markers were assessed: T lymphocytes activation

markers (HLA DR+, CD28+, chemokine receptors CXCR3 and CCR5), surface molecules CD4, CD8, CD3, CD19, costimulation molecule CD80 on B lymphocytes, and intracellular cytokines: interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), interleukin 2 (IL-2), interleukin 4 (IL-4) in CD3+ T cells, and interleukin 10 (IL-10) and interleukin 12 (IL-12) in monocytes.

We found higher IL-2 and activation markers on T lymphocytes (DR+) and B lymphocytes (CD80+) in the patients when compared with healthy subjects. This activation of the immune system persisted even during remission. The IL-2 production was significantly lower in MPA when compared with WG suggesting less inflammation in MPA. On the other hand, the patients had lower IL-10 and IL-12, most probably as a result of previous or ongoing immunosuppressive treatment. Morover, the patients had higher levels of IFNγ and CCR5 when compared with healthy controls, which represents a significant shift towards Th1 population. The higher number of CD8 positive cells and lower number of CD4 positive cells indicates that cytotoxic T lymphocytes are involved in the pathogenesis of AAV. The number of DR positive activated T lymphocytes and TNFα production increased with advanced renal failure. In conclusion, our study supported the hypothesis of AAV being a Th1 mediated disease. The difference in the IL-2 production between WG and MPA patients suggests a different cytokine regulation of immune reaction in these patients.

4. Although the incidence of AAV is increasing, it is still low and therefore necessitates multicentric studies on its treatment

and prognosis to responsibly address these issues. We discussed the treatment of AAV in two papers. The subclassification of AAV based on the disease severity at presentation, and the two EUVAS waves of clinical trials were reviewed in "Current treatment strategies in ANCA-positive renal vasculitis - lessons from European randomized trials." The first wave of randomized clinical trials had the aim of optimizing the existing therapeutic regimens. The second wave concentrated on testing of some newer therapeutic approaches.

Our centre closely cooperates with European Vasculitis Study Group (EUVAS) and repeatedly includes a considerable number of patients in the studies. Between years 1995 and 2001 we contributed a total of 40 patients to the trials CYCAZAREM (cyclophosphamide versus azathioprine during remission for generalised vasculitis), MEPEX (plasma exchange versus methylprednisolone for severe renal vasculitis) and CYCLOPS (daily oral versus pulse cyclophosphamide during induction phase for generalised vasculitis). We continue to randomize our patients to subsequent studies such as the IMPROVE trial comparing mycophenolate and azathioprine as a maintenance therapy in patients in remission. So far, most of the patients have been included in the international randomized trial CYCLOPS, the aim of which was to optimise the treatment of induction of remission in patients with generalized, but not immediately life-threatening AAV, which is the stage of the disease where most of our patients are diagnosed. The intent was to reduce the toxicity of induction therapy by reducing the overall dose of CYC during the induction period by using it in intermittent pulsed form.

We published the results of patients included in our center in

"Daily oral versus pulse intravenous cyclophosphamide in the therapy of ANCA-associated vasculitis – preliminary single center experience" in Prague Medical Report. Our center included 28 patients from Dec. 1999-Nov. 2001. Eighteen (12 women, 6 men) were evaluable at the moment of analysis. The mean age at presentation was 54.6 years. Seven patients were randomised to the pulse limb. Eleven patients were randomised to the oral limb. The cumulative dose of CYC in pulse limb was approximately 150 mg/kg body weight/6 months. In the oral limb the cumulative dose of CYC was more than two-fold, 315 mg/kg body weight/6 months. All the patients in the pulse limb (100%) achieved remission, contrary to only 55% of patients in the oral limb. During the whole period studied, there was only one relapse in the pulse limb, which occurred 5 months after the cessation of immunosuppressive treatment. The number of infectious complications in immunosuppressive treatment was comparable in both limbs (pulse 43% vs. oral 45%). However, there were only 14% _ one patient _ of serious (i.e. requiring hospitalization) events in the pulse limb. The patient had leukopenia ($1{,}7.10^9$ /L) caused by CMV infection and was succesfully treated with ganciclovir. In the oral limb, 27% of infectious complications _ two patients _ were severe and unfortunately resulted in the death of both. All three patients died during the induction treatment, even though CYC was always stopped as soon as leukopenia and infection were ascertained. The overall mortality was also higher in the oral limb (36%) compared to the pulse limb (14%). Apart from the three patients from the oral limb who died of infectious complications there was one more death in this group, not related to the diagnosis or therapy. This patient died in a local

hospital due to bleeding caused by an overdose of coumarine. In the pulse limb, only one death was recorded. This patient died of pulmonary embolism while on a maintenance dose of CS (prednisolone 10mg/day). We cannot confidentaly exclude that the therapy did not contribute to the fatal complication.

