Clinical and Histologic Determinants of Renal Outcome in ANCA-Associated Vasculitis: A Prospective Analysis of 100 Patients with Severe Renal Involvement


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This study aimed to identify clinical and histologic prognostic indicators of renal outcome in patients with ANCA-associated vasculitis and severe renal involvement (serum creatinine >500 μmol/L). One hundred patients who were enrolled in an international, randomized, clinical trial to compare plasma exchange with intravenous methylprednisolone as an additional initial treatment were analyzed prospectively. Diagnostic renal biopsies were performed upon entry into the study. Thirty-nine histologic and nine clinical parameters were determined as candidate predictors of renal outcome. The end points were renal function at the time of diagnosis (GFR₀) and 12 mo after diagnosis (GFR₁₂), dialysis at entry and 12 mo after diagnosis, and death. Multivariate analyses were performed. Predictive of GFR₀ were age (r = −0.40, P = 0.04), arteriosclerosis (r = −0.53, P = 0.01), segmental crescents (r = 0.35, P = 0.07), and eosinophilic infiltrate (r = −0.41, P = 0.04). Prognostic indicators for GFR₁₂ were age (r = −0.32, P = 0.01), normal glomeruli (r = 0.24, P = 0.04), tubular atrophy (r = −0.28, P = 0.02), crescentic glomeruli (r = −0.26, P = 0.03), and GFR₀ (r = 0.29, P = 0.01). Fibrous crescents (r = 0.22, P = 0.03) were predictive of dialysis at entry. Normal glomeruli (r = −0.30, P = 0.01) and treatment arm (r = −0.28, P = 0.02) were predictive of dialysis after 12 mo. No parameter predicted death. Both chronic and acute tubulointerstitial lesions predicted GFR₁₂ in severe ANCA-associated glomerulonephritis, whereas plasma exchange was a positive predictor of dialysis independence after 12 mo for the entire patient group. Plasma exchange remained a positive predictor of dialysis independence and improved renal function after 12 mo, indicating that the unaffected part of the kidney is vital in determining renal outcome.


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to moderate renal involvement (serum creatinine <500 \mu mol/L) and received standardized treatment in an attempt to identify clear clinical and histologic predictors (20). We demonstrated that renal function at diagnosis, in combination with chronic renal lesions identified by histology, is predictive of renal function after 18 mo, and active lesions are associated with renal function recovery. In this study, performed within the framework of the European Vasculitis Study (EUVAS) group (21), we investigated the distribution of acute and chronic lesions in renal biopsies and evaluated clinical and histologic predictors of outcome in patients with severe renal involvement (serum creatinine >500 \mu mol/L).

Materials and Methods

Patients

Patients were derived from 29 hospitals located in 11 European countries. Patients were enrolled in the Methylprednisolone versus Plasma Exchange (MEPEX) trial, which is a randomized trial to evaluate adjunctive therapy for severe glomerulonephritis in ANCA-associated systemic vasculitis (21). Patients who had a serum creatinine of 500 \mu mol/L or more were included. The local ethics committees approved the study, and all patients gave written informed consent for participation. Inclusion criteria for MEPEX are listed in Table 1. Exclusion criteria of this study are described extensively elsewhere (21). All patients followed a standard treatment regimen. For adjunctive therapy, they were randomly assigned either to receive intravenous methylprednisolone or to undergo plasma exchange. Standard therapy consisted of oral corticosteroids, which started at 1.0 mg/kg per d and was tapered down within the first 6 mo, and cyclophosphamide 2.5 mg/kg per d, which at 3 mo was replaced by the less toxic azathioprine. Patients who were randomly assigned to receive intravenous methylprednisolone were administered 1000 mg/d for 3 consecutive days, starting directly after diagnosis. The patients in the plasma exchange arm received seven plasma exchanges of 60 ml/kg during the first 14 d after diagnosis. Patients were included in this analysis only when both histologic data, obtained from renal biopsy at the time of study entry, and clinical data were available.

Disease definitions were adopted from the 1992 Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis (5) and a previous European Union study (22). The diseases were distinguished on the basis of criteria that were published previously (21), and determinations were made by local physicians.

ANCA Testing

Indirect immunofluorescence (IIF) and ELISA for ANCA testing were performed locally at all participating centers. The staining pattern in the IIF test was scored as perinuclear (P-ANCA), cytoplasmic (C-ANCA), atypical, or negative. Positive sera for ANCA directed against myeloperoxidase (MPO) or proteinase-3 (PR3) were reported as MPO-ANCA and PR3-ANCA, respectively.

