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Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis

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Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis.

Background. In kidney biopsies of patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis, a variety of histopathological lesions occur, and their relationship to renal outcome is virtually unknown. This multicenter European study reports a clinicopathological analysis of biopsies from 157 patients with systemic vasculitis.

Methods. The biopsies were evaluated according to a previously standardized scoring protocol. Serum creatinine values were measured at the time of biopsy and one year later. In addition, the lowest creatinine level during follow-up was taken into account as the optimum level of renal function recovery. The clinical prognostic value of the histopathological parameters was analyzed with the Kruskal–Wallis one-way analysis of variance and the Mann–Whitney *U*-test.

Results. The percentage of normal glomeruli correlated most significantly with renal outcome at all points of measurement (all $P < 0.001$). Other lesions predicting for renal function were glomerular sclerosis ($P < 0.0005$ at one year after the biopsy), diffuse interstitial infiltrates ($P < 0.0001$ at entry, $P < 0.0003$ at one year), tubular necrosis ($P < 0.0025$ at entry), and tubular atrophy ($P < 0.002$ at entry, $P < 0.0002$ at one year).

Conclusion. Traditionally, attention is focused on the extent of active lesions in the renal biopsy in order to determine the severity of renal disease and its implication for renal outcome. Because of their significant impact on renal function, combined with their easy recognition, we recommend the use of the percentage of normal glomeruli in an adequate biopsy in predicting renal function of patients with systemic vasculitis.

Key words: renal biopsy, systemic vasculitis, tubular necrosis, ANCA, fibrinoid necrosis, rapidly progressive glomerulonephritis.

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Renal involvement in systemic vasculitis is histopathologically characterized by a number of lesions such as extracapillary proliferation and fibrinoid necrosis. Nonactive lesions such as focal or global glomerular sclerosis also may be seen. In the interstitium, tubular damage, infiltrates, and vasculitis of arteries and arterioles may be found. Among patients with systemic vasculitis, there is a wide variety in the incidence of these lesions. Next to active and chronic lesions, unaffected glomeruli may occur as well.

Renal failure as a cause of death in systemic vasculitis has decreased in frequency over the past decades; however, it still attains a number one position as a fatal factor [1], especially because of adverse effects of treatment. Previous research addressing the relationship in systemic vasculitis between histopathological renal lesions and renal outcome has produced contradictory results [2–8]. Alternatively, extracapillary proliferation, fibrinoid necrosis, glomerular sclerosis, interstitial inflammation, and acute tubular damage have been put forward as the most likely candidates to predict the degree of renal function. It has also been claimed that the severity of histological alteration has no relationship at all to renal outcome [9]. A comparison between studies is difficult because of large differences in entrance criteria, the type of lesions evaluated, and the way renal outcome was measured.

We currently report the results of the largest study of systemic vasculitis described to date. Renal biopsy results from 157 patients with systemic vasculitis from 14 European centers were assembled together with their clinical data, and their renal histopathology was correlated to renal function both at the time of biopsy and during follow-up.

METHODS

This study was part of the EC/BCR Project for ANCA-Assay Standardisation [10], a multicenter European study in which patients were entered both retrospectively and prospectively. From each center, the last 15 to 20 patients and the first 15 new patients with Wegener's granulomatosis (WG), microscopic polyangiitis, Churg–Strauss syndrome (CSS), or idiopathic rapidly progressive glomerulonephritis (iRPGN) seen in a period of two years were included.

One hundred and ninety-three renal biopsies from 178 patients were available for evaluation. Follow-up biopsies ($N = 15$) and biopsies with less than three glomeruli ($N = 16$) were excluded. Five patients were lost to follow-up. This left us with a total number of 157 biopsies for the clinicopathological analysis. Seventy-seven biopsies were from "historical" patients, and 80 were from "new" patients. Because the aim of this study was to correlate renal function at the time of biopsy and during follow-up, the historical and the new patients were not analyzed separately because the data from both groups were comparable. The number of glomeruli in all patients ranged from 4 to 132, with a mean of 15 glomeruli and a median of 11 glomeruli in the biopsy.

