Relapse Rate, Renal Survival, and Cancer Morbidity in Patients with Wegener's Granulomatosis or Microscopic Polyangiitis with Renal Involvement

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Abstract. Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are both frequently associated with antineutrophil cytoplasmic autoantibodies (ANCA). Immunosuppressive treatment has dramatically improved outcome for these patients, but today we have to deal with the problems of relapses, cases refractory to treatment, and long-term side effects of therapy. This study comprises a consecutive series of 123 patients with WG (n = 56) or MPA (n = 67) with biopsy-confirmed renal involvement, followed up for a median of 55 mo (range, 0.1 to 273.2 mo). ANCA was detected by enzyme-linked immunosorbent assay in 97% of patients. Nearly half of the patients (46%) relapsed. There was no statistically significant difference in overall relapse rate according to type of ANCA. Renal survival was 78% in patients alive at the end of follow-up. Three variables seemed important for renal survival: serum creatinine, the titer of proteinase 3-ANCA measured by capture enzyme-linked immunosorbent assay, and B thrombocyte count, at time of referral. Cancer incidence data were obtained from the population-based South Swedish Regional Tumor Registry. Standardized morbidity ratio was calculated using expected values from the health care region. We found an 11-fold increase in risk for bladder cancer in patients treated with cyclophosphamide for at least 12 mo. Skin carcinoma had the strongest relationship with azathioprine use for at least 12 mo and with corticosteroid therapy for at least 48 mo. In addition, four patients developed myelodysplastic syndrome and five had carcinoma *in situ* of the skin. Because the therapeutic regimen used today is not efficient enough to prevent relapses and is associated with a host of side effects, of which the risk for cancer is by far the most important, improved therapy and medical care are needed for patients with WG and MPA. (J Am Soc Nephrol 9: 842–852, 1998)

Systemic small vessel vasculitis comprises a heterogeneous group of diseases that are generally considered uncommon. According to the Chapel-Hill consensus, the group of systemic small vessel vasculitides strongly associated with antineutrophil cytoplasmic autoantibodies (ANCA) include Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and the Churg–Strauss syndrome (1). Since the association of these diseases with ANCA was demonstrated (2,3), the reported incidence of WG and MPA seems to have increased during the past decade. The annual incidence is now approximately 11 per million (4,5). The clinical picture varies and depends on the severity and extent of organ involvement, with WG most often associated with respiratory and renal involvement (6).

Renal involvement may be absent in the initial phase of WG and MPA, but it is well known that, in most cases, renal involvement develops over time (7,8), most often as a pauci-immune glomerulonephritis (GN).

Since the introduction of cyclophosphamide and corticosteroids in the treatment of WG, mortality has decreased from 82% in 1 yr to 20% in a 63-mo follow-up period (9), and remission rates have increased to 93% (8,10). Relapses do occur, however; in the series of patients presented by Fauci and Wolff (9), the relapse rate was 32% and in the material presented by Hoffman *et al.* (8) it was even higher (50%). Furthermore, side effects of therapy were noted: hemorrhagic cystitis in 34% and malignant lymphoma in one of 85 patients (10). The risk of malignancy, however, was found to be considerable in the material presented by Hoffman *et al.* (8). In 158 patients with WG followed up for more than 6 mo, there was a 2.4-fold overall increase in malignancies, a 33-fold increase in bladder cancer, and an 11-fold increase in lymphomas. Cyclophosphamide-induced cystitis has recently been reported in 50% of cyclophosphamide-treated patients with WG (11).

Because the clinical setting is similar in WG and MPA, we decided to retrospectively investigate the clinical outcome in a consecutive series of patients with small vessel vasculitis and pauci-immune GN, focusing on relapse rate, morbidity in end-stage renal disease (ESRD), mortality, and findings of malignancy during follow-up. Furthermore, we assessed the prevalence of ANCA and anti-glomerular basement membrane (anti-GBM) antibodies by enzyme-linked immunosorbent assay

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(ELISA) in stored sera drawn at the time of referral to our department.

Materials and Methods

Patients

The study included 123 consecutive patients (44 women and 79 men) admitted to the Department of Nephrology, University Hospital of Lund, between 1971 and 1993 with a clinical diagnosis of small vessel vasculitis, including histologically confirmed renal involvement. Our catchment area comprises approximately 1.2 million inhabitants. The median duration of follow-up was 55 mo (range, 0.1 to 273.2 mo).

Clinical Diagnosis

Patients with a small vessel vasculitis affecting the kidneys were subclassified into WG and MPA, respectively, according the Chapel-Hill consensus conference (1). Other small vessel vasculitic diseases such as systemic lupus erythematosus, the Churg–Strauss syndrome, cryoglobulinemia, Henoch–Schönlein purpura, and hepatitis small vessel vasculitis were excluded, as were patients with Goodpasture's syndrome. For WG, the criteria and definitions established by the American College of Rheumatology (12) were used. Only cases with a granulomatous disease either histology-proven or strongly indicated by noninvasive diagnostic procedures were classified as WG. Thus, patients with involvement of the upper or lower respiratory tract, without granulomatous inflammation either verified by histology or strongly indicated by noninvasive diagnostic procedures, were classified as MPA. The patients' medical records were investigated, and clinical diagnoses were reevaluated in all cases by two nephrologists. There were 56 patients (15 women, 41 men) with WG and 67 patients (29 women, 38 men) with MPA.

Morphologic Diagnosis

Renal biopsy was performed in all of the patients and was scrutinized by C. Brun and S. Larsen (Kommunehospitalet, Copenhagen, Denmark) before 1985, and after 1985 by Dr. H. Henriksson and P. Alm (Department of Pathology, University Hospital of Lund). Extracapillary GN was taken to include all cases with at least one crescent present in the kidney biopsy specimen. Necrotizing GN comprised signs of vasculitic disease with necrosis but without any crescents present. Immunofluorescence staining showed no or minor deposits of immune complexes (pauci-immune). Ninety-seven patients had an extracapillary GN, 13 a necrotizing GN, 12 a proliferative GN (one in which granulomata were found), and one a sclerosing GN, according to renal histology. Clinical and laboratory data of the patients at time of referral to our department are given in Table 1.

