Renal pathology in idiopathic membranous nephropathy: A new perspective

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Histology findings in idiopathic membranous nephropathy (MGN) have been associated with the risk of renal failure, but whether they are independent of the clinical variables at the time of biopsy, predict rate of progression, or should guide therapy is uncertain. Renal biopsies of 389 adult MGN patients were evaluated semiquantitatively for interstitial fibrosis, tubular atrophy, vascular sclerosis, focal and segmental glomerulosclerosis lesions (FSGS), complement deposition, and stage and synchrony of deposits by electron microscopy (EM). Associations were tested between these findings and the rate of renal function decline (slope), renal survival, remission in proteinuria, and response to immunosuppression. Patients with a greater degree of tubulo-interstitial disease, vascular sclerosis, and secondary FSGS were older, had a higher mean arterial pressure, and a lower creatinine clearance at presentation. Although these histologic features were associated with a reduced renal survival, they did not predict this outcome independently of the baseline clinical variables nor did they correlate with the rate of decline in function or with baseline proteinuria. Furthermore, the severity of tubulo-interstitial and vascular lesions did not preclude a remission in proteinuria in those who received immunosuppressive therapy. Neither stage nor synchronicity of EM deposits nor the amount of complement deposition predicted renal survival but the latter did correlate with progression rate. In MGN, certain histologic changes are associated with renal survival outcome. However, the indicators of chronic injury are associated with age, blood pressure, and creatinine clearance at presentation and not with rate of disease progression or initial proteinuria.

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Idiopathic membranous nephropathy (MGN) is the second most common cause of end-stage renal disease in the primary glomerulonephritis group in adults.[1] The literature has consistently reported that the long-term outcome is quite variable and that reliable early predictors are still needed to ensure that potent immunosuppressive treatment targets patients that are at the greatest risk of progression.[2–9]

The level of renal function, degree of hypertension, and severity of proteinuria are the most common and routinely measured clinical predictors. Certain standard histologic features on renal biopsy have also long been associated with a poor outcome, particularly the severity of tubulo-interstitial damage and vascular sclerosis.[3,4,6,9–16] A correlation between the amount of complement deposition on immunofluorescence and the severity of proteinuria has also been reported.[17] Most recently, the presence of the lesion of focal and segmental glomerulosclerosis and the findings of heterogeneous vs homogeneous (synchronous) morphology of the subepithelial electron-dense deposits have been linked to a poor outcome.[18–22] Whether these histology features predict outcome independently of the clinical parameters remains unclear.

We examined these histologic features and the clinical findings at the time of renal biopsy to determine their independent value in predicting outcome in terms of not only renal survival, but also the rate of progression (slope of creatinine clearance (CrCl)), remissions in proteinuria, and on response to therapy.

RESULTS

There were 520 patients with a diagnosis of MGN with clinical information available in the Toronto Glomerulonephritis registry from 1974 to the end of March 2003. Exclusions were <12 months follow-up (94), secondary membranous (20), $<$age 16 years at presentation (8), and miscellaneous (9).

The remaining 389 subjects' baseline characteristics, follow-up, and outcome are summarized in Table 1. Sixty-eight percent of patients were male, the average age was 48 years, and patients were followed for a median of 60 months. Two hundred and twenty patients received immunosuppressive treatment and 50 patients reached renal failure. On average, the first clinical assessment available predated the renal biopsy by 2 months. Only 31 patients had their biopsy

Table 1 | Baseline and follow-up clinical variables (*n*=389)

At onset	
Age (years)	48 ± 16
Sex (% female)	33
Ethnicity (%)	
Caucasian/African American/Asian/other	70/5/4/10
MAP (mmHg)	102 ± 14
CrCl (ml/min/1.73 m^2)	76 ± 29
Proteinuria (g/day)	4.7 (0.3–31.3)
Follow-up	
Duration of follow-up (months)	58 (12–400)
MAP (mmHg)	100 ± 9
Proteinuria (g/day)	3.9 (0.3–24.2)
Immunosuppression (%)	56
ACEI or ARB therapy (%)	37
Outcomes	
Rate of change in renal function[a]	−0.31 ± 0.68
Renal failure (%)	14

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CrCl: creatinine clearance; MAP: mean arterial pressure.
[a]Measured as change in the slope of CrCl (ml/min//1.73 m^2/months).
Results are expressed as mean (±s.d.) or median (range).