Patient classification

As the majority of patients from this study were recruited before the Chapel Hill Consensus Conference in the nomenclature of systemic vasculitis [10, 11], after study entrance, they were reclassified according to definitions based on this classification system. The following diagnostic groups were formed:

Wegener's granulomatosis (WG). These patients were divided into three subgroups. (*1*) Patients with histologically proven vasculitis with granuloma and/or giant cells in a biopsy specimen (either renal or extrarenal location); (*2*) patients with clinical evidence of at least one airway symptom or sign compatible with WG (pulmonary nodules or fixed infiltrates, sinusitis, purulent or bloody discharge from the nose, saddle nose, otitis media, orbital pseudotumor, tracheal stenosis), with renal histology showing crescentic and/or necrotizing glomerulonephritis with few or no immune deposits; and (*3*) patients with clinical evidence of airway symptoms compatible with WG, with no histological evidence of the vasculitic nature of the disease.

Microscopic polyangiitis (MPA). Inclusion criteria for patients were histologically proven crescentic and/or necrotizing glomerulonephritis with few or no immune deposits, or histologically proven vasculitis of small vessels and systemic manifestations of disease compatible with vasculitis, but no airway symptoms compatible with WG (discussed earlier in this section).

Idiopathic rapidly progressive glomerulonephritis. This classification included patients with histologically proven crescentic and/or necrotizing glomerulonephritis with few or no immune deposits, without systemic disease manifestations.

Classical polyangiitis nodosa (classical PAN). This encompassed patients with proof of arterial vasculitis (either by angiography or by biopsy), and criteria of small vessel vasculitis at any biopsy location or crescentic glomerulonephritis excluded the patient from this category, and moved him or her into the MPA group (discussed earlier in this section).

Churg–Strauss syndrome (CSS). The following were classified with CSS: patients with histologically proven vasculitis or crescentic glomerulonephritis or giant cells/granuloma formation in combination with asthma and eosinophilia.

All patients were followed up for at least one year.

Therapy

At study entrance, 42 patients received intravenous pulsed regimen therapy with prednisone ($N = 16$), cyclo-

phosphamide ($N = 10$), or prednisone and cyclophosphamide ($N = 16$), mostly in combination with oral doses of prednisone and/or cyclophosphamide. Of the other patients, 102 were on oral therapy with prednisone ($N = 28$), cyclophosphamide ($N = 6$), prednisone and cyclophosphamide ($N = 52$), prednisone and azathioprine ($N = 14$), prednisone, azathioprine, and cotrimoxazole ($N = 1$), or azathioprine ($N = 1$). In addition to intravenously pulsed or oral therapy, 17 patients were on hemodialysis. Thirteen patients received no therapy at study entrance.

During the follow-up, intravenously pulsed therapy was discontinued in 28 patients and was newly started in 8. All other patients received oral therapy consisting of combinations of prednisone, cyclophosphamide, azathioprine, and cotrimoxazole.

Scoring renal function

The serum creatinine was used as an indicator of renal function. Serum creatinine was measured both at entry and at one-year follow-up. Furthermore, creatinine values were determined at three-month intervals. A continuous quality control on serum creatinine measurement among 425 European centers, including those participating in this study, guaranteed a deviation from the mean of less than 5%. For this analysis, serum creatinine values were categorized into three groups: below 150 μmol/liter, between 150 and 500 μmol/liter, and above 500 μmol/liter. The histopathological findings in the kidney were correlated with the serum creatinine at entry and at one year, and they were also correlated with the lowest serum creatinine level (referred to as the optimum level) measured during follow-up, to exclude the distortion caused by relapses that could have occurred within the first year of follow-up.