Organ Involvement

All of the included patients had histologically verified renal involvement. The majority of patients with WG (70%) had involvement of at least two other organ systems; the corresponding value for patients with MPA was 50%. Organ involvement was only recorded as such if registered in the medical record, if the clinical presentation was obvious at the time of referral, and if it was assessed to be caused by active vasculitis.

Laboratory Investigation

Routine laboratory tests were performed at the Department of Clinical Chemistry, University Hospital of Lund. Serum creatinine was measured by routine enzymatic methods with inter- and intra-assay coefficients of variation of <5% (reference interval, 60 to 115 μmol/L). B thrombocytes were analyzed with a Coulter or a Sysmex analyzer. If aggregates were found, a manually performed method was used. Inter- and intra-assay coefficients of variation with the Sysmex analyzer were 8 and 4%, respectively (reference interval, 140 to 400 × 10^9/L).

ANCA Analysis

Sera were drawn at the time of admittance to our department, *i.e.*, at the time of renal biopsy, and stored at −20°C until analyzed in February 1996. Sera from 120 of the 123 patients (98%) were available for analysis.

Proteinase 3-ANCA ELISA and Myeloperoxidase-ANCA ELISA

Microtiter plates coated with isolated human proteinase 3 (13) and with human granulocyte myeloperoxidase (MPO) (14) were obtained

Table 1. Clinical and laboratory data, according to clinical diagnosis for 123 patients with small vessel vasculitis affecting the kidneys[a]

Variable	Ref. Range	WG (n = 56)	MPA (n = 67)
Female/Male		15 /41	29 /38
Age median, yr (range)		58 (30 to 77)	65 (11 to 85)
Duration of follow up, median, mo (range)		67.1 (0.1 to 169)	47.3 (0.2 to 273.2)
PR3-ANCA[b] n (%)		47 (87)	26 (39)
MPO-ANCA n (%)		6 (11)	37 (56)
B hemoglobin (g/L) ± SD	113 to 166	101 ± 20	102 ± 21
B leukocytes (×10^9/L) ± SD	4.0 to 10.0	12.5 ± 5.4	10.3 ± 4.1
B thrombocytes (×10^9/L) ± SD	140 to 400	359 ± 148	361 ± 133
ESRD (mm/h)	2 to 32	82 ± 43	74 ± 41
CRP (mg/L)	<5	70 ± 102	69 ± 91
S-creatinine (μmol/L)	45 to 116	393 ± 316	389 ± 286

[a] Ref., reference; WG, Wegener's granulomatosis; MPA, microscopic polyangiitis; PR3, proteinase 3; ANCA, antineutrophil cytoplasmic autoantibodies; MPO, myeloperoxidase; ESRD, end-stage renal disease; CRP, C-reactive protein.

from Wieslab AB (Lund, Sweden), and the assays were performed as described elsewhere (15).

Capture Proteinase 3-ANCA ELISA

The method described by Baslund *et al.* was followed (16). A monoclonal antibody (4A3) was used to coat microtiter plates and allowed to capture purified proteinase 3 (PR3). Sera were incubated, and bound IgG were detected by alkaline phosphate-conjugated anti-human IgG. To exclude nonspecific binding and rheumatoid factor binding, a background control plate was coated with a nonspecific monoclonal antibody of the same subclass, and the absorbance values for this plate were subtracted from the absorbance values obtained for the anti-PR3-coated plate for each individual well. A serum was regarded as positive if the absorbance was >3 SD higher than values obtained for donor control subjects. On each plate, a calibrator set was incubated to allow quantification of the values. If the absorbance in a sample was higher than the highest calibrator, it was diluted 10× and reanalyzed before calculation of the unit value.

Anti-GBM ELISA

Microtiter plates (Nunc, Roskilde, Denmark) coated with purified Goodpasture antigen (*i.e.*, alpha-3-IV NC1) were obtained from Wieslab AB (Lund, Sweden), and the ELISA was performed as described elsewhere (17).

Treatment Regimen

When a systemic small vessel vasculitis was diagnosed or strongly suspected, our policy was to institute a daily therapy of cyclophosphamide (2 mg/kg orally) and prednisolone (1 mg/kg orally). In cases presenting as a rapidly progressive GN or pulmonary renal syndrome, pulse-methylprednisolone or plasmapheresis was added. The dosage of cyclophosphamide was reduced if there was a tendency to reduction in the leukocyte count ($<4 \times 10^9$). After 3 to 6 mo of remission, a switch from cyclophosphamide to azathioprine (1 to 2 mg/kg orally) was usually made. The steroid dosage was tapered and was usually 15 to 20 mg/d at 3 mo after the initiation of therapy.

Remission

Remission was defined as complete if there was no evidence of active vasculitic disease and complete resolution of pulmonary infiltrates, improvement of renal function, and resolution of extrarenal manifestations of vasculitis, and as partial if there was a nonprogressive disease with a tendency to remission, *i.e.*, stabilization in renal function, measured by serum creatinine, or regression, although not complete, of pulmonary infiltrates or other extrarenal and extrapulmonary manifestations. Reliable data were missing for two patients.

Relapse

Relapse was defined as the reappearance of activity of the vasculitic disease in a patient who had achieved remission, with the occurrence of at least one of the following signs: rapid rise in serum creatinine (without any other cause for the deterioration of renal function); hemoptysis; pulmonary hemorrhage or new appearance of pulmonary infiltrates without evidence for infection, iritis, or uveitis; new mononeuritis multiplex or vasculitis of the central nervous system; or active necrotizing vasculitis identified by biopsy in any tissue. Relapses were recorded in three cases with a smoldering disease, in which remission was not achieved within the first year. Elevated inflammatory activity and rapid deterioration of organ function, due to vasculitis and recorded as relapse, occurred after 23, 60, and 89 mo, respectively, in these three cases.

ESRD

ESRD was defined as loss of renal function necessitating renal replacement therapy for sustaining life.

