ANCA-associated glomerulonephritis : insights into etiology, pathogenesis, and prognosis

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Education is when you read the fine print.
Experience is what you get if you don't.
Pete Seeger

Chapter 2

Clinical and histological determinants of renal outcome in ANCA-associated vasculitis: a prospective analysis of 100 patients with severe renal involvement

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Abstract

This study aimed to identify clinical and histological prognostic indicators of renal outcome in ANCA-associated vasculitis patients with severe renal involvement (serum creatinine > 500 μmol/L). One hundred patients enrolled in an international randomized clinical trial comparing plasma exchange with intravenous methyl prednisolone as an additional initial treatment were prospectively analyzed. Diagnostic renal biopsies were performed upon entry into the study. Thirty-nine histological and 9 clinical parameters were determined as candidate predictors of renal outcome. The end-points were renal function at the time of diagnosis (GFR_0), 12 months after diagnosis (GFR_{12}), dialysis at entry and 12 months after diagnosis, and death. Multivariate analyses were performed. Predictive of GFR_0 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 GFR_{12} were age ($r = -0.32$; $p = 0.01$), normal glomeruli ($r = 0.24$; $p = 0.04$), tubular atrophy ($r = -0.28$; $p = 0.02$), intra-epithelial infiltrate ($r = -0.26$; $p = 0.03$), and GFR_0 ($r = 0.29$; $p = 0.01$). Fibrous crescents ($r = 0.22$; $p = 0.03$) were predictive of dialysis at entry. Normal glomeruli ($r = -0.30$; $p = 0.01$) and treatment limb ($r = -0.28$; $p = 0.02$) were predictive of dialysis after 12 months. No parameter predicted death. Both chronic and acute tubulo-interstitial lesions predicted GFR_{12} in severe ANCA-associated glomerulonephritis, while plasma exchange was a positive predictor of dialysis-independency after 12 months for the entire patient group. Plasma exchange remained a positive predictor if patients who were dialysis-dependent at presentation were analyzed separately ($r = -0.36$; $p = 0.01$). Normal glomeruli were a positive predictor of dialysis-independency and improved renal function after 12 months indicating that the unaffected part of the kidney is vital in determining renal outcome.

Introduction

Rapidly progressive deterioration of renal function is a common and usually severe clinical feature of anti-neutrophil cytoplasm autoantibody (ANCA)-associated vasculitis, which may lead to end-stage renal failure or death [1]. Histopathology of renal biopsies shows pauci-immune crescentic glomerulonephritis [2] with variable amounts of extracapillary proliferation, fibrinoid necrosis, and glomerulosclerosis [3]. The most frequently occurring forms of ANCA-associated vasculitis are microscopic polyangiitis (MPA) and Wegener's granulomatosis (WG), while renal limited vasculitis (RLV) occurs less frequently [4]. Despite the existence of clear definitions [5], the diagnosis of individual patients may be difficult. Patients with ANCA-associated glomerulonephritis are currently treated similarly regardless of which form is diagnosed. European trials are aimed at developing treatment that corresponds to disease severity [6].

Therapy is associated with severe and potentially lethal adverse effects for a substantial number of patients [7-9]; nearly 90% of patients experience persistent morbidity despite adequate treatment [10]. Therefore, there is a need for prognostic markers of renal patient outcome to help modify therapy for patients suffering from ANCA-associated vasculitis [11]. Several studies searching for clinical and histological predictors of renal outcome have provided contradictory results [11-19]. Meta-analyses are difficult to conduct because there is substantial heterogeneity with regard to study designs, inclusion criteria, scoring methods, treatment strategies, and end-points [11-19]. We have previously studied ANCA-associated vasculitis patients with mild to moderate renal involvement (serum creatinine < 500 μmol/L) receiving standardized treatment in an attempt to identify clear clinical and histological 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 months and active lesions are associated with renal function recovery.

In the present 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 histological predictors of outcome in patients with severe renal involvement (serum creatinine > 500 μmol/L).