Antineutrophil cytoplasmic antibody testing

An indirect immunofluorescence (IIF) test was performed locally by all participating centers by using an IgG-specific FITC conjugate. The locally used IIF methodology has proven to be comparable among the centers with respect to the antineutrophil cytoplasmic antibody (ANCA) pattern scored [12]. The IIF test was scored as cytoplasmic (cANCA), perinuclear (pANCA), atypical, or negative staining [13] using the cut-off screening dilution used routinely in each laboratory.

Sera were tested for the presence of anti-PR3 and anti-myeloperoxidase (anti-MPO) antibodies in an enzyme-linked immunosorbent assay (ELISA) format. For anti-PR3 antibody detection, three antigens were used and isolated from human neutrophils by three different methods [14]. For anti-MPO detection, one antigen was used. ELISAs were performed in a standardized manner that had previously shown acceptable results and reproducibly low variation between the participating centers [12]. Cut-off values for assay positivity were derived from receiver operating characteristic (ROC) curves. The cut-off value for PR3 antibody positivity was set at 500 and for MPO antibody positivity at 1500.

Immunofluorescence

A scoring form for immunofluorescence (IF) data was created to be filled in by the participating centers. IgG, IgA, IgM, C3, C1Q, and fibrin/fibrinogen were scored on aspect (granular or linear), intensity (0 = absent; 1 = positive; 2 = strongly positive), and site (mesangial, peripheral, or outside the glomerular tuft, including crescents and/or necrosis). Also, deposits in arteries, arterioles, and the tubular basement membrane were scored.

Scoring renal histopathology

Four specialized renal pathologists (J.A.B., F.F., L.H.N., and R.W.) evaluated the biopsies according to a scoring protocol; the standardization was described in a previous study [15]. Within a renal biopsy specimen, each glomerulus had to be scored separately on the presence of fibrinoid necrosis, extracapillary proliferation (cellular/fibrous and segmental/circumferential), sclerosis (focal, segmental, or global), or any other lesion, which was to be specified by the reviewing pathologist. Apart from these categories, granulomatous reaction (defined as destruction of the glomerulus with an accumulation of epithelioid cells and/or giant cells) and periglomerular infiltrates (defined as a dense infiltrate around a glomerulus without granuloma, with or without destruction of Bowman's capsule) were marked. Consequently, the percentage of glomeruli with any of these features could be calculated as a fraction of the total number of nonsclerotic glomeruli in the biopsy. Subsequently, the percentages were recoded on a three-point scale: 0 for 0%, 1 for a percentage $<50\%$, and 2 for a percentage $\geq 50\%$. Each biopsy was scored by two pathologists independently. Disagreements between the pathologists on the final three-point scale were solved in plenary sessions in which they had to re-review the biopsies and come to agreement. Interstitial data were evaluated according to a two-point ($-/+$) or three-point ($-/+/++$) scale. For these data, a final scoring was established.

Statistics

All statistical analyses were carried out with the SPSS package for Windows. The Kruskal–Wallis one-way analysis of variance was used to evaluate serum creatinine in case of histopathological parameters measured on a three-point scale. The Mann–Whitney *U*-test was applied for interstitial data scored on a two-point scale. Both tests were used to correlate the pathological renal findings with the serum creatinine at entry, at one year after the biopsy, and at the time of the optimum (that is, the lowest) creatinine level. The patients were divided

Table 1. Patient characteristics

Diagnosis	*N*	Male:female	Mean age	Percentage of patients with entry serum creatinine		
				<150	150–500	>500 or on HD
Wegener's granulomatosis	88	38:50	58	19	49	32
MPA	43	27:16	62	16	58	26
iRPGN	16	9:7	59	6	44	50
CSS	7	2:5	61	29	42	29

Diagnosis	Percentage of patients with optimum serum creatinine			Percentage of patients with serum creatinine at 1 year		
	<150	150–500	>500 or on HD	<150	150–500	> 500 or on HD
Wegener's granulomatosis	63	25	12	43	42	15
MPA	55	37	8	33	56	11
iRPGN	44	38	18	44	31	25
CSS	71	29	0	42	58	0

Abbreviations are: HD, hemodialysis; MPA, microscopic polyangiitis; iRPGN, idiopathic rapidly progressive glomerulonephritis; CSS, Churg Strauss syndrome.

into three clinical groups according to the following criteria: serum creatinine less than 150 μmol/liter, between 150 and 500 μmol/liter, and above 500 μmol/liter. Patients on hemodialysis were registered into the last group, irrelevant of their actual serum creatinine.