Malignancy During Follow-Up

Cancer incidence data were obtained from the South Swedish Regional Tumor Registry. Last date of follow-up was June 1996. The expected cancer incidence was calculated using data from the same registry, covering the same catchment area as our studied population. The person-years method was used in calculations, classifying individuals into 5-yr age groups with single calendar years as the unit cell size. Only cancer detected and registered after the diagnosis of vasculitis was included in the analysis. For some of the analyses, a latency time of ≥60 mo was used. Latency time was defined as the time from referral to the Department of Nephrology when the diagnosis of vasculitis was made to the time of diagnosis of malignancy.

Statistical Analyses

The Kaplan–Meier method was used to estimate survival, relapse-free survival, and renal (ESRD-free) survival. Comparisons between groups were done by the Generalized Wilcoxon statistic. Cox's regression model was used for multivariate adjustment of potential confounding, and was used to investigate the predictive value of selected variables for relapse rate, renal survival, and cumulative patient survival. Relapse-free survival was investigated only in patients who lived long enough to be able to relapse, *i.e.*, who survived the first 3 mo ($n = 112$).

Comparisons between subcohorts of patients according to type induction therapy with regard to relapse were done with the χ^2 test, and with regard to entry characteristics such as laboratory parameters and age with the Mann–Whitney U test, Stat-View 4.0 for Macintosh (Abacus Concepts, Berkeley, CA). Simple proportions of events occurring during follow-up have been presented for descriptive purposes.

Subcohorts were created for different exposure to azathioprine, cyclophosphamide, and corticosteroids, when analyzing the malignancy data. Furthermore, a latency period of 60 mo was used in some of the analyses. The latency period was defined as the time of referral to our department to the time of diagnosis of malignancy. Case-specific standardized morbidity ratios (SMR) and 95% confidence intervals (CI) were calculated. *P* values were calculated by the Poisson distribution or the χ^2 distribution if the expected values were greater than 10. $P < 0.05$ was considered significant. The Poisson and χ^2-calculated *P* values were used to test whether the corresponding SMR differed significantly from 1. All tests were two-tailed.

Results

Demographics and Entry Characteristics

Clinical and laboratory data of the 56 patients with WG and the 67 patients with MPA at time of referral to our department are given in Table 1.

ANCA (PR3- or MPO-ANCA) were found in sera from 116 patients, *i.e.*, in 97% of the 120 sera tested. Four patients (3%) had no detectable ANCA at the time of admittance. PR3-ANCA was detected in 73 patients by the capture ELISA. Six of these were negative by the direct-binding ELISA, and two additional patients had a PR3-ANCA titer in the lower limit

zone by the direct ELISA assay. PR3-ANCA was found in 87% (47 of 54) of the patients with WG and in 39% (26 of 66) of those with MPA. MPO-ANCA was found in 43 patients: in 11% (6 of 54) with WG and in 56% (37 of 66) with MPA (Table 1).

One of the 120 patient sera tested was found to be positive for anti-GBM; this sera was from a man with the clinical picture of MPA and a high titer of MPO-ANCA.

The extent of organ involvement at time of referral to our department, according to clinical diagnosis and type of ANCA, respectively, is presented in Table 2. Involvement of the lower respiratory tract was recorded in 70% (39 of 56) of patients with WG and in 33% (22 of 67) of patients with MPA, and in 55% (40 of 73) of patients with PR3-ANCA and 46% (20 of 43) of patients with MPO-ANCA. Upper respiratory involvement was recorded in 55% (31 of 56) of patients with WG and in 28% (19 of 67) of patients with MPA.

The majority of patients had no immunosuppressive treatment at time of referral; 68% (38 of 56) of the patients with WG and 76% (51 of 67) of patients with MPA were nontreated. Seven patients with WG started treatment with cyclophosphamide and oral corticosteroids, nine with corticosteroids alone, and another two with corticosteroids and azathioprine. Two of the patients with MPA started treatment with cyclophosphamide and corticosteroids and another 14 with corticosteroids alone.

There was no statistically significant difference in B hemoglobin level, B thrombocyte count, or serum creatinine level at time of referral between those who had started immunosuppressive therapy and those not treated before referral. A slightly higher B leukocyte count was noted for patients who had started immunosuppressive therapy before referral (13.3×10^9/L; mean value, SD ± 4.9) *versus* those who had not (10.6×10^9/L; mean value, SD ± 4.7) ($P = 0.006$).

Induction therapy with oral corticosteroids and cyclophosphamide was started within the first days after referral in 90 patients, of whom 63 (70%) went into complete remission and another 18 (20%) into partial remission. Nine patients did not achieve remission. Five died within 1 mo after referral, and four had a progressive deterioration or went into a smoldering state of the inflammatory disease. Twenty-two patients received induction therapy with azathioprine and corticosteroids; 13 (59%) went into complete remission, and six (27%) partial remission. Eight of these 22 patients, given induction therapy with azathioprine, were further treated with cyclophosphamide during the course of follow-up. Four patients received only oral corticosteroids, one only azathioprine, and six did not receive any treatment. In the total cohort, complete remission was achieved in 80 (66%) patients and partial remission in another 26 (21%) patients. Thus, the overall remission rate, including partial remission, was 87%. There was no statistically significant difference between those who had received induction therapy with cyclophosphamide *versus* azathioprine, according to entry data on age at referral, B hemoglobin, B leukocyte count, B thrombocyte count, serum creatinine, or to type or titers of ANCA.

In our present material, 115 patients received corticosteroids for a median duration of 36 mo (range, 0.5 to 168 mo), 99 received cyclophosphamide for a median duration of 8 mo (range, 0.25 to 75 mo), and 80 patients received azathioprine for 36 mo (range, 0.5 to 168 mo). Adjunct therapy with plasmapheresis or pulse intravenous methylprednisolone was administered in 51% of cases. Immunoadsorption with Protein A (Excorim, Lund, Sweden) was used in seven of the patients.

Relapses

In the total cohort, one to seven relapses were recorded in 56 patients (46%) with a median time to the first relapse of 27 mo (range, 2 to 168 mo). The mean number of relapses per patient in the relapsing subgroup was 1.6 (range, 1 to 7).