Materials and Methods

Patients

Patients were derived from 29 hospitals located in 11 European countries. Patients were enrolled in the MEPEX trial, which is a randomized trial evaluating adjunctive therapy for severe glomerulonephritis in ANCA-associated systemic vasculitis [21]. Patients were included who had a serum creatinine of 500 µmol/L or more at entry. 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 randomized to either receive intravenous methyl prednisolone or undergo plasma exchanges. Standard therapy consisted of oral corticosteroids, which started at 1.0 mg/kg daily and was tapered down within the first six months, and cyclophosphamide 2.5 mg/kg daily, which at three months was replaced by the less toxic azathioprine. Those patients that were randomized to receive intravenous methyl prednisolone, were administered three times 1,000 mg daily for three consecutive days, starting directly after diagnosis. The patients in the plasma exchange limb, received seven plasma exchanges of 60 mL/kg during the first 14 days after diagnosis. Patients were only included in this analysis if both histological data, obtained from renal biopsy at the time of study entry, and clinical data were available.

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

1	New diagnosis of WG, 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 necrotising glomerulonephritis on renal biopsy.
2	ANCA-positivity for one of the following: a) C-ANCA pattern by IIF b) positivity in the PR3 ELISA c) positivity in the MPO ELISA, with or without P-ANCA ANCA-negativity is allowed if the disease is confirmed histologically
3	Biopsy-proven necrotising and/or crescentic glomerulonephritis, in the absence of another defined glomerulopathy, with severe renal impairment defined by: (i) oliguria (< 400 ml/24hr) *or* (ii) intention to commence dialysis within 48 hours of admission *or* (iii) creatinine > 500 µmol/L

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 based on criteria previously published [21] and determinations were made by local physicians.

ANCA Testing

Indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (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 MPO or 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 (GFR_0), dialysis status at entry, age, gender, quantitatively assessed proteinuria at entry, diagnosis (WG, MPA, or RLV), ANCA-antigen specificity (PR3-ANCA or MPO-ANCA), IIF pattern (C-ANCA or P-ANCA), and treatment limb (intravenous methyl prednisolone or plasma exchange). Candidate parameters for histological predictors were determined from paraffin sections of renal biopsies stained with silver, periodic acid-Schiff, haematoxylin and eosin, and trichrome. Sections were reviewed by two of five participating pathologists (IMB, FF, LHN, RW, or JAB). 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 semi-quantitatively. Granulomas were scored quantitatively. In total, thirty-nine histological 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 (GFR_0), renal function at 12 months (GFR_{12}), dialysis-independency at diagnosis, dialysis-independency at 12 months, relapse, and death. Renal function was defined as the glomerular filtration rate, 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 histological parameters and GFR_{12} after correction for GFR_0. The latter value was expressed as the corrected GFR_{12} ($CORGFR_{12}$), defined as the difference between the observed GFR_{12} and its linear prediction based upon GFR_0. This correction created a corrected value that was statistically independent of the starting value [28].

Statistical Analyses

The software used for statistical analyses was the SPSS 10.0 standard version for Windows (SPSS, Inc., Chicago, IL, USA). Correlation of the quantitative and dichotomous candidate predictors with GFR_0, GFR_{12}, and $CORGFR_{12}$ was determined by using Pearson's correlation test. The Spearman's 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's correlation test. Phi-values were used to correlate dichotomous and categorical candidate predictors with the occurrence of dialysis and death at 12 months. 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 based on a binary logistic regression analysis was used for dialysis-dependency and death at 12 months. Each parameter that correlated with a P value of 0.10 or less was entered in the model as possible predictor of renal outcome. The group was analyzed both as a whole, and subgroup analyses were made for those patients that were dialysis-dependent at entry and those who were not. The values of exponent β were used for the expression of odds ratios. Correlation coefficients were noted as r and predictive values as r^2.

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 over 500 mg intravenous methyl prednisolone; these patients were excluded. None of the remaining 137 patients were lost to follow-up or withdrawn from the study. Renal biopsies were obtained from 102 patients for re-evaluation. 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.

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

Age (range) [yr]	64.1 (26.8-80.7)
Gender (male/female)	63 / 37
Diagnosis (WG/MPA/RLV)	33 / 57 / 10
GFR_0 (mL/min) [mean ± SD]	10 ± 4
GFR_{12} (mL/min) [mean ± SD]	32 ± 13
Proteinuria (mg/24h)	30 ± 6
Adjunctive treatment (IVMeP/PE)* [%]	49 / 51

* IVMeP = intravenous methyl prednisolone, PE = plasma exchange.