Because an extensive number of histopathological parameters were correlated with the same clinical values, the *P* value at which differences were regarded as statistically significant was set to 0.0025, according to the Bonferroni formula [16].

RESULTS

Patient classification and extrarenal symptoms

Of the 157 patients entered into this study, 88 were diagnosed with WG, 43 with MPA, 16 with iRPGN, and 7 with CSS (Table 1). Their mean age at entrance was not significantly different among the four groups. Subcategorization according to serum creatinine values at entry and during follow-up is listed in Table 1. Except for the patients with iRPGN, all patients had extrarenal manifestations (Fig. 1).

Antineutrophil cytoplasmic antibody test results

Table 2 lists the results of the ELISA and the IIF test for ANCA testing. Of seven historical patients, serum was not available. Because the ELISA for anti-PR3 antibodies was performed three times for each patient, it would be conceivable that only one or two of the test results were positive. However, this occurred only in a minority of cases; there was concordance between the three PR3 ELISAs in all but two cases (who tested positive on 2 out of 3 ELISAs). All pANCA patients with anti-MPO antibodies had negative ELISA test results on all three anti-PR3 assays. Of those patients marked as double negatives, six were positive on an anti-PR3 ELISA in one out of three cases, and one had a positive anti-MPO ELISA at a later time. In the group of patients with double positivity for both anti-PR3 and anti-MPO antibodies, seven had a positive anti-PR3 assay in three cases, four in two, and one in one case. We analyzed whether significant differences in renal histopathology occurred based on the ANCA test results but found only minor differences between the patient groups (abstract; Bajema et al, *J Am Soc Nephrol* 8:532A, 1997).

Immunofluorescence

In 22 of 157 cases, IF was either not performed or the specimen contained insufficient material for evaluation. Of the remaining 135 cases, 25 were negative on all categories. Results of biopsies positive for either Ig, complement, or fibrin/fibrinogen are given in Table 3. Ig deposits in glomeruli were never scored as strongly positive (++) but, if present, as positive (+); only in arterioles did infrequent strongly positive deposits of IgM, C3, C1Q, and fibrin/fibrinogen occur. In 48 patients, crescents and areas of fibrinoid necrosis showed positive or strongly positive staining for fibrin/fibrinogen. Glomerular IgA deposits were only reported in combination with at least two other categories positive for IF. A very weak linear staining for either IgG or IgA was reported in five and three patients, respectively; however, in view of the focality of the staining pattern, its weak intensity, and the absence of anti-GBM antibodies in their sera, anti-GBM nephritis was not considered likely.

Histopathology

Glomerular data. As is illustrated in Figure 2, the percentage of normal glomeruli in the biopsy proved to be a highly predictive parameter to estimate renal function, both at the time of biopsy and during follow-up. At the time of biopsy (Fig. 2A), those patients with more than 50% normal glomeruli in the renal biopsy also clinically fell into the lower creatinine levels. Most of the patients