Candidate Predictors of Renal Outcome

Candidate parameters for clinical predictors of renal outcome in this study were renal function at entry (GFR0), dialysis status at entry, age, gender, quantitatively assessed proteinuria at entry, diagnosis (Wegener’s granulomatosis, microscopic polyangiitis, or renal limited vasculitis), ANCA-antigen specificity (PR3-ANCA or MPO-ANCA), IIF pattern (C-ANCA or P-ANCA), and treatment arm (intravenous methylprednisolone or plasma exchange). Candidate parameters for histologic predictors were determined from paraffin sections of renal biopsies that were stained with silver, periodic acid-Schiff, hematoxylin and eosin, and trichrome. Sections were reviewed by two of five participating pathologists (I.M.B., F.F., L.H.N., R.W., or J.A.B.). Both pathologists, blinded to patient data and the other observer’s results, scored the biopsies separately and according to a previously standardized protocol (23,24). Briefly, each glomerulus had to be scored separately for the presence of fibrinoid necrosis, crescents (cellular/fibrous and segmental/circumferential), sclerosis (local, segmental, or global), periglomerular infiltrates, granulomatous reactions, and other lesions. The number of glomerular lesions was reported as the percentage of glomeruli in a biopsy. Most interstitial, tubular, and vascular lesions were scored dichotomously, except for interstitial infiltrates, type of cellular infiltrates (neutrophils, mononuclear cells, and eosinophils), interstitial fibrosis, and tubular atrophy, which were scored semiquantitatively. Granulomas were scored quantitatively. In total, 39 histologic parameters were examined. Discrepancies between observers were resolved by conference during central reviews to achieve a consensus for each biopsy.

Clinical Outcome Parameters

Clinical outcome parameters were renal function at diagnosis (GFR0), renal function at 12 mo (GFR12), dialysis dependence at diagnosis, dialysis dependence at 12 mo, relapse, and death. Renal function was defined as the GFR, which was determined using the equation developed by Cockcroft and Gault (25). Renal function at entry has been shown to be a major predictor of renal outcome for a number of renal diseases (11,26,27). Therefore, we also investigated the correlation between clinical and histologic parameters and GFR12 after correction for GFR0. The latter value was expressed as the corrected GFR12 (CORGFRI2), defined as the difference between the observed GFR12 and its linear prediction on the basis of GFR0. This correction created a corrected value that was statistically independent of the starting value (28).

Table 1. Inclusion criteria for MEPEX (1, 2, and 3 are required)³

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. New diagnosis of WG or MPA or its renal-limited variant, in accordance with the Chapel Hill consensus criteria (7), with active vasculitis, as indicated by the presence of active necrotizing glomerulonephritis on renal biopsy.</td>
<td></td>
</tr>
<tr>
<td>2. ANCA positivity for one of the following:</td>
<td></td>
</tr>
<tr>
<td>a. C-ANCA pattern by IIF</td>
<td></td>
</tr>
<tr>
<td>b. in the PR3 ELISA</td>
<td></td>
</tr>
<tr>
<td>c. in the MPO ELISA, with or without P-ANCA ANCA</td>
<td></td>
</tr>
<tr>
<td>negativity is allowed if the disease is confirmed histologically</td>
<td></td>
</tr>
<tr>
<td>3. Biopsy-proven necrotizing and/or crescental glomerulonephritis, in the absence of another defined glomerulopathy, with severe renal impairment defined by:</td>
<td></td>
</tr>
<tr>
<td>a. oliguria (&lt;400 ml/24 h) or intention to commence dialysis within 48 h of admission</td>
<td></td>
</tr>
<tr>
<td>c. creatinine &gt;500 \mu mol/L</td>
<td></td>
</tr>
</tbody>
</table>

³C-ANCA, cytoplasmic ANCA; IIF, indirect immunofluorescence; MPA, microscopic polyangiitis; MPO, myeloperoxidase; P-ANCA, perinuclear ANCA; PR3, proteinase-3; WG, Wegener’s granulomatosis.
Statistical Analyses
The software used for statistical analyses was the SPSS 10.0 standard version for Windows (SPSS, Inc., Chicago, IL). Correlation of the quantitative and dichotomous candidate predictors with $GFR_0$, $GFR_{12}$, and $CORGFR_{12}$ was determined using Pearson correlation test. The Spearman rank correlation test was used to correlate categorical variables with $GFR_0$, $GFR_{12}$, and $CORGFR_{12}$. Correlations of quantitative candidate predictors with the occurrence of dialysis and death were assessed by the Pearson correlation test. Phi values were used to correlate dichotomous and categorical candidate predictors with the occurrence of dialysis and death at 12 mo. A model for the estimation of $GFR_0$, $GFR_{12}$, and $CORGFR_{12}$ was designed using a stepwise linear multiple regression analysis. An estimation model that was based on a binary logistic regression analysis was used for dialysis dependence and death at 12 mo. Each parameter that correlated with patient relapse were calculated because they were of low statistical value.