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. 1,000 mg of methyl prednisolone was administered intravenously for three times to patients assigned to this treatment limb.

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

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.

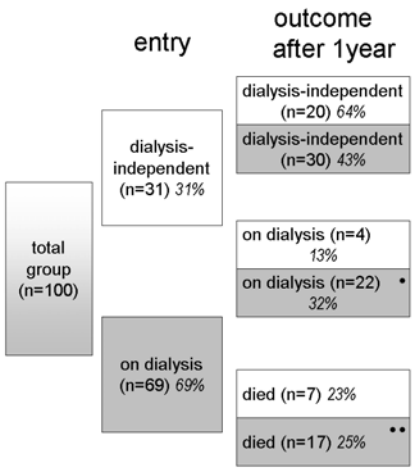

Figure 1. Flow chart of the clinical courses of patients. Gray boxes represent patients who were on dialysis at entry, white boxes represent patients who were dialysis-independent at entry. Numbers and percentages are listed. The black dots represent the patients who experienced a relapse.

Histological Features

The occurrence of the main histological lesions was analyzed in order to explore the extent found at the entry period in this group of clinically severely renal impaired patients. The frequencies of the glomerular and tubulo-interstitial lesions are presented in Table 3. The majority of the non-sclerotic 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 histological 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 Figures 2 and 4. When the number of variables is high (48 in this study) and the number of cases 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 tenth of the number of cases to prevent "over fitting". Therefore, the number of variables 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.

Table 3. Average distribution of most characteristic glomerular and tubulo-interstitial lesions

Histologic lesion	Percentage
% normal glomeruli	12.8 ± 15.3
% fibrinoid necrosis	25.5 ± 25.2
% crescents	56.0 ± 28.8
* segmental crescents	25.9 ± 27.3
* circumferential crescents	74.1 ± 49.5
# cellular crescents	90.6 ± 26.1
# fibrous crescents	9.4 ± 2.7
% global sclerosis	26.4 ± 25.7
Interstitial edema (0/1)	0.5 ± 0.5
Interstitial infiltrates (0/1/2/3)	1.8 ± 0.7
neutrophils (0/1/2)	0.7 ± 0.5
monocytes (0/1/2)	1.8 ± 0.4
eosinophils (0/1/2)	0.4 ± 0.5
Interstitial fibrosis (0/1/2)	1.2 ± 0.6
Tubular casts (0/1)	0.9 ± 0.3
Tubular necrosis (0/1)	0.8 ± 0.4
Tubular atrophy (0/1/2)	1.1 ± 0.6
Intra-epithelial infiltrates (0/1)	0.8 ± 0.4
Small-vessel vasculitis (0/1)	0.1 ± 0.3
Arteriosclerosis (0/1)	0.8 ± 0.4
Arteriolosclerosis (0/1)	0.5 ± 0.5

Glomerular lesions are expressed as a mean percentage of the total number of glomeruli per patient together with the standard deviation.
* all crescents were scored as either segmental or circumferential. Segmental and circumferential crescents are expressed as percentage of total no. of crescents.
# all crescents were scored as either cellular or fibrous. Cellular and fibrous crescents are expressed as percentage of total no. of crescents.
Numbers behind tubulo-interstitial lesions indicate the categorical scoring system.

Predictors of GFR_0 for Patients Not on Dialysis

Patients on dialysis at entry (N=69) were excluded from this analysis because their GFR was not measurable. Arteriosclerosis was the best predictor of GFR_0 ($r = -0.53$; $p = 0.01$). Other clinical and histological parameters that showed relationships to GFR_0 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). Females had a significantly worse GFR_0 than males. Higher patient age, the presence of tubular casts and arteriosclerosis, and a predominant eosinophilic infiltrate correlated with a worse GFR_0. It appeared from the regression analysis that arteriosclerosis in combination with age, segmental crescents, and eosinophilic infiltrates was predictive for GFR_0 (Table 5). The univariate relationship of these variables with GFR_0 is shown in Figure 2A-D.