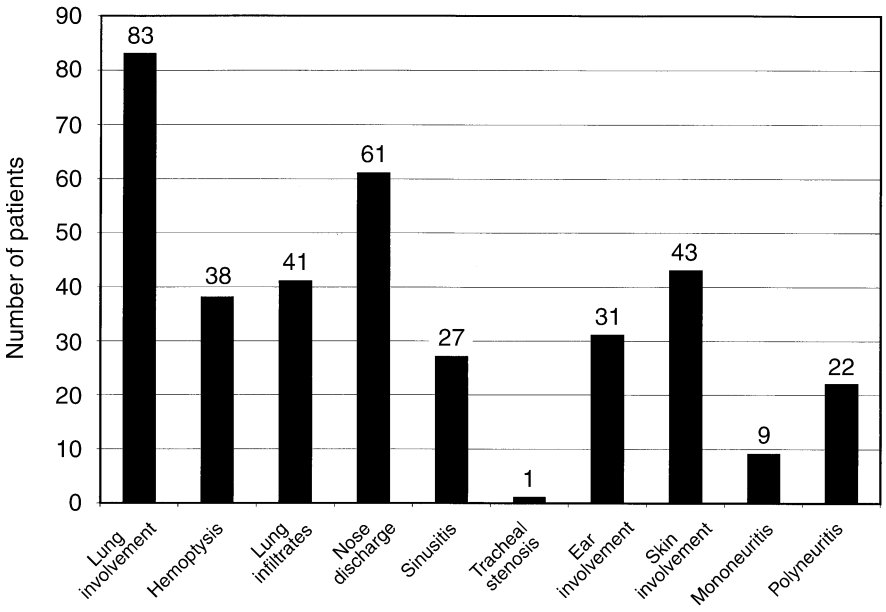

Fig. 1. Extrarenal symptoms.

Table 2. ANCA test results in 150 patients

IIF	ELISA	Number of patients
Not performed	Anti-PR3 antibodies	1
	Anti-MPO antibodies	2
cANCA	Anti-PR3 antibodies	39
	Anti-MPO antibodies	3
	Double positive	5
	Double negative	8
pANCA	Anti-PR3 antibodies	1
	Anti-MPO antibodies	50
	Double positive	7
	Double negative	5
Atypical	Anti-PR3 antibodies	2
	Anti-MPO antibodies	2
	Double negative	19
Negative	Anti-PR3 antibodies	2
	Anti-MPO antibodies	3
	Double negative	1

with only pathological glomeruli were found in the group with the highest creatinine values. The same pattern was seen one year after the biopsy was taken (Fig. 2B) and at the optimum point during follow-up where the lowest creatinine levels of all patients were combined (Fig. 2C).

No significant correlations were observed between the amount of extracapillary proliferation and renal function at entry ($P < 0.03$) or during follow-up ($P < 0.015$ both at one year and at the optimum level). If a distinction was made between cellular and fibrous extracapillary proliferation, the results remained statistically insignificant (data not shown). Also, the amount of fibrinoid necrosis did not influence renal function in terms of serum creatinine either at entry ($P < 0.03$) or during follow-up ($P < 0.4$ and $P < 0.2$).

At one year after the renal biopsy, the amount of glomerular sclerosis in the initial biopsy proved to be predictive of renal function, with higher creatinine values in those patients whose biopsy had shown the highest amounts of glomerular sclerosis (Fig. 3).

Interstitial data. Three interstitial histopathological parameters showed a clear relationship to renal outcome: diffuse interstitial infiltrates, tubular necrosis, and tubular atrophy (Table 4).

The amount of diffuse interstitial infiltrates distinguished the histopathological groups at both the time of entry and one year later, but not at the optimum point during follow-up. At the time of biopsy, serum creatinine was higher in those patients with more tubular necrosis. However, because of a significantly higher decrease in creatinine in the group with the highest amount of tubular necrosis (Kruskal–Wallis one-way analysis of variance, $P = 0.002$ and $P = 0.001$, respectively), the groups became clinically indistinguishable during follow-up, irrespective of whether this was measured at the optimum creatinine level or one year after the biopsy was taken. The amount of tubular atrophy proved to be a consistent indicator for renal function, both at entry and during follow-up.