The final results of the multicentric randomized trial CYCLOPS have not been yet published. Our preliminary results of a small group of patients confirm the higher toxicity of oral CYC that resulted in higher morbidity and mortality in this group. Surprisingly, in our hands, the efficacy of pulse CYC seemed to be better. This was certainly due to the small number of patients and the high mortality in the oral limb. There was no early relapse in the followed-up period (18 months after enrollment). In the pulse limb, one patient relapsed later on. The preliminary results of the patients from all centers proved the intermittent pulse administration of CYC to be as efficient as the pulse administration with a significant reduction of the cumulative dose and both short-term and long-term toxicity of CYC.

5. The presenting features, response to therapy and the overall and renal survival of patients with AAV with renal involvement were studied in "Long-Term Outcome of Patients with Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitis with Renal Involvement" published in Kidney and Blood Pressure Research. This retrospective analysis involved patients who were diagnosed and followed in our center between 1986 and 1997. Special attention was paid to the impact of age, diagnostic subgroups, level of renal function and type of ANCA on the outcome of the patients.

Sixty-one patients were included in the study (54.1% had WG, 23% RLV, 16.4% MPA and 4.9% CSS). The median age was 54 years, and 60.7% were men. Patients with RLV and MPA were significantly older then patients with WG. ANCA was detected in 85.2% (C-ANCA in 57.4%, P-ANCA in 42.6%). The median follow-up was 90.5 (range 1–168) months. All patients received homogenous induction treatment according to the treatment guidelines in the respective years. It consisted of oral continuous CYC and CS. Plasma exchange was used in 29.3% of patients. In 45.2% of patients CYC was switched to AZA after reaching stable remission. More than three quarters of the patients had generalized disease at presentation. By definition, all patients had a renal involvement, which was biopsy-proven in 87%. The median serum creatinine level of patients dialysis-independent at diagnosis was 221.5 μmol/l, and 32.8% were dialysis-dependent. Initial renal function did not differ according to sex, age or C-ANCA/P-ANCA.

Remission was achieved in 87% of the patients, more often in patients with independent renal function at presentation. All the patients who did not achieve remission died. Relapses occurred in 44.7%. The median disease-free interval was 58 months, the median renal disease-free interval was 62.5 months. The relapse rate was higher in C-ANCA-associated disease when compared to P-ANCA, although the difference was of borderline significance. For the group of 61 patients, the estimated patient survival at 5 and 10 years was 78.3 and 62.2%, respectively. The estimated survival depended on age. I did not depend on sex, initial proteinuria, Creactive protein or hemoglobin level. Patients who had to be initially hemodialyzed had significantly worse estimated survival

compared to patients who were dialysis-independent. Pulmonary involvement did not have any impact on estimated survival, nor did the use of plasma exchange in the initial treatment. Nineteen of 61 patients (31%) died. The median time to death was 41.3 months. Six patients died of infectious complications related to immunosuppressive treatment; 7 patients due to cardiovascular events; 2 of exsanguination from a gastric ulcer; 2 of cancer; 1 of multi-organ failure, and 1 cause of death is unknown. No patient died of active vasculitis. Nine patients presented with lung hemorrhage, and none of them died of this condition. The estimated renal survival at 5 and 10 years was 69.2 and 55.8%, respectively. None of the variable studied had any impact on the renal prognosis. Twenty-five of 61 patients (41%) suffered from adverse effects of their therapy. A severe bacterial infection requiring hospital admission occurred in 16 patients (26%). A severe viral infection requiring hospital admission occurred in 5 patients (8%). Solid tumors occurred in 3 patients. However, in only 1 of them (lung cancer 5 years after the diagnosis of WG) the malignancy may have been secondary to the immunosuppression.

In conclusion, our retrospective study comprised a high number of patients from one center treated in principle with a uniform induction regimen and followed for a long period of time. All patients had a relatively severe renal involvement, most of them were biopsied. The demographic data in our cohort confirmed that at presentation, patients with RLV and MPA were older compared to WG patients, possibly due to the more indolent course of the disease leading also to the higher serum creatinine level at presentation in the RLV group. AAV is a life-

threatening disease that requires prompt recognition and therapy. Prognosis is an especially important issue as the disease process is aggressive and the therapeutic options are inherently dangerous. The cumulative 5- and 10-year patient survival in our cohort was 78.3 and 62.2%, respectively, which is comparable to the findings of other investigators. Mortality was associated with age over 50 and advanced renal failure at presentation. No patient died of active vasculitis. The causes of early mortality were related to the adverse effects and toxicity of the treatment (infections, gastrointestinal bleeding). Infections remained the main cause of morbidity as well. Late mortality was mainly due to cardiovascular events. In view of the severity of the renal disease at presentation and the length of follow-up, the renal survival in our study was very satisfactory. In this study, a relapse rate of 44.7% was noted with the median renal disease-free interval of more than 5 years; the vast majority of patients were no longer on immunosuppressive treatment at the time of relapse. Renal relapses probably have a major impact on the loss of independent renal function in the course of the disease. However, some of our patients progressed to end-stage renal failure without experiencing any relapse. In these cases, the non-immunologic progression of renal disease was probably involved, which highlights the importance of optimal conservative care, in particular careful blood pressure control, preferentially using ACE inhibitors, or angiotensin-II antagonists. Consistent with previous reports we noticed an increased relapse rate in C-ANCA-associated disease, which should probably be taken into consideration, especially in the length of maintenance treatment.