Table 4. Correlation of clinical and histological parameters with GFR_0, GFR_{12}, $CORGFR_{12}$, dialysis at entry, dialysis at 12 months, and death

	GFR_0 of patients not on dialysis		GFR_{12}		$CORGFR_{12}$		Dialysis at entry *(b)*		Dialysis at 12 months *(b)*		Death *(d)*	
	r	p	r	p	r	p	r	p	r	p	r	p
Clinical variables												
GFR_0 of patients not on dialysis	-	-	0.255	0.230	0.048	0.824	-	-	0.067	0.754	-0.127	0.520
GFR_0 of all patients	-	-	0.290	0.013*	0.000	1.000	-0.918	<0.001*	-0.221	0.060	-0.117	0.262
Gender *(a)*	-0.454	0.015*	-0.137	0.248	-0.070	0.526	0.066	0.510	-0.092	0.424	0.151	0.130
Age	-0.399	0.035*	-0.321	0.006*	-0.319	0.006*	0.017	0.868	0.083	0.476	0.132	0.192
Microscopic polyangiitis (-/+)	-0.105	0.595	-0.129	0.276	-0.071	0.553	0.117	0.244	0.165	0.149	0.110	0.273
Renal limited vasculitis (-/+)	0.250	0.199	0.029	0.808	0.015	0.903	0.019	0.428	-0.038	0.741	0.047	0.640
Wegener's granulomatosis (-/+)	-0.025	0.900	0.116	0.329	0.064	0.592	-0.173	0.083	-0.148	0.196	-0.145	0.146
Dialysis at entry *(b)*	0.135	0.495	-0.274	0.019*	-0.002	0.987	-	-	0.251	0.029*	0.022	0.826
Treatment limb *(c)*	0.227	0.245	0.125	0.291	0.121	0.309	-0.008	0.935	-0.277	0.016*	0.036	0.722
Glomerular lesions												
No abnormalities	-0.094	0.635	0.239	0.042*	0.245	0.038*	-0.031	0.761	-0.303	0.008*	0.102	0.312
Fibrinoid necrosis	0.126	0.523	0.152	0.199	0.081	0.498	-0.166	0.100	-0.133	0.253	0.021	0.833
Crescents	-0.069	0.729	0.072	0.545	0.049	0.681	-0.043	0.669	-0.025	0.827	-0.043	0.674
Fibrous crescents	0.052	0.794	-0.156	0.187	-0.106	0.376	0.220	0.028*	0.080	0.492	-0.030	0.763
Glomerulosclerosis	0.047	0.813	-0.274	0.019*	-0.269	0.022*	0.023	0.817	0.207	0.073	-0.014	0.894
Global sclerosis	0.044	0.826	-0.282	0.016*	-0.273	0.020*	0.035	0.732	0.214	0.064	-0.002	0.985
Interstitial lesions												
Neutrophilic infiltrate	0.114	0.579	-0.037	0.758	-0.010	0.931	0.288	0.097	0.221	0.299	0.373	0.010*
Eosinophilic infiltrate	-0.412	0.037*	0.169	0.155	0.157	0.190	0.114	0.744	0.126	0.757	0.170	0.431
Interstitial fibrosis	-0.007	0.971	-0.244	0.037*	-0.233	0.049*	0.235	0.239	0.234	0.382	0.155	0.662
Tubular atrophy	0.035	0.858	-0.279	0.017*	-0.311	0.008*	0.241	0.213	0.307	0.128	0.115	0.857
Intra-epithelial infiltrates	-0.105	0.597	-0.260	0.026*	-0.310	0.008*	0.116	0.512	0.308	0.027*	0.088	0.679
Vascular lesions												
Small-vessel vasculitis	-0.256	0.189	-0.047	0.695	-0.045	0.707	0.028	0.777	-0.007	0.953	-0.048	0.632
Arteriosclerosis	-0.531	0.009*	-0.131	0.310	-0.142	0.275	-0.201	0.059	0.160	0.196	0.164	0.123

* correlation with a P value < 0.05.

(a) male was coded '0' and female '1' in this analysis.

(b) dialysis-independency was coded '0' and dialysis-dependency '1' in this analysis.