Results
Patients
Patients were enrolled in the MEPEX trial between March 25, 1995, and October 29, 2001. Four of the 151 patients who entered the trial declined further participation, nine were found to have circulating anti–glomerular basement membrane antibodies, and one had already received $>500 \mu g$ of intravenous methylprednisolone; these patients were excluded. None of the remaining 137 patients was lost to follow-up or withdrawn from the study. Renal biopsies were obtained from 102 patients for reevaluation. Two biopsies were excluded because of the absence of cortical tissue, meaning that 100 biopsies were available for the final analysis. Clinical characteristics of the patients are depicted in Table 2.

The average cumulative dose of cyclophosphamide was 18 g. On average, 4.8 L was exchanged during every plasma exchange; this was performed seven times. A total of 1000 mg of intravenous methylprednisolone was administered intravenously three times to patients who were assigned to this treatment arm.

Focusing on the 69 patients who were on dialysis at entry, 51% received plasma exchange; of these, 54% became dialysis independent, 17% were on dialysis, and 29% were dead at 12 mo. Of the 69 patients who were on dialysis at entry, 49% received intravenous methylprednisolone; of these, 32% became dialysis independent, 47% were on dialysis, and 21% were dead at 12 mo. These data show that patients who were on dialysis at entry were equally distributed over the two additional therapy arms and that in both groups, a similar percentage of patients died. However, patients who received plasma exchange had a better prognosis than those who received intravenous methylprednisolone, in terms of dialysis independence.

An overview of patient courses, from study entry to outcome, is shown in Figure 1. Only three patients experienced a relapse. Correlations with patient relapse were not calculated because they were of low statistical value.

Histologic Features
The occurrence of the main histologic lesions was analyzed to explore the extent found at the entry period in this group of patients with clinical severe renal impairment. The frequencies of the glomerular and tubulointerstitial lesions are presented in Table 3. The majority of the nonsclerotic glomeruli contained crescents; only very few were unaffected, irrespective of the presence of focal or diffuse glomerulosclerosis. In other words, severe and extensive acute lesions were characteristic of the renal biopsies from this patient group, whereas the extent of chronic changes in the form of global glomerulosclerosis was mild to moderate.

Predictors of Outcome
Correlation coefficients of the variables in relation to the outcome parameters are presented in Table 4. A poor correlation was obtained for the histologic parameters that were excluded from Table 4. Models for the estimation of outcome parameters were designed using binary logistic and stepwise linear multiple regression analyses and are reported in Table 5. These models showed that a combination of parameters predicted the outcome parameter best. The univariate correlation of these predictors with the outcome parameters is shown in

Table 2. Clinical characteristics of the whole patient group ($n = 100$)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr; range)</td>
<td>64.1 (26.8 to 80.7)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>63/37</td>
</tr>
<tr>
<td>Diagnosis (WG/MPA/RLV)</td>
<td>33/57/10</td>
</tr>
<tr>
<td>GFR$_0$ (ml/min; mean $\pm$ SD)</td>
<td>10 $\pm$ 4</td>
</tr>
<tr>
<td>GFR$_{12}$ (ml/min; mean $\pm$ SD)</td>
<td>32 $\pm$ 13</td>
</tr>
<tr>
<td>Proteinuria (mg/24 h)</td>
<td>30 $\pm$ 6</td>
</tr>
<tr>
<td>Adjunctive treatment (IVMeP/PE; %)</td>
<td>49/51</td>
</tr>
</tbody>
</table>

aIVMeP, intravenous methylprednisolone; PE, plasma exchange; RLV, renal-limited vasculitis.

Figure 1. Flow chart of the clinical courses of patients. □, Patients who were on dialysis at entry; □, patients who were dialysis independent at entry. Numbers and percentages are listed. Black dots represent patients who experienced a relapse.
relationship of these variables with GFR₀ is shown in Figure 2, A through D.