Relapses were found in 32 of the 81 patients (40%) who went into remission (complete or partial) on cyclophosphamide and oral corticosteroids, whereas the relapse rate for those with azathioprine- and corticosteroid-induced remission were 17 of 19 treatment responders (89%; $P = 0.0004$).

At the time of the first relapse, a majority of the patients (69%) had ongoing immunosuppressive therapy: 27% prednisolone + azathioprine, 25% prednisolone, 13% prednisolone

Table 2. Distribution of organ system involvement at time of referral for 56 patients with WG and 67 patients with MPA and according to type of ANCA, PR-3-ANCA, and MPO-ANCA, respectively[a]

Distribution of Organ System Involvement at Referral	WG *n* (%)	MPA *n* (%)	PR3-ANCA *n* (%)	MPO-ANCA *n* (%)
Renal	56 (100%)	67 (100%)	73 (100%)	43 (100%)
Upper respiratory	31 (55%)	19 (28%)	38 (52%)	9 (21%)
Pulmonary	39 (70%)	22 (33%)	40 (55%)	20 (46%)
Ocular	11 (20%)	8 (12%)	16 (22%)	1 (2%)
Musculoskeletal	27 (48%)	22 (33%)	36 (49%)	11 (26%)
Neurologic	7 (12%)	11 (16%)	11 (15%)	7 (16%)
Gastrointestinal	2 (4%)	11 (16%)	6 (8%)	4 (9%)
Cardiac	0	6 (9%)	1 (1%)	3 (7%)
Skin	3 (5%)	2 (3%)	3 (4%)	1 (2%)

[a] Abbreviations as in Table 1.

+ cyclophosphamide, and 4% azathioprine. Ninety-one percent of the patients with WG were receiving immunosuppressive therapy at the time of the first relapse compared with 53% of those with MPA ($P = 0.002$). No such difference was found between patients with PR3-ANCA *versus* MPO-ANCA.

There was no statistically significant difference in overall relapse rate according to type of ANCA ($P = 0.975$), but as shown in Figure 1, there was a tendency to higher relapse rates in patients with PR3-ANCA. Forty-seven percent of the patients with PR3-ANCA relapsed, as did 40% of those with MPO-ANCA.

Cox regression analysis of patients surviving the first 3 mo ($n = 112$) showed a significantly lower relapse rate in those who presented with B thrombocyte counts of 288 to 407 $\times 10^9$ compared to those with lower counts (RR = 0.3; $P = 0.011$; 95% CI, 0.15 to 0.78).

Statistical analysis of possible prognostic factors for relapses within the follow-up period, excluding those patients who died during the first 3 mo, showed no significant correlation to gender, age, clinical diagnosis, number of organ systems involved at presentation, or to serum creatinine, B hemoglobin, and B leukocytes at presentation.

Renal Status at Follow-Up

Among the 85 patients alive at the end of follow-up, 78% had preserved renal function and 22% had reached ESRD; 12 patients were on hemodialysis, six had a functioning kidney transplant, and one was going to start renal replacement therapy. The corresponding values for those who died during follow-up were 58% with preserved renal function and 42% with ESRD.

Three variables seemed important for renal survival: First, as shown in Figure 2A, there was a significantly better renal survival rate in patients presenting with low serum creatinine than in patients with high serum creatinine at referral to our department (overall comparison, $P = 0.002$). Second, the PR3-ANCA titer measured by capture ELISA was of significant prognostic value for renal survival ($P = 0.004$) (Figure 2B). Patients with a very high titer of PR3-ANCA (>550 U) in the capture assay had a renal survival rate at 5 yr of 60%, compared with 85% for those with lower titers of PR3-ANCA (<550 U) ($P = 0.001$). No significant differences in renal survival according to titers of PR3-ANCA with conventional ELISA ($P = 0.310$), or to MPO-ANCA titers ($P = 0.5440$), were found. ESRD developed in 32% of patients with PR3-ANCA and in 21% of those who had MPO-ANCA; however, the difference was not significant. Third, patients with low B thrombocytes at presentation had a lower renal survival rate compared to those with higher levels ($P = 0.028$) (Figure 2C).

When analyzing renal status at the end of follow-up and controlling for serum creatinine, there was no statistically significant relation to age, gender, clinical diagnosis, number of organs involved at presentation, hemoglobin or leukocytes. Nor was there any statistically significant difference in renal survival between those who had received induction therapy with cyclophosphamide and corticosteroids *versus* those with azathioprine and corticosteroids.

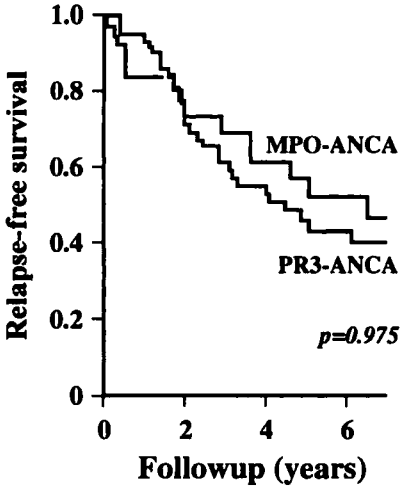

Figure 1. Relapse-free survival for patients with proteinase 3 (PR3) antineutrophil cytoplasmic antibodies (ANCA) and myeloperoxidase (MPO)-ANCA, respectively.

Mortality

In the whole material, 38 patients (31%) died during follow-up. Eleven patients (9%) died within 3 mo of referral to our department. Significant prognostic factors for mortality were age and serum creatinine levels at the time of referral to our department. Figure 3A shows the cumulative survival for the patient material subdivided into three age groups of equal size regarding numbers of patients included ($P = 0.0002$).

Serum creatinine at referral was of prognostic significance for mortality ($P = 0.028$) (Figure 3B). Mortality in the early phase was high in patients with high serum creatinine levels at referral. There was no significant difference in mortality rate according to gender or clinical diagnosis; 32% of the patients with WG died, as did 30% of the patients with MPA ($P = 0.944$).