CONCLUSION

AAV is a multi-factorial disease with an increasing incidence. Although our knowledge of etiopathogenesis is increasing rapidly, the environmental and genetic factors involved in the etiology and the exact pathogenetic mechanisms remain to be elucidated. In a case-control study, we were able to show a significantly higher anamnestic exposure to silicon-containing compounds in our group of AAV patients with pulmonary involvement compared to the control group. In keeping with the published data, we found a tendency to higher exposure in the P-ANCA subgroup. None of the other factors studied (sex, diagnosis, smoking) were significant. The question of familial predisposition in AAV was raised in the first two familial cases of AAV from the Czech Republic described so far. The families shared a similar genetic background with the HLA haplotype A 01, B 57, Cw 06, DRB1 07/11 in the affected members of the first family and A 02/30, B 8/62, Cw 03, DRB1 04 in the second. The patients described in our study did not share the same environment. The fact that two members of the two families fell ill with the same disease and their similar HLA typing seem to favor the role of a genetic predisposition to AAV.

The issue of AAV pathogenesis was addressed in a study comparing cytokine profile in patients and healthy controls. The higher activation of the immune system observed in AAV patients persists even during remission. The IL-2 production was significantly lower in MPA when compared with WG suggesting less inflammation in MPA. The patients had higher levels of IFNγ and CCR5 when compared with healthy

controls, which represents a significant shift towards Th1 population. The higher number of CD8 positive cells and lower number of CD4 positive cells indicates that cytotoxic T lymphocytes are involved in the pathogenesis of AAV.

The last two decades, with the advent of cytotoxic therapy, have brought greatly increased survival probability, but a significant risk of infective complications in particular and many other problems with the management of chronic grumbling and relapsing disease with accumulating morbidity and mortality. The vasculitides are relatively rare and are heterogeneous in their presentation; hence the importance of well conducted, multi-centre collaborative trials to identify promising new therapies and to maximize the benefit of existing treatment regimens. Our preliminary results of patients recruited for the CYCLOPS trial confirm the higher toxicity of daily oral CYC with a double cummulative dose of the drug when compared to the intermittent pulse administration. In our hands, the efficacy of pulse CYC was at least as good as in the oral limb.

In our outcome analysis of 61 patients with renal AAV we were able to demonstrate a difference in age and initial renal function between the patients with RLV, MPA and WG. The estimated cumulative 5- and 10-year patient survival in our cohort was 78.3 and 62.2%. Mortality was associated with age and renal failure at presentation. Infection were the main cause of early morbidity and mortality. Late mortality was mainly due to cardiovascular events. The estimated renal survival at 5 and 10 years was 69.2 and 55.8%, respectively. In this study, a relapse rate of 44.7% was noted with the median renal disease-free interval of more than 5 years. Consistent with previous reports

we noticed an increased relapse rate in C-ANCA-associated disease, which should probably be taken into consideration, especially in the length of maintenance treatment. Our data confirm that despite effective induction treatment, the long-term outcome of patients with AAV remains unsatisfactory with a relatively high overall mortality (with standardized mortality ratio compared to the common population of about 2.5) and dialysis dependence due to a high relapse rate and toxicity of current treatment. Search for newer, more effective and less toxic modes of treatment is therefore warranted. As the outcome in AAV was found to be related to age and the level of renal function at presentation, a diagnostic delay may have a major influence on the outcome. An increased awareness of AAV with subsequent rapid ANCA testing, recognition of the presence of organ involvement and a quick referral of the patient to a specialist is therefore warranted.

The study of AAV therefore remains a challenge in all aspects.

LIST OF SUBJECT-RELATED PUBLICATIONS

Rihova Z, Honsova E, Zavada J, et al. Two familial cases of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Rheumatology (Oxford). 2006 Mar;45(3):356-7.

Rihova Z, Maixnerova D, Jancova E, et al. Silica and asbestos exposure in ANCA-associated vasculitis with pulmonary involvement. Renal Fail 2005;27(5):605-8.

Rihova Z, Jancova E, Merta M, et al. Long-term outcome of patients with antineutrophil cytoplasmic antibody-associated vasculitis with renal involvement. Kidney Blood Press Res. 2005, 28(3): 144-152.

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