Predictors of GFR₁₂ and CORGFR₁₂
Age ($r = -0.32, P = 0.01$), GFR₀ ($r = 0.29, P = 0.01$), dialysis at entry ($r = -0.27, P = 0.02$), tubular atrophy ($r = -0.28, P = 0.02$), normal glomeruli ($r = 0.24, P = 0.04$), interstitial fibrosis ($r = -0.24, P = 0.04$), and intraepithelial infiltrate ($r = -0.26, P = 0.03$) showed a relationship with GFR₁₂ (Table 4). Although the univariate correlation of some predictive variables with GFR₁₂ is weak (Figure 2, E through I), the combination of age, normal glomeruli, tubular atrophy, intraepithelial infiltrate, and GFR₀ showed a reasonable correlation with GFR₁₂ ($r² = 0.491, r = 0.701$) as shown in Figure 3 and Table 5.

An analysis was performed to determine which parameters independent of GFR₀ correlated with GFR₁₂ (the so-called CORGFR₁₂), which could be regarded as renal function recovery. The same parameters that were predictive of GFR₁₂, except for dialysis at entry and GFR₀ as defined, also were predictive of CORGFR₁₂ (Table 5). The univariate correlation of these variables with CORGFR₁₂ is shown in Figure 2, J through M.

Predictors of Dialysis Dependence at Entry and at 12 Mo
A prognostic indicator of dialysis dependence at entry was the percentage of fibrous crescents ($r = 0.22, P = 0.03$). There was an increased chance for being dialysis dependent with an increased percentage of fibrous crescents, although the predictive value was moderate (Table 5). The percentage of normal glomeruli ($r = -0.30, P = 0.01$), dialysis dependence at entry ($r = 0.25, P = 0.03$), intraepithelial infiltrates ($r = 0.31, P = 0.03$), and treatment arm ($r = -0.28, P = 0.02$) showed a relationship with dialysis dependence at 12 mo (Table 5). In the logistic regression analysis, the combination of normal glomeruli and treatment arm was predictive of dialysis at 12 mo. Univariate relationships of these predictors are shown in Figure 4. In clinical terms, the higher the percentage of normal glomeruli, the lower the chance for developing dialysis dependence. Plasma exchange for treatment arm was clinically favorable over intravenous methylprednisolone as adjunctive therapy.

Analyzing the subgroups, only for 69 patients who were dialysis dependent could statistical significance be reached. For this subgroup, the same parameters correlated with dialysis dependence at 12 mo: The percentage of normal glomeruli ($r = -0.31, P = 0.03$), treatment arm ($r = -0.36, P = 0.01$), and intraepithelial infiltrates ($r = 0.32, P = 0.07$). In addition, more glomerulosclerosis ($r = 0.27, P = 0.05$) and the presence of arteriosclerosis ($r = 0.32, P = 0.03$) correlated with a higher chance for dialysis dependence at 12 mo.

Predictors of Death
Only two parameters correlated with death: ANCA directed against MPO ($r = 0.24, P = 0.04$) and the amount of neutrophils in the interstitial infiltrate ($r = 0.37, P = 0.01$; Table 4). However, there were no parameters that were predictive of death as determined by regression analysis. Twenty-four patients died within the first year of follow-up. Two deaths clearly were

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Table 3. Average distribution of most characteristic glomerular and tubulointerstitial lesions

<table>
<thead>
<tr>
<th>Histologic Lesion</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal glomeruli</td>
<td>12.8 ± 15.3</td>
</tr>
<tr>
<td>Fibrinoid necrosis</td>
<td>25.5 ± 25.2</td>
</tr>
<tr>
<td>Crescents</td>
<td>56.0 ± 28.8</td>
</tr>
<tr>
<td>segmental crescents</td>
<td>56.0 ± 27.3</td>
</tr>
<tr>
<td>circumferential crescents</td>
<td>74.1 ± 49.5</td>
</tr>
<tr>
<td>cellular crescents</td>
<td>90.6 ± 26.1</td>
</tr>
<tr>
<td>fibrous crescents</td>
<td>9.4 ± 2.7</td>
</tr>
<tr>
<td>Global sclerosis</td>
<td>26.4 ± 25.7</td>
</tr>
<tr>
<td>Intersitial edema (0/1)</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>Intersitial infiltrates (0/1/2/3)</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>neutrophils (0/1/2)</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td>monocytes (0/1/2)</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>eosinophils (0/1/2)</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Intersitial fibrosis (0/1/2)</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Tubular casts (0/1)</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Tubular necrosis (0/1)</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Tubular atrophy (0/1/2)</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>Intraepithelial infiltrates (0/1)</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Small-vessel vasculitis (0/1)</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Arteriosclerosis (0/1)</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Arteriolosclerosis (0/1)</td>
<td>0.5 ± 0.5</td>
</tr>
</tbody>
</table>

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*aGlomerular lesions are expressed as a mean percentage of the total number of glomeruli per patient together with the SD. Numbers after tubulointerstitial lesions indicate the categroical scoring system.