Regarding the type and titer of ANCA, mortality in patients with PR3-ANCA was 34%, compared to 23% with MPO-ANCA (NS, $P = 0.258$). When analyzing the titers of PR3-ANCA assessed by the capture technique, there was a tendency, although not statistically significant, to higher mortality in patients with high titers: 19 of 45 patients (42%) with a PR3-ANCA titer >550 U died *versus* 6 of 28 (21%) of those with a titer <550 U ($P = 0.069$) (Figure 3C). A similar tendency, although not statistically significant, was noted for PR3-ANCA titers measured by conventional ELISA: 9 of 35 (26%) patients with a titer of <92 U died *versus* 16 of 38 (42%) of those with a titer ≥92 U ($P = 0.207$). No correlation was found between MPO-ANCA titers and mortality. There was no statistically significant difference in patient survival between those who had received induction therapy with cyclophosphamide and corticosteroids *versus* those with azathioprine and corticosteroids.

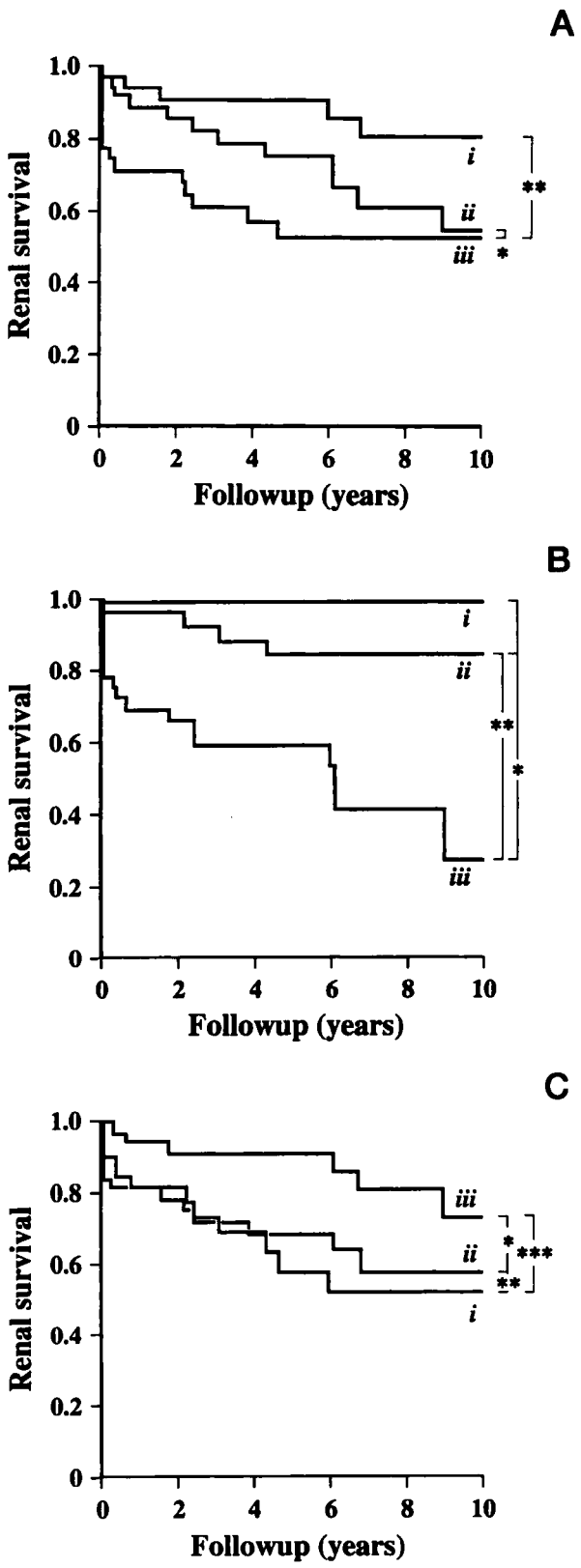

Figure 2. Long-term renal survival in 123 patients with Wegener's granulomatosis (WG) or microscopic polyangiitis (MPA); all patients with renal involvement at time of referral. (A) With regard to serum creatinine at time of referral, comparison with three subgroups of patients subdivided according to serum creatinine values. *i* = serum creatinine < 202 μmol/L, *n* = 41; *ii* = 202 to 429 μmol/L, *n* = 41; *iii* > 429 μmol/L, *n* = 41. Overall comparison, *P* = 0.002. **P* = 0.027; ***P* = 0.002. (B) With regard to titer of PR3-ANCA in 73 patients with PR3-ANCA at time of referral, measured by the capture enzyme-linked immunosorbent assay, comparison with subgroups with low and high titers, respectively. *i* = PR3-ANCA titer < 27 U, *n* = 2; *ii* = PR3-ANCA titer 27 to 550 U, *n* = 32; *iii* = PR3-ANCA titer > 550 U, *n* = 39. Overall comparison, **P* = 0.004; ***P* = 0.001. (C) According to B thrombocytes at time of referral, comparison with subgroups with low, middle, and high B thrombocyte count. *i* = B thrombocyte count 10 to 288 × 10^9/L, *n* = 40; *ii* = B thrombocyte count 288 to 407 × 10^9/L, *n* = 41; *iii* = B thrombocytes count > 407 × 10^9/L, *n* = 40. **P* = 0.028; ***P* = 0.028; ****P* = 0.008.

Malignancies

Fifteen patients developed a malignancy after the time of diagnosis of the vasculitis. The data are presented in Tables 3 and 4. As shown in Table 3, there was a 10.4 times higher risk of developing skin cancer in our studied cohort of patients with small vessel vasculitis, compared with the expected cancer incidence.

As shown in Table 4, when calculating morbidity ratio for malignancies with a latency of 60 mo or more after the diagnosis of vasculitis, treatment with cyclophosphamide for more than 12 mo resulted in an SMR for cancer of 3.7, which was highly significant, as was the respective SMR for treatment with azathioprine for more than 12 mo (3.0). The SMR for treatment with corticosteroids for more than 48 mo was 2.2.

Cyclophosphamide therapy during ≥12 mo and a latency time for diagnosis of cancer of 60 mo or more were strongly associated with vulva cancer (International Classification of Diseases-7 176), with an SMR of 181.3 and 95% CI of 4.59 to 1010.10 (P = 0.005), and urinary bladder carcinoma with SMR 20.2 and 95% CI 4.17 to 59.13 (P = 0.0004). Skin carcinoma had the strongest relationship with long-term azathioprine (≥12 mo) and corticosteroid therapy (>48 mo) (SMR of 24.7 and 20.8, respectively; see Table 4).