(c) plasma exchange treatment was coded '0' and intravenous methyl prednisolone was coded '1'.

(d) being alive was coded '0' and dead '1'.

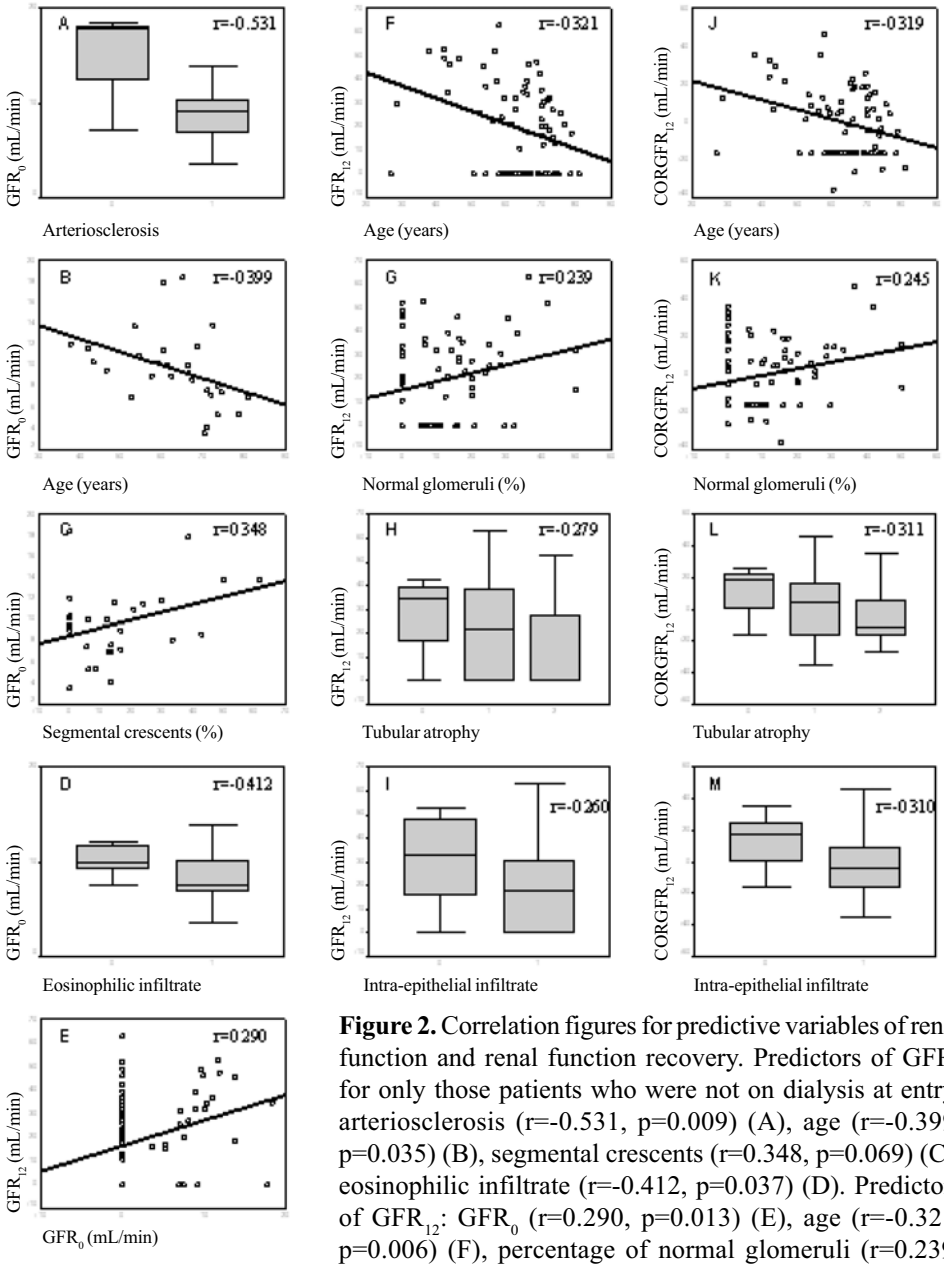

Figure 2. Correlation figures for predictive variables of renal function and renal function recovery. Predictors of GFR_0 for only those patients who were not on dialysis at entry: arteriosclerosis (r=-0.531, p=0.009) (A), age (r=-0.399, p=0.035) (B), segmental crescents (r=0.348, p=0.069) (C), eosinophilic infiltrate (r=-0.412, p=0.037) (D). Predictors of GFR_{12}: GFR_0 (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), intra-epithelial infiltrate (r=-0.260, p=0.026) (I). Predictors of $CORGFR_{12}$: 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), intra-epithelial 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).