*bAll crescents were scored as either segmental or circumferential and as either cellular or fibrous and are expressed as percentage of total number of crescents.

Figures 2 and 4. When the number of variables is high (48 in this study) and the number of cases is relatively low (100 in this study), inclusion of all variables in the regression analysis is not statistically relevant. The number of variables should be lower than one 10th of the number of cases to prevent “over fitting.” Therefore, the number of variables that were taken into account was limited to only those that exhibited reasonable correlations with the outcome parameter and with $P < 0.10$. The formulas for the predictive variables with the odds ratios are reported in Table 5.

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Predictors of GFR₀ for Patients Not on Dialysis
Patients who were on dialysis at entry ($n = 69$) were excluded from this analysis because their GFR was not measurable. Arteriosclerosis was the best predictor of GFR₀ ($r = -0.53, P = 0.01$). Other clinical and histologic parameters that showed relationships to GFR₀ were gender ($r = -0.45, P = 0.02$), age ($r = -0.40, P = 0.04$), tubular casts ($r = -0.47, P = 0.01$), and eosinophilic infiltrates ($r = -0.41, P = 0.04$; Table 4). Women had a significantly worse GFR₀ than men. Higher patient age, the presence of tubular casts and arteriosclerosis, and a predominant eosinophilic infiltrate correlated with a worse GFR₀. It seemed from the regression analysis that arteriosclerosis in combination with age, segmental crescents, and eosinophilic infiltrates was predictive for GFR₀ (Table 5). The univariate
that patients who present with severe renal dysfunction must present with acute severe renal dysfunction. It is widely assumed that patients who have ANCA-associated glomerulonephritis and have an extensive amount of chronic lesions for which treatment would not likely to be successful. This study shows that the majority of patients have extensive acute lesions and that a significant number of patients benefit from treatment, in particular when plasmapheresis is given. The combination of normal glomerular, acute and chronic tubulointerstitial damage, age, and treatment was predictive of renal outcome at 12 mo. A worse outcome in patients with a low percentage of normal glomeruli, more acute and chronic tubulointerstitial lesions, and intravenous methylprednisolone as adjunctive treatment was observed. A greater extent of acute and chronic glomerular and interstitial lesions predicted a worse renal function and higher chance for dialysis dependence at entry.

Most biopsies at entry showed extensive acute lesions in these patients, who had serum creatinine levels >500 μmol/L, whereas the number of globally sclerosed glomeruli was relatively low. This indicated that the severely disturbed renal function that was observed at entry was not due to a low-level disease that led to extensive chronic damage but rather to an acute onset of disease that was characterized by extensive acute lesions in the form of crescents and fibrinoid necrosis. The acute

death\textsuperscript{a}

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>(GFR_{0}) of Patients Not on Dialysis</th>
<th>(GFR_{12})</th>
<th>(CORGFR_{12})</th>
<th>Dialysis at Entry\textsuperscript{b}</th>
<th>Dialysis at 12 Mo\textsuperscript{c}</th>
<th>Death\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
<td>(P)</td>
</tr>
</tbody>
</table>

\(GFR_{0}\) of patients not on dialysis

-0.454 0.015\textsuperscript{f} 0.127 0.520

GFR\textsubscript{0} of all patients

-0.901 <0.01\textsuperscript{f} -0.221 0.600 -0.117 0.262

gender\textsuperscript{d}

-0.520 0.014 0.000 0.110 0.900 0.117 0.273

age

-0.173 0.083 0.148 0.196

MPA (−/+)

-0.010 0.931 0.288 0.097

RLV (−/+)

-0.010 0.931 0.288 0.097

WG (−/+)