Table 2 | Histology findings

	Score			
	0	1+	2+	3+
Tubulo-interstitial disease[a] (%)	34	48	11	7
Vascular sclerosis (%)	46	29	16	9
Complement deposition (C3) (%)	32[b]	33	22	13

[a]Interstitial fibrosis and tubular atrophy scores were almost identical. These two categories were combined and categorized according to the highest score.
[b]11% were scored negative and 21% were scored trace complement deposition.

delayed for more than 12 months after the first assessment. At presentation, their median proteinuria was 3.2 g/day (0.6–27.0), mean arterial pressure (MAP) was 103 ± 14 mmHg, and CrCl was 71 ± 21 ml/min/1.73 m^2.

Histology findings are summarized in Table 2. Interstitial fibrosis and tubular atrophy scores were almost identical and hence these two features were scored as a single category and assigned the highest of the two individual scores. Twenty-five percent of biopsies showed focal and segmental glomerulosclerosis lesions (FSGS) lesions. On EM, 11, 32, 22, and 35% of biopsies showed stage 1, 2, 3, and 4, respectively, with 48% showing a homogeneous and 52% showing a heterogeneous pattern.

The κ-score was excellent for FSGS superimposed on MGN and good for interstitial fibrosis and vascular disease ($\kappa = 0.86$, 0.67, and 0.60, respectively). Ninety-seven percent of the interobserver error was by a single grade (i.e. 0 vs 1, 1 vs 2, or 2 vs 3). We used the score from initial biopsy report in the analyses.

Correlations among the histology findings

Vascular and tubulo-interstitial changes were strongly correlated (spearman's ρ 0.50, $P<0.001$). The presence of FSGS lesions also correlated with vascular sclerosis and tubulo-interstitial lesions (spearman's ρ of 0.28 and 0.36, respectively, all $P<0.001$). The amount of C3 deposition showed no correlation with any of these histology findings.

Tubulo-interstitial disease, vascular sclerosis, FSGS lesions, and their association with clinical findings

At presentation. Patients with tubulo-interstitial lesions, vascular sclerosis, and FSGS lesions were older, and had a higher MAP and a lower CrCl at onset (Table 3). Women had a higher percent with no fibrosis (48% of women compared to 35% of men, $P=0.02$) and no vascular sclerosis (63% of women compared to 52% of men, $P=0.04$). In contrast, no association was seen between these histology findings and presenting proteinuria: for example, in patients with >8 g/day proteinuria, 24% had ⩾moderate tubulo-interstitial fibrosis and 26% had ⩾moderate vascular sclerosis and this was not significantly different from those with ⩽3.5 g/day at 15 and 25%, respectively ($P=$NS, χ^2).

Race and body mass index at onset were not associated with any of the light microscopic findings.

During follow-up. The severity of lesions by light microscopy also predicted the MAP during follow-up. The MAP in those with *severe* tubulo-interstitial disease was 105 ± 9 mmHg with a median of 1 antihypertensive medication (range 0–3) compared to those with a 0 grade of 96 ± 8 mmHg with a median of 0 antihypertensive medication (range 0–2) ($P<0.001$, analysis of variance and Kruskal–Wallis test). The severity of lesions also had an impact on the class of antihypertensive treatment prescribed during follow-up. Patients with severe vascular or FSGS lesions were more likely to receive an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) during follow-up (46% with FSGS lesions compared to 33% without such lesions, $P=0.05$, χ^2).

The proportion of patients given dual immunosuppression within each histology group was similar. A total of 14, 16, 18, and 17% of patients with 0, 1+, 2+, and 3+ tubulo-interstitial scores were given such therapy and 16, 15, 16, and 16% of patients with 0, 1+, 2+, and 3+ vascular sclerosis scores were so treated. Similarly, 21% of patients with FSGS lesions vs 13% without these lesions were given dual therapy ($P=$NS, χ^2).

On outcomes. *Renal survival:* Tubulo-interstitial and vascular sclerosis score did predict a lower renal survival (Figures 1 and