Table 5. Formulas for estimated outcomes

Formulas	Label of values	r^2	Exp β	Chance
*Estimated GFR_0 (mL/min) = 23.0 – 4.9 x arteriosclerosis – 0.15 x age + 0.13 x segmental crescents – 3.1 x eosinophilic infiltrate	arteriosclerosis: –/+ age: years segmental crescents: % eosinophilic infiltrate: –/+/++	0.678		
Estimated GFR_{12} (mL/min) = 79.1 – 0.63 x age + 0.58 x normal glomeruli – 14.4 x tubular atrophy – 14.2 x intra-epithelial infiltrate + 0.95 x GFR_0	age: years normal glomeruli: % tubular atrophy: –/+/++ intra-epithelial infiltrate: –/+ GFR_0: mL/min	0.491		
Estimated $CORGFR_{12}$ (mL/min) = 62.1 – 0.63 x age + 0.58 x normal glomeruli – 14.5 x tubular atrophy – 14.1 x intra-epithelial infiltrate	See box above	0.443		
Estimated dialysis at entry: β = 0.081	fibrous crescents: %	0.212	1.09	OR = Exp (β x Δfibrous crescents) = $(Exp\ \beta)^{\Delta fibrous\ crescents}$
Probability dialysis at 12 months: y = – 0.64 – 0.070 x normal glomeruli + 1.3 x limb	normal glomeruli: % limb: 0 = plasma exchange, 1 = iv. methylprednisolone	0.309	0.93 3.52	p = Exp (y) / (1 + Exp (y))

Odds ratios (OR) are expressed as exponent Beta (exp β). Values in the formulas are βs. Predictive values of the models are expressed as r^2.
* Estimated GFR_0 only goes for patient not on dialysis.

Predictors of GFR_{12} and $CORGFR_{12}$

Age (r = -0.32; p = 0.01), GFR_0 (r = 0.29; p = 0.01), dialysis at entry (r = -0.27; p = 0.02), tubular atrophy (r = -0.28; p = 0.02), (global) glomerulosclerosis (r = -0.27; p = 0.02), normal glomeruli (r = 0.24; p = 0.04), interstitial fibrosis (r = -0.24; p = 0.04) , and intra-epithelial infiltrate (r = -0.26; p = 0.03) showed a relationship with GFR_{12} (Table 4). Although the univariate correlation of some predictive variables with GFR_{12} is weak (Figure 2E-I), the combination of age, normal glomeruli, tubular atrophy, intra-epithelial infiltrate, GFR_0 showed a reasonable correlation with GFR_{12} (r^2 = 0.491, r = 0.701) as shown in Figure 3 and Table 5.

An analysis was performed to determine which parameters independent of GFR_0 correlated with GFR at 12 months (the so-called $CORGFR_{12}$), which could be regarded as renal function recovery. The same parameters predictive of GFR_{12}, except for dialysis at entry and GFR_0 as defined, were also predictive of $CORGFR_{12}$ (Table 5). The univariate correlation of these variables with $CORGFR_{12}$ is shown in Figure 2J-M.