In addition to the 15 cases with registered malignant tumor, four patients developed myelodysplastic syndrome (MDS) and another five developed carcinoma *in situ* of the skin during follow-up. These premalignant conditions were not included in the present calculations of SMR. Data on these nine patients with MDS and carcinoma *in situ* are presented separately in Table 5.

Discussion

Our findings regarding the prevalence of PR3-ANCA and MPO-ANCA in patients with WG and MPA are in accordance with the findings of others (reviewed in reference 18), with a prevalence of PR3-ANCA of 85% in patients with WG, 45% in patients with MPA and a prevalence of MPO-ANCA of 10% in patients with WG and 45% in patients with MPA. The sensitivity of the ELISA for ANCA was high; only 3% of our patients with renal small vessel vasculitis were found to be negative at the time of referral to the Department of Nephrology. On the other hand, in our series of patients, PR3-ANCA was more often found in patients with MPA than in the series

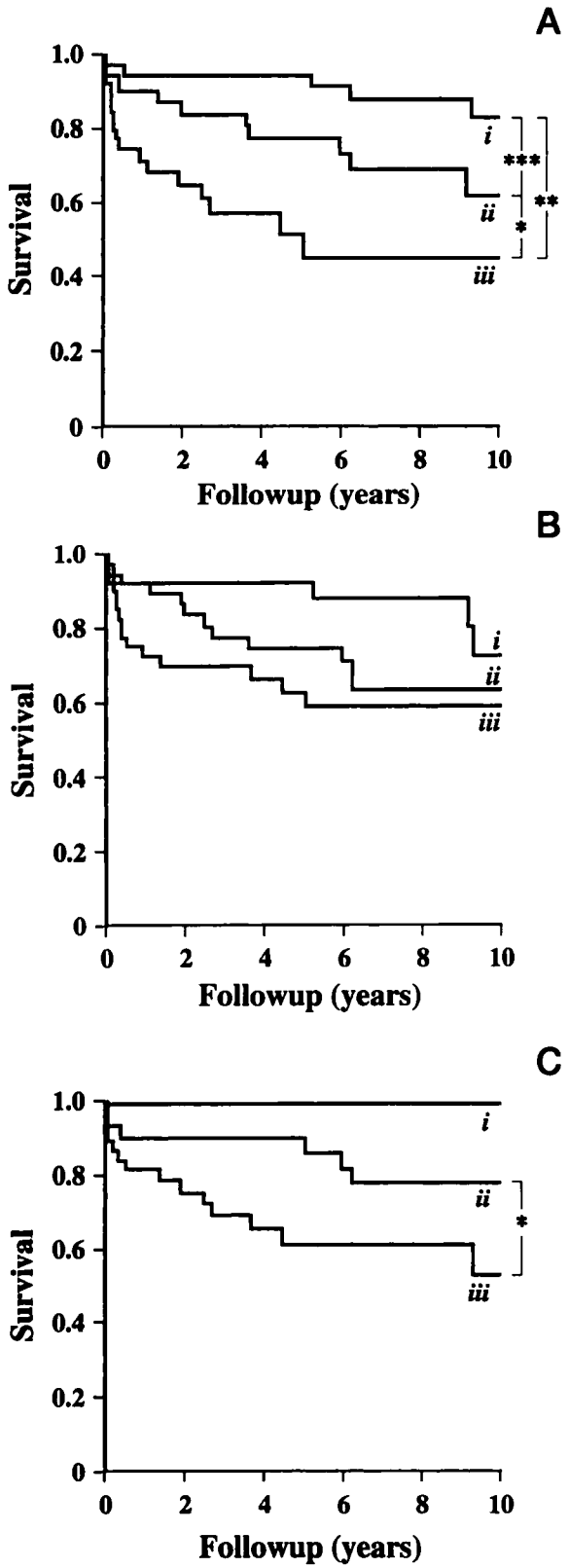

Figure 3. Long-term patient survival in 123 patients with WG or MPA; all patients with renal involvement at time of referral, followed-up for a mean time of 64 mo (range, 0.07 to 273 mo). (A) According to age at time of referral, cohort of patients subdivided into three equally sized groups. *i* = 11 to 55.9 yr, $n = 41$; *ii* = 56 to 68.3 yr, $n = 42$; *iii* > 68.3 yr, $n = 40$. *$P = 0.015$; **$P = 0.0002$; ***$P = 0.023$. Overall comparison, $P = 0.0002$. (B) With regard to serum creatinine at time of referral, comparison with three subgroups of patients, subdivided according to serum creatinine values. *i* = serum creatinine < 202 μmol/L, $n = 41$; *ii* = 202 to 429 μmol/L, $n = 41$; *iii* > 429 μmol/L, $n = 41$. Overall comparison, $P = 0.028$. (C) With regard to titer of PR3-ANCA at time of referral in those 73 patients with detectable PR3-ANCA, measured by the capture ELISA, comparison with subgroups with low and high titers, respectively. *i* = PR3-ANCA titer < 27 U, $n = 2$; *ii* = PR3-ANCA titer 27 to 550 U, $n = 32$; *iii* = PR3-ANCA titer > 550 U, $n = 39$. Overall comparison, $P = 0.123$. *$P = 0.069$.

presented by Jayne *et al.*, who found 13% PR3-ANCA but 33% unclassified ANCA in ELISA (19).

Clinical improvement was seen in 87% of the patients in our study, corresponding fairly well to the data obtained by others (8,20). Complete remission was obtained in 70% of patients who received induction therapy by cyclophosphamide and corticosteroids. This is somewhat lower than the results presented by Jayne *et al.* (19), who found a complete remission rate of 93% in a similar material, although with a lower percentage of renal involvement in their cohort (49% of patients with WG and 93% of patients with MPA).