-0.010 0.931 0.288 0.097

dialysis at entry\textsuperscript{b}

0.003 0.761 0.000 0.761

treatment arm\textsuperscript{c}

0.464 0.032 0.221 0.060

Glomerular lesions

-0.241 0.213 0.307 0.128 0.115 0.857

no abnormalities

-0.274 0.019 0.000 0.732

fibrinoid necrosis

-0.127 0.520

crecents

-0.027 0.681

fibrous crescents

-0.102 0.312

glomerulosclerosis

-0.110 0.273

global sclerosis

-0.020 0.817

Intestinal lesions

-0.007 0.593 0.250 0.117

neutrophilic infiltrate

-0.145 0.146

eosinophilic infiltrate

-0.145 0.146

intestinal fibrosis

-0.145 0.146

tubular atrophy

-0.145 0.146

intraepithelial infiltrates

-0.145 0.146

Vascular lesions

-0.274 0.019 0.000 0.732

Small-vessel vasculitis

-0.007 0.593 0.250 0.117

arteriosclerosis

-0.145 0.146

\(\text{CORGFR}_{12}\), corrected GFR at 12 mo; \(GFR_{0}\), renal function at time of diagnosis; \(GFR_{12}\), renal function at 12 mo.

\(\text{Dialysis independence was coded 0, and dialysis dependence was coded 1.}\)

\(\text{Being alive was coded as 0, and being dead was coded as 1.}\)

\(\text{Male was coded as 0, and female was coded as 1.}\)

\(\text{Plasma exchange treatment was coded 0, and intravenous methylprednisolone was coded as 1.}\)

\(\text{Correlation with } P < 0.05.\)
lesions did not correlate with $GFR_0$, or with dialysis at entry. We also found this in a previous study (29), and we think that this phenomenon may be because although, histologically, acute lesions seem to be similar to each other, they are in fact a heterogeneous group of lesions of which some are in a healing process and others are on their way to irreversible damage. This could explain their lack of predictive value for renal function at time of biopsy.

A relationship existed between $GFR_0$ and age, gender, arteriosclerosis, tubular casts, and interstitial infiltrates (in particular of eosinophilic granulocytes) for patients who were not on dialysis at entry. Three of these variables, age, tubular casts, and interstitial infiltrates, also showed a relationship with $GFR_0$ in a previous study of patients with mild to moderate renal involvement (serum creatinine $< 500 \mu\text{mol/L}$) (20). This indicated that these parameters are important for renal impairment in ANCA-associated glomerulonephritis, both at a high and a low range of renal dysfunction. Age and gender were already known to have a high impact on renal function in ANCA-associated renal disease; worse renal function in elderly patients with acute disease and a relative benefit for men was observed (30–32).

Arteriosclerosis was not associated with age and had prognostic value for determining renal outcome in this analysis. This possibly reflects a component of chronic renal vascular disease (11). It is interesting that the severity of tubular casts and interstitial infiltrates reflected the level of renal dysfunction at entry in this patient group, as well as in our previously described patient group with ANCA-associated glomerulonephritis with moderate renal involvement. The amount of tubular casts may reflect the degree of obstruction (33). The interstitial infiltrate, in part consisting of eosinophilic granulocytes, may be indicative of ongoing chronic interstitial fibrosis and could account for the increased intrarenal collagen synthesis as has been shown for lupus nephritis (34) and renal allograft fibrosis (35).

The percentage of fibrous crescents was the only parameter that was predictive of dialysis at entry. It is tempting to hypothesize that patients who are in need of dialysis at the time of diagnosis have had the disease for some time, which is reflected by the fibrous crescents. However, approximately half of the patients who were on dialysis at entry were no longer on dialysis at 12 mo. This suggests that fibrous crescents should not be a contraindication to start therapy. One parameter that predicted dialysis at 12 mo was the treatment arm. The adjunctive treatment of preference was plasma exchange, which had a favorable effect on dialysis independence after 1 yr. Therefore, plasma exchange seems to be the preferred additional form of therapy for patients who have ANCA-associated glomerulonephritis and present with severe renal failure. The trial report on the MEPEX data revealed that the addition of plasma exchange to oral cyclophosphamide led to an increased chance of renal recovery compared with the addition of intravenous methylprednisolone (unpublished data). This beneficial effect was sustained throughout the 12-mo study period. The mortality rate was comparable between the two treatment groups.