DISCUSSION

We report the results of a clinicopathological analysis of 157 renal biopsies from patients with systemic vasculitis. After study entrance, patients were classified as having WG, MPA, iRPGN, classic PAN, or CSS. Except for the patients with iRPGN, all had extrarenal symptoms

Table 3. Immunofluorescence data from the renal biopsies

Site of deposits	IgG		IgA		IgM		C3		C1Q		Fibin/ fibrinogen	
Mesangial	5	(5%)	2	(2%)	14	(13%)	16	(15%)	7	(6%)	6	(5%)
Peripheral	20	(18%)	6	(5%)	34	(31%)	53	(48%)	21	(19%)	12	(11%)
Crescents/areas of necrosis	1	(1%)	0	(0%)	2	(2%)	6	(5%)	2	(2%)	48	(44%)
Arterioles	0	(0%)	1	(1%)	9	(8%)	46	(42%)	9	(8%)	11	(10%)
Arteries	1	(1%)	0	(0%)	0	(0%)	9	(8%)	1	(1%)	1	(1%)
Tubular basement membrane	1	(1%)	0	(0%)	0	(0%)	6	(5%)	1	(1%)	0	(0%)

Not performed or insufficient material: 22
Negative on all categories: 25
Pauci-immune IF pattern: 110

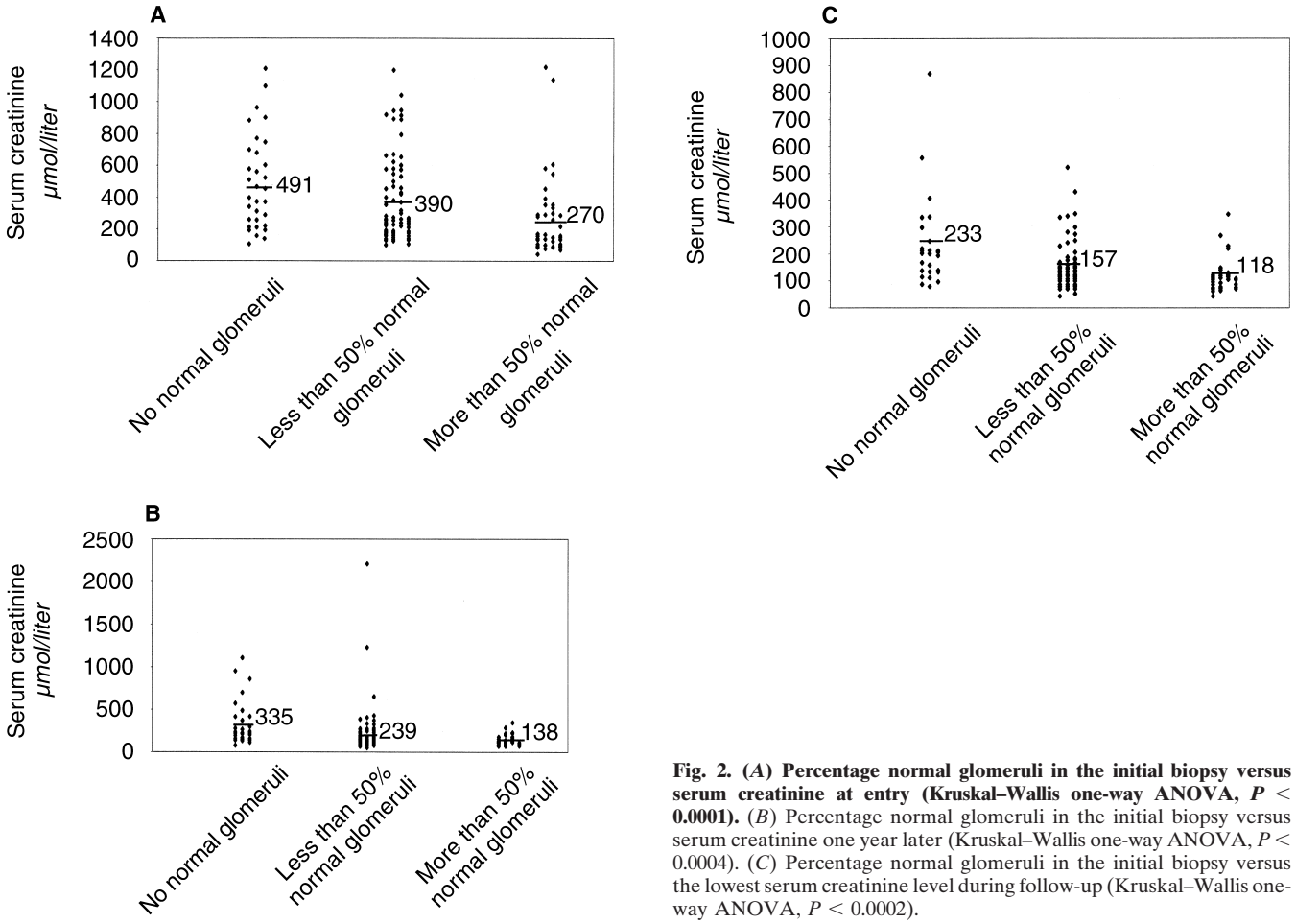

Fig. 2. (*A*) Percentage normal glomeruli in the initial biopsy versus serum creatinine at entry (Kruskal–Wallis one-way ANOVA, $P < 0.0001$). (*B*) Percentage normal glomeruli in the initial biopsy versus serum creatinine one year later (Kruskal–Wallis one-way ANOVA, $P < 0.0004$). (*C*) Percentage normal glomeruli in the initial biopsy versus the lowest serum creatinine level during follow-up (Kruskal–Wallis one-way ANOVA, $P < 0.0002$).