The relapse rate is still high in spite of immunosuppressive therapy. Several studies have revealed a relapse rate of 20 to 50% (5,8,10,19–22). Few studies have shown any relation to immunosuppressive therapy administered just before or at the time of relapse (10,19,21). In the 85 patients with WG presented by Fauci *et al.* in 1983 (10), 22 of 25 patients were on immunosuppressive therapy, although mostly in tapering doses, at the time of relapse. In another study, four of 23 patients who relapsed were on continuous cyclophosphamide therapy (19). Our results revealed that the majority of patients (69%) had ongoing therapy with immunosuppressive agents, although in tapering dosages, at the time of the first relapse.

Our study showed that there was no significant difference in relapse rate between those with WG and those with MPA. However, patients with WG and PR3-ANCA seemed to be more aggressively treated than the patients with MPA. In fact, 91% of patients with WG were receiving immunosuppressive therapy at the time of first relapse, compared to 53% of those with MPA. Because immunosuppressive therapy should reduce the risk of relapse (23), this could explain our findings of a nearly equal relapse rate for WG and MPA. The overall relapse rate for patients with PR3-ANCA and MPO-ANCA was not significantly different (47 and 40%, respectively), but we cannot rule out a difference between these two. Similar results were obtained by DeOliviera *et al.*, who found a relapse rate of 44% in patients with PR3-ANCA and 13% in patients with MPO-ANCA, although the difference was not statistically significant (24). In their study, patients with limited WG, as well as those with Churg–Strauss syndrome, were included, and therefore their results are not comparable with ours. But interestingly, in their series of patients the mean time to relapse was 64 mo (approximately 5 yr), at which time it may be possible to detect a difference in relapse rate according to type of ANCA, as shown by our results presented in Figure 1. How-

Table 3. Cancer morbidity in 123 patients with small vessel vasculitis involving the kidneys[a]

ICD-7 Codes	Type of Cancer	Observed No. of Cases	Expected No. of Cases	SMR	95% CI
140 to 209	All tumors	15	9.27	1.6	0.9–2.7
170	Breast	1	0.69	1.5	0.04–8.1
176	Vulva	1	0.03	32.9	0.8–183.5
177	Prostate	2	1.67	1.2	0.1–4.3
178	Testicular	1	0.02	45.7	1.2–254.7
180	Renal	1	0.30	3.3	0.1–18.4
181	Urinary bladder	3	0.63	4.8	1–13.9
191	Skin	5	0.48	10.4	3.4–24.3
200 to 202	Non-Hodgkin lymphoma	1	0.27	3.7	0.1–20.5

[a] First and second tumor registered, with a total of 944.4 person-years of observation, type of cancer, observed and expected number of cases in the studied cohort with vasculitis, and standardized morbidity rate (SMR) with 95% confidence interval (95% CI). Cancer morbidity is only demonstrated for ICD-7 codes with observed cases. ICD, International Classification of Diseases.

ever, a recent preliminary report has demonstrated that relapse is four times more likely in patients with PR3-ANCA than in those with MPO-ANCA (25). Future prospective studies are needed to clarify this finding.

The morbidity in ESRD correlated with serum creatinine levels at presentation, stressing the importance of early diagnosis and a thorough examination regarding renal involvement in the initial phase. Renal survival in those patients presenting with a serum creatinine <200 μmol/L appeared excellent (approximately 90% after 10 yr), regardless of the renal biopsy findings. Our results reveal a possible dose–response relationship between serum creatinine at presentation and renal outcome. To our knowledge, this is the first study to show a relationship between high B thrombocyte counts and good renal survival, and also the prognostic importance of PR3-ANCA titers, assessed by the capture technique for PR3-ANCA detection, for renal survival. In cases with poor prognosis, the lower B thrombocyte count may, to some degree, be due to consumption or to the development of secondary thrombotic microangiopathy caused by widespread endothelial damage. Another explanation could be bone marrow suppression due to uremia or to treatment. We have found no other accounts in which uremia was considered the reason; besides, the uremia in the studied population was acute, not long-standing, and so could hardly explain this finding. Analysis of patients with low B thrombocyte counts did not show any correlation to low B leukocyte count or to low B hemoglobin levels (data not presented).

The 5-yr survival in our present material corresponds fairly well to that presented by others (26). Mortality was found to be influenced by age, as expected. Our results did not reveal a statistically significant difference in mortality rate when comparing patients with PR3-ANCA and MPO-ANCA, a finding that is in accordance with other studies (27). This contrasts with the findings of Hogan *et al.*, although their studied population exclusively comprised patients with MPA (28). Our results could be explained by the fact that all of our patients had renal involvement at the start, *i.e.*, at the time of referral, compared with 69% in the material presented by Hogan.

Furthermore, high titers of PR3-ANCA, detected by capture ELISA, were associated with a significantly poorer renal survival and, although not statistically significant, possibly with a higher mortality. To our knowledge, no such correlation has been described for PR3 titers before, although there has been a report on a faster deterioration of renal function in patients with PR3-ANCA than in those with MPO-ANCA-associated renal disease (27). Our findings thus strengthen the likelihood that PR3-ANCA is involved in the pathogenesis of WG and MPA with renal involvement. Moreover, our results indicate that different epitopes of PR3 may have a different impact on the clinical outcome, with the epitope detected by the capture technique being more important for renal outcome.

Thirteen percent (15 cases) of our patients developed cancer, and another nine developed MDS and carcinoma *in situ* of the skin during follow-up. The highest risk for cancer morbidity was associated with testicular and vulva cancer, although only detected in sporadic cases. Of greater clinical importance would seem to be the 10-fold increase in risk for skin cancer, and 4.8-fold increase in risk for urinary bladder carcinoma. The cancer morbidity in our material was lower than that presented by Hoffman *et al.* (8). This could be due to milder or shorter duration of immunosuppression in our material; the material presented by Hoffman *et al.* was made up exclusively of patients with WG. Furthermore, MDS and carcinoma *in situ* were not registered as cancer in our material. When analyzing cancer morbidity in relation to duration and type of immunosuppression, our results seemed to be compatible regarding the risk for lymphoma. We found an 8.5-fold increase in patients who received cyclophosphamide for more than 12 mo *versus* an 11-fold increase in the material by Hoffman *et al.* (8). The incidence of urinary bladder cancer in our material seemed to be lower than that presented by others. We found three cases with urinary bladder cancer, all with cyclophosphamide treatment for more than 12 mo, and when comparing the found