Age, the percentage of normal glomeruli, intraepithelial infiltrates, tubular atrophy, and $GFR_0$ predicted $GFR_{12}$. Age has been shown to be important for renal outcome before (11,36). We reported on the importance of normal glomeruli for renal recovery in patients with ANCA-associated glomerulonephritis in 1999 (23) and in 2002 (20). Also in several other studies,

Table 5. Formulas for estimated outcomes$^a$

<table>
<thead>
<tr>
<th>Formulas</th>
<th>Label of Values</th>
<th>$r^2$</th>
<th>Exponent $\beta$</th>
<th>Chance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated $GFR_{12}$ (ml/min) = $23.0 - 4.9 \times \text{arteriosclerosis} - 0.15 \times \text{age} + 0.13 \times \text{segmental crescents} - 3.1 \times \text{eosinophilic infiltrate}$</td>
<td>Arteriosclerosis: $-/+$, Age: years, Segmental crescents: $%$, Eosinophilic infiltrate: $-/+/+/+$</td>
<td>0.675</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated $GFR_{12}$ (ml/min) = $79.1 - 0.63 \times \text{age} + 0.58 \times \text{normal glomeruli} - 14.4 \times \text{tubular atrophy} - 14.2 \times \text{intraepithelial infiltrate} + 0.95 \times \text{GFR}_0$</td>
<td>Age: years, Normal glomeruli: $%$, Tubular atrophy: $-/+/+/+$, Intraepithelial infiltrate: $-/+$, GFR$_c$ (ml/min) See box above</td>
<td>0.491</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated $GFR_{12}$ (ml/min) = $62.1 - 0.63 \times \text{age} + 0.58 \times \text{normal glomeruli} - 14.5 \times \text{tubular atrophy} - 14.1 \times \text{intraepithelial infiltrate}$</td>
<td></td>
<td>0.443</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated dialysis at entry: $\beta = 0.081$</td>
<td>Fibrous crescents: $%$</td>
<td>0.212</td>
<td>1.09</td>
<td>OR = $\text{Exp} (\beta \times \Delta \text{fibrous crescents}) = (\text{Exp } \beta)^{\Delta \text{fibrous crescents}}$</td>
</tr>
<tr>
<td>Probability dialysis at 12 months: $y = -0.64 - 0.070 \times \text{normal glomeruli} + 1.3 \times \text{arm}$</td>
<td>Normal glomeruli: $%$, Arm: $0 = \text{plasma exchange}, 1 = \text{intravenous methylprednisolone}$</td>
<td>0.309</td>
<td>0.93</td>
<td>$p = \text{Exp} (y)/(1 + \text{Exp} (y))$</td>
</tr>
</tbody>
</table>

$^a$Odds ratios (OR) expressed as exponent $\beta$. Values in formulas are $\beta$. Predictive values of the models are expressed as $r^2$.

$^b$Estimated $GFR_0$ applies only to patients who were not on dialysis.
Figure 2. Correlation figures for predictive variables of renal function and renal function recovery. Predictors of renal function at the time of diagnosis (GFR₀) for only patients who were not on dialysis at entry: Arteriosclerosis (r = −0.531, P = 0.009; A), age (r = −0.399, P = 0.035; B), segmental crescents (r = 0.348, P = 0.069; C), and eosinophilic infiltrate (r = −0.412, P = 0.037; D). Predictors of renal function at 12 mo (GFR₁₂): GFR₀ (r = 0.290, P = 0.013; E), age (r = −0.321, P = 0.006; F), percentage of normal glomeruli (r = 0.239, P = 0.042; G), tubular atrophy (r = −0.279, P = 0.017; H), and intraepithelial infiltrate (r = −0.260, P = 0.026; I). Predictors of corrected GFR₁₂ (CORGFR₁₂): Age (r = −0.319, P = 0.006; J), percentage of normal glomeruli (r = 0.245, P = 0.038; K), tubular atrophy (r = −0.311, P = 0.008; L), and intraepithelial infiltrate (r = −0.310, P = 0.008; M). All correlations are visualized either as scatter plots (continuous variables) or as box plots (categorical or dichotomous variables).
in the interstitial infiltrate. It is interesting that leucocytosis was demonstrated previously as a predictor of death in patients with idiopathic renal vasculitis (41), although a causal relationship between leukocyte count and progression of injury could not be established. We think that a possible link could be leucocytosis as one of the first signs of an infection, which, in combination with immunosuppressive treatment, would be a high-risk factor for death. Our study shows that the most important cause of death of patients who died between 3 and 12 mo of follow-up was therapy-related infection. Recent studies also showed that the main cause of death in ANCA-associated glomerulonephritis is treatment-related infectious complication (42,43).