such as upper airway disease, pulmonary disease, arthralgia, and skin involvement. ANCA test results at the time of biopsy were available of 150 patients but had no relationship to renal histopathology. All biopsies for which IF data were available ($N = 135$) showed a so-called pauci-immune staining pattern, with positive or no staining for Ig (abstract; Jennette et al, *J Am Soc Nephrol* 7:1735, 1996). All patients received oral and/or intravenously pulsed therapy with prednisone and/or cyclophosphamide. Renal histopathological parameters were quantitatively evaluated by a group of four experienced renal pathologists. Each biopsy was individually scored by two pathologists, and discrepancies were resolved during plenary sessions. Thus, a high level of reliability for renal morphological scoring was guaranteed. Serum creatinine levels at the time of biopsy and during follow-up served as indicators for renal function.

Although all patients in this study were treated with immunosuppressive therapy, their regimens were not standardized, and therefore, an analysis of the effect

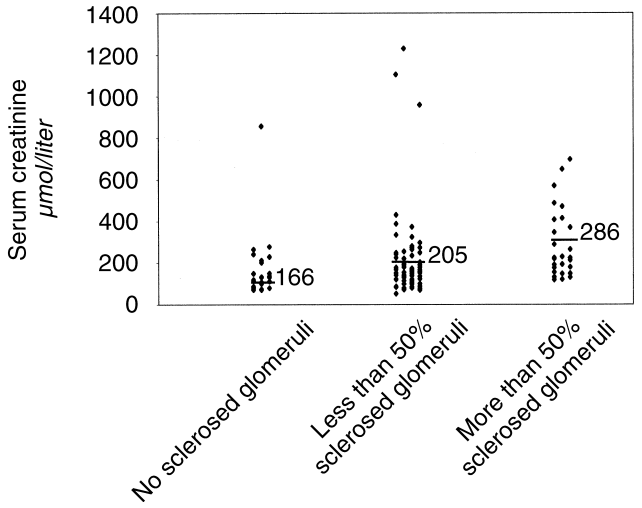

Fig. 3. Percentage sclerosed glomeruli in the initial biopsy versus serum creatinine one year later (Kruskal–Wallis one-way ANOVA, $P < 0.0005$).