Table 4. Analysis of cancer morbidity in 123 patients with small vessel vasculitis involving the kidneys, divided into subgroups according to type and duration of immunosuppressive treatment given during follow-up, type of cancer, observed and expected number of cases, SMR, 95% CI, including data on these cases with a malignancy diagnosed exclusively 60 mo or later after the time of diagnosis of vasculitis (latency time)

Group and ICD-7 Code	Type of Cancer	Observed No. of Cases	Expected No. of Cases	SMR	95% CI
Cyclophosphamide ≥ 12 mo: 45 patients, 10 cases with cancers, Σ 438.1 person-years of observation				2.8	1.4 to 5.0
190 to 191	Skin	2	0.19	10.5	1.3 to 37.9
176	Vulva	1	0.01	82.8	2.1 to 461.5
178	Testes	1	0.01	76.3	1.9 to 425.2
181	Urinary bladder	3	0.26	11.5	2.4 to 33.6
200 to 202	Non-Hodgkin lymphoma	1	0.12	8.5	0.2 to 47.2
Cyclophosphamide ≥ 12 mo and ≥ 60 mo latency time: 37 patients, 8 cases with malignancies, Σ 222.3 person-years of observation				3.7	1.6 to 7.4
190 to 191	Skin	1	0.11	8.9	0.2 to 49.3
176	Vulva	1	0.01	181.3	4.6 to 1010.1
181	Urinary bladder	3	0.15	20.2	4.2 to 59.1
200 to 202	Non-Hodgkin lymphoma	1	0.07	15.3	0.4 to 85.5
180	Renal carcinoma	1	0.07	14.3	0.4 to 79.7
Azathioprine ≥ 12 mo: 54 patients, 10 cases with cancer, Σ 577.5 person-years of observation				2	0.9 to 3.6
190 to 191	Skin	4	0.25	16.1	4.4 to 41.1
178	Testes	1	0.02	57.6	1.5 to 320.9
181	Urinary bladder	2	0.33	6.1	0.7 to 21.9
Azathioprine ≥ 12 mo and ≥ 60 mo latency time: 49 patients, 9 cases with cancer, Σ 311.7 person-years of observation				3.0	1.4 to 5.7
190 to 191	Skin	4	0.16	24.7	6.7 to 63.2
200 to 202	Non-Hodgkin lymphoma	1	0.09	10.7	0.3 to 59.5
181	Urinary bladder	2	0.20	10.0	1.2 to 36.1
Corticosteroids >48 mo: 42 patients, 9 cases with cancer, Σ 467.2 person-years of observation					
190 to 191	Skin	4	0.19	20.8	5.7 to 53.3
176	Vulva	1	0.01	94.2	2.4 to 524.8
181	Urinary bladder	2	0.29	6.9	0.8 to 25

incidence with a reference population, analyzing data from the South Swedish Tumour Registry, an 11-fold increased risk was obtained. In the material presented by Talar-Williams *et al.*, seven patients with urinary bladder cancer were recorded; in six of the seven cases, cyclophosphamide was administered during more than 12 mo (range, 2.7 to 8 yr), and they reported a 31-fold increased risk for urinary bladder cancer (95% CI, 13 to 65) (11). In the present material, the risk for skin carcinoma was significantly associated with azathioprine therapy for more than 12 mo (16-fold increase) and with corticosteroids for more than 48 mo (20-fold increase). Of course, there is a correlation between long-term use of azathioprine or cyclophosphamide and corticosteroids, and we cannot draw any conclusions from our present results regarding which drug is most responsible for increasing the risk for skin cancer.

One could criticize the value of this retrospective study, particularly with regard to the fact that not all of the included patients received a uniform induction therapy. Of the 116 patients who received induction treatment, the majority (90) received oral cyclophosphamide and corticosteroids, the minority azathioprine and corticosteroids, and only four corticosteroids alone. However, there were no differences between those who had received induction with cyclophosphamide *versus* those who had received azathioprine regarding entry data (age, type of ANCA, blood chemistry). Thus, all of our cases were included to allow for statistical analyses of age and laboratory data at entry. The strength of the present study is that the total cohort is derived from a defined area with a standard registry for cancer, and that all of the included patients had biopsy-confirmed renal involvement.

Because the therapeutic regimen used today is not efficient enough to prevent relapses and is associated with a multitude

Table 5. Nine patients with small vessel vasculitis and carcinoma *in situ* of the skin (n = 5) or MDS (n = 4) during follow-up[a]

Gender	Age	Clinical Diagnosis	ANCA Type	Malignant Disease	Cyclophosphamide (mo)	Lag Time (mo)	Azathioprine (mo)	Lag Time (mo)	Steroids (mo)	Lag Time (mo)
F	68	WG	PR3	Carcinoma *in situ* + cervix uteri	13	29	37	0	51	0
F	72	WG	PR3	Carcinoma *in situ*	6	52	46	0	39	18
M	73	MPA	MPO	Carcinoma *in situ*	20	0	2	10	14	0
F	52	WG	PR3	Carcinoma *in situ*	20	107	107	0	60	66
M	21	MPA	PR3	Carcinoma *in situ*	17	116	93	59	144	0
M	51	MPA	MPO	MDS	1, 28	2	1	0, 5	4	0
F	74	MPA	PR3	MDS	14	62, 5	57	0	64	72, 5
M	30	WG	PR3	MDS	36	30 to 42	0		>66	0
M	63	WG	PR3	MDS	0.6	0	0		0.6	0

[a] Gender, age at presentation with renal vasculitis, clinical diagnosis, type of ANCA, type and duration of oral immunosuppression (months), and time from cessation of immunosuppression to diagnosis of carcinoma *in situ* and MDS (lag time, months). MDS, myelodysplastic syndrome. Other abbreviations as in Table 1.

of side effects, of which the risk of malignancy is by far the most important, there is a need to improve therapy and medical care for patients with ANCA-associated small vessel vasculitis, *i.e.*, WG and MPA.

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