Predictors of Dialysis-dependency at Entry and at 12 Months

A prognostic indicator of dialysis-dependency at entry was the percentage of fibrous crescents (r = 0.22; p = 0.03). There was an increased chance of 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-dependency at entry (r = 0.25; p = 0.03), intra-epithelial infiltrates (r = 0.31; p = 0.03), and treatment limb (r = -0.28; p =

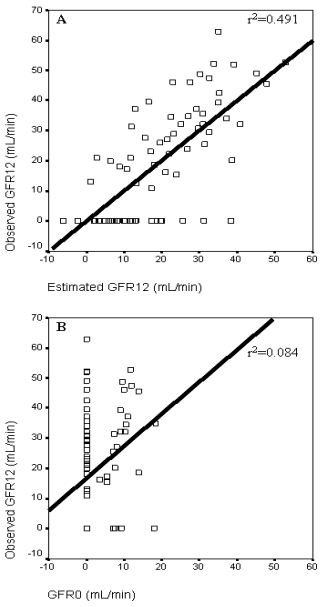

Figure 3. Scatterplot of observed GFR_{12} versus estimated GFR_{12} (A). The estimated GFR_{12} was calculated on the basis of the following formula: estimated GFR_{12} = 79.1 – 0.63 x age (years) + 0.58 x normal glomeruli (%) – 14.4 x tubular atrophy (–/+/++) – 14.2 x intra-epithelial infiltrate (–/+) + 0.95 x GFR_0 (mL/min). Scatterplot of measured GFR_{12} versus GFR_0 (B).

0.02) showed a relationship with dialysis-dependency at 12 months (Table 5). In the logistic regression analysis, the combination of normal glomeruli and treatment limb was predictive of dialysis at 12 months. Univariate relationships of these predictors are shown in Figure 4A-C. In clinical terms, the higher the percentage of normal glomeruli, the lower the chance of developing dialysis-dependency. Plasma exchange for treatment limb was clinically favorable over intravenous methyl prednisolone as adjunctive therapy.

Analyzing the subgroups, only for those 69 patients that were dialysis-dependent, statistical significance could be reached. For this subgroup, the same parameters correlated with dialysis-dependency at 12 months: the percentage of normal glomeruli (r = -0.31; p = 0.03), the treatment limb (r = -0.36; p = 0.01), and intra-epithelial 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) also correlated with a higher chance of dialysis-dependency at 12 months.

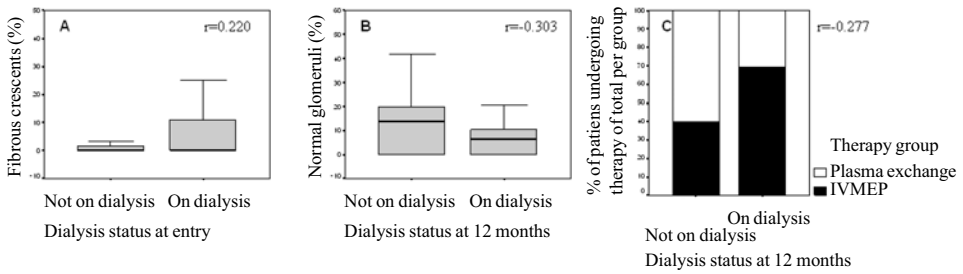

Figure 4. Correlation figures for predictive variables of dialysis status. Predictor of dialysis status at entry: percentage of fibrous crescents (r=0.220, p=0.028) (A). Predictors of dialysis status at 12 months: percentage of normal glomeruli (r=-0.303, p=0.008) (B) and treatment limb (r=-0.277, p=0.016) (C). All correlations are visualized either as box plots, or bar diagrams. IVMEP = intravenous methyl prednisolone.

Predictors of Death

There were only two parameters that 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 were clearly disease-related. These deaths were due to pulmonary vasculitis, and vasculitic gastro-intestinal bleeding with pulmonary capillaritis and myocardial infarction. Three deaths were probably disease-related: these patients died from pulmonary hemorrhage, which was not further specified. Eleven deaths were therapy-related. These were due to infections, such as pneumocystis carinii pneumoniae and cytomegalovirus. One patient died of a cerebral abscess. Whether this death was due to therapy or disease is unclear. Furthermore, three patients died of vascular causes, such as myocardial infarction and stroke. Finally, four patients died of unknown causes.