Baseline renal function was found previously to be a predictor of renal outcome in retrospective studies (11,36,44). In addition, our previous study of patients with mild to moderate renal involvement in ANCA-associated vasculitis showed that GFR_0 is important for predicting GFR_{12} (20). CORGFR_{12} was used to determine the influence of the GFR_{0}-independent variables. This measure for renal function recovery enabled us to study the difference between the measured GFR_{12} and the expected GFR_{12} on the basis of GFR_{0}. However, the same parameters, except for GFR_{0} and dialysis at entry, as expected, predicted GFR_{12} and CORGFR_{12}. This means that age, the percentage of normal glomeruli, tubular atrophy, and intraepithelial infiltrates indeed are important predictors of renal function recovery and are independent of renal function at entry.

The reason that some of the parameters that correlated univariately with GFR_{12} were not predictive of GFR_{12}, as resulting from the regression model, could be that some of the parameters correlated with each other. For instance, glomerulosclerosis and interstitial fibrosis were strong correlates with GFR_{12} in the univariate analysis, but because of their positive relationship with tubular atrophy, they did not turn out to be predictors of GFR_{12} in the regression model.

The models that were obtained from regression analysis are useful to the clinician for estimating renal status after 12 mo. In addition, the maximum chance for dialysis could be deducted from one of these models. If patients have the worst phenotype possible in this model—that is, no normal glomeruli in the renal biopsy and intravenous methylprednisolone as adjunctive treatment—then their chance for being on dialysis after 12 mo is 50%. Taking into account the chance of 24% for dying, this means that even in the worst case, there is still a 26% chance for recovery. Despite that the predictive values of the models postulated are limited (r^2 for GFR_{12} = 0.491; r^2 for dialysis at 12 mo = 0.309), these models clearly showed that consideration of several clinical and histologic parameters results in a better prediction of renal outcome after 12 mo than evaluation of GFR_{0} (r^2 for GFR_{12} = 0.084; r^2 for dialysis at 12 mo = 0.073) or dialysis dependence at entry (r^2 for GFR_{12} = 0.075; r^2 for dialysis at 12 mo = 0.091) alone.

Predictive parameters in this study were defined for different outcome parameters in patients with ANCA-associated glomerulonephritis and severe renal involvement. All patients who participated in the study presented with severe renal

Normal glomeruli were shown to predict renal outcome over time (36–40). In follow-up biopsies of patients with ANCA-associated glomerulonephritis, the percentage of normal glomeruli did not change over time (29). Therefore, apart from reflecting the functioning part of the kidney, normal glomeruli also are a relatively constant parameter, the combination of which may explain their strength as a predictive parameter. Also intraepithelial tubular infiltrates are predictive of GFR_{12}, and their presence may well be related to the development of tubular atrophy, a widely known parameter of chronic renal failure in general and associated with worse renal outcome in ANCA-associated vasculitis (11,18).

No parameters predicted death; however, one of the parameters that correlated with death was the amount of neutrophils

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disease and were treated according to protocol. However, it has to be noted that the results of this study must be interpreted with the understanding that every patient had severe renal disease with a serum creatinine >500 μmol/L and that extrapolation of these results to patients who do not meet this or any of the other inclusion criteria is not advisable. Further studies are required to determine whether these results can be extrapolated to long-term follow-up. Another point of consideration is that the r values of the univariate analyses could raise some doubt on the clinical applicability of the findings. A possible explanation for the relatively low r values is that the patient cohort is a predefined group of patients with bad renal function at entry, with parameters in a relatively tight range, which is bound to lead to relatively low r values. Therefore, it was necessary to analyze the data not only in a univariate manner but also in a multivariate manner, because the combination of parameters predicts much better for outcome than single parameters alone. Another issue concerns that the patients in this study were randomly assigned into two treatment arms, which could have confounded the renal outcome data. However, the treatment arms proved to predict only dialysis dependence at 12 mo, whereas no correlation was found for the other outcome parameters.

Conclusion
This study identified determinants of renal outcome in patients with ANCA-associated vasculitis and severe renal involvement. The prospective design, the homogeneity of the population, the population size, the standardization of patient treatment, and the detailed scoring system provided optimal conditions for this analysis. Our data suggest that in severe ANCA-associated glomerulonephritis, the combination of renal function at diagnosis, the percentage of normal glomeruli, age, and acute and chronic tubulointerstitial lesions predict GFR\textsubscript{12}. The prediction was much more accurate than that based on GFR at entry alone. The percentage of normal glomeruli at diagnosis combined with adjuvant treatment predicted dialysis dependence at 12 mo. The regression model provides a tool to the clinician for estimating the chances for a favorable outcome for patients.

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