Table 4. *P*-values for correlations between interstitial renal data and serum creatinine at entry and during follow-up

Histopathological category	Creatinine at entry	Optimum creatinine	Creatinine one year after biopsy
Interstitium			
Edema	0.02	0.15	0.13
Focal infiltrates	0.49	0.04	0.08
Diffuse infiltrates (mainly neutrophils)	0.0001	0.01	0.0003
Interstitial fibrosis	0.03	0.04	0.008
Granulomas	0.09	0.36	0.24
Tubuli			
Casts	0.2	0.17	0.03
Necrosis	0.0025	0.46	0.3
Atrophy	0.002	0.0008	0.0002
Intra-epithelial infiltrates	0.003	0.02	0.01
Arteries			
Sclerosis	0.006	0.05	0.07
Necrosis	0.94	0.75	0.41
Infiltrates (mainly mononuclears)	0.67	0.47	0.97
Thrombosis (only seen in 3 cases)	—	—	—
Scarring	0.09	0.66	0.88
Arterioles			
Hyalinosis	0.66	0.93	0.85
Necrosis	0.05	0.45	0.44
Infiltrates	0.04	0.02	0.42
Thrombosis	0.04	0.12	0.64

of therapy on renal outcome in combination with the histopathological data was not performed. A standardized mode of treatment for patients with systemic vasculitis is not available, and few randomized trials have been performed. However, prospective clinical trials are in progress in which therapeutic regimens are prescribed for clinical subgroupings of vasculitis, according to diagnosis, disease extent, and severity [17].

Both at the time of biopsy and during follow-up, the percentage of normal glomeruli in the biopsy turned out to be the most reliable predictor for renal outcome (Fig. 2). By contrast, extracapillary proliferation and fibrinoid necrosis, the active glomerular lesions that serve to establish the diagnosis of systemic vasculitis, proved not to be indicative for renal function, either at entry or during follow-up. This is in line with a recent study of 107 patients with ANCA-associated MPA and glomerulonephritis—but excluding WG—which reported that the lesions in the renal biopsy were not predictive of renal survival, except for interstitial sclerosis [18].

In this study, the only active lesions predictive of renal function were not from a glomerular origin but from the interstitium. The amount of diffuse interstitial infiltrates correlated with serum creatinine values at entry and during follow-up. Furthermore, tubular necrosis was significantly more prominent in patients with high levels of serum creatinine at entry. Although tubular necrosis is not a specific ANCA-associated lesion, apparently its presence is related to acute renal failure. Also nonspecific for systemic vasculitis syndromes are tubular atrophy and glomerular sclerosis, so-called chronic lesions, which came out as long-term predictors of renal function: The more they were present in the biopsy at entry, the higher the serum creatinine was one year later.

From the five parameters correlating with serum creatinine levels—normal glomeruli, sclerotic glomeruli, diffuse interstitial infiltrates, tubular necrosis, and tubular atrophy—we find the percentage of normal glomeruli the most useful predictor for renal function. *P* values for its correlation to outcome were below 0.001 both at entry and during follow-up. Normal glomeruli are easy to recognize, and their percentage of the total number of glomeruli in the biopsy is easily calculated, in contrast to interstitial lesions, where it can be troublesome to decide in what quantity they occur. Of course, it could be argued that the fraction of normal glomeruli is complementary to the fraction of glomeruli with active lesions and sclerosis, or, alternatively, that an index could be calculated with the vasculitic lesions as main parameters to predict for renal outcome. Conceptually, such an index supports the idea that it is the activity of disease that ultimately determines how much of the kidney is spared. However, active lesions such as extracapillary proliferation and fibrinoid necrosis in our study did not show a correlation with renal function. It is conceivable that these lesions are partly reversible and responsive to therapy, whereas at some point, they become irreversible, thus leading to glomerulosclerosis [19]. This may account for the fact that in our study, active lesions were less reliable parameters for renal outcome than normal glomeruli.

From the present study, we conclude that the best predictor for renal function in systemic vasculitis is the percentage of normal glomeruli in the initial renal

biopsy. Because this fraction can be easily calculated, we advocate its usage to predict renal outcome of patients with systemic vasculitis.

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