Discussion

In this study, clinical and histological prognostic indicators of outcome for ANCA-associated vasculitis patients with severe renal involvement (serum creatinine > 500 μmol/L) were determined. This was a prospective study in which patients were treated uniformly, investigating for the first time the subgroup of patients with ANCA-associated glomerulonephritis that present with acute severe renal dysfunction. It is widely assumed that patients presenting with severe renal dysfunction must 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 if plasmapheresis is given. The combination of normal glomeruli, acute and chronic tubulo-interstitial damage, age, and treatment was predictive of renal outcome at 12 months. A worse outcome in patients with a low percentage of normal glomeruli, more acute and chronic tubulo-interstitial lesions, and intravenous methyl prednisolone as adjunctive treatment was observed. A greater extent of acute and chronic glomerular and interstitial lesions predicted a worse renal function and higher chance of dialysis-dependency at entry.

Most biopsies at entry showed extensive acute lesions in these patients, which had serum creatinine levels higher than 500 μmol/L, whereas the number of globally sclerosed glomeruli was relatively low. This indicated that the severely

disturbed renal function observed at entry was not due to a low level disease leading to extensive chronic damage, but rather to an acute onset of disease characterized by extensive acute lesions in the form of crescents and fibrinoid necrosis. The acute 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 due to the fact that 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 for 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 at entry who were not on dialysis. 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 lower than 500 μmol/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 males 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]. Interestingly, 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 predictive of dialysis at entry. It is tempting to hypothesize that patients 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 on dialysis at entry are no longer on dialysis at 12 months. This implies that fibrous crescents

should not be a contra-indication to start therapy. One parameter that predicted dialysis at 12 months was the treatment limb. The adjunctive treatment of preference was plasma exchange, which had a favorable effect on dialysis-independency after one year. Therefore, plasma exchange seems to be the preferred additional form of therapy for patients with ANCA-associated glomerulonephritis presenting 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 to the addition of intravenous methyl prednisolone (unpublished data). This beneficial effect was sustained throughout the 12 months study period. The mortality rate was comparable between the two treatment groups.

Age, the percentage of normal glomeruli, intra-epithelial 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, 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 are also a relatively constant parameter, the combination of which may explain their strength as a predictive parameter. Also intra-epithelial tubular infiltrates are predictive of GFR_{12}, and their presence may well be related to the development of tubular atrophy, a well-known parameter of chronic renal failure in general, and associated with worse renal outcome in ANCA-associated vasculitis [11,18].

No parameters predicted for death; however, one of the parameters that correlated with death was the amount of neutrophils in the interstitial infiltrate. Interestingly, leucocytosis was previously demonstrated 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 that 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 months 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 previously found 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]. A corrected GFR_{12}, designated $CORGFR_{12}$, 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 intra-epithelial infiltrates are indeed 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 correlators 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 obtained from regression analysis are useful to the clinician for estimating renal status after 12 months. In addition, the maximum chance of 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 methyl prednisolone as adjunctive treatment, their chance of being on dialysis after 12 months is 50%. Taking into account the chance of 24% on dying, this means that even in the worst case, there is still a 26% chance on recovery. Despite the fact that the predictive values of the models postulated are limited (r^2 for GFR_{12} = 0.491 and r^2 for dialysis at 12 months = 0.309), these models clearly showed that consideration of several clinical and histological parameters results in a better prediction of renal outcome after 12 months than evaluation of GFR_0 (r^2 for GFR_{12} = 0.084 and r^2 for dialysis at 12 months = 0.073) or dialysis dependency at entry (r^2 for GFR_{12} = 0.075 and r^2 for dialysis at 12 months = 0.091) alone.

Predictive parameters in this study were defined for different outcome parameters in ANCA-associated glomerulonephritis patients with severe renal involvement. All patients who participated in the study presented with severe renal 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 not meeting 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 could be 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 not only to analyze the data in a univariate manner, but also in a multivariate manner, since the combination of parameters predicts much better for outcome than single parameters alone. Another issue concerns the fact that the patients in this study were randomized into two treatment arms, which could have confounded the renal outcome data. However, the treatment arms only proved to predict dialysis-dependency at 12 months, while no correlation was found for the other outcome parameters.

In summary, this study identified determinants of renal outcome in ANCA-associated vasculitis patients with severe renal involvement. Both the prospective design, the homogeneity of the population, 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 tubulo-interstitial lesions, predicts GFR after 12 months. 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-dependency at 12 months. The regression model provides a tool to the clinician for estimating the chances of a favorable outcome for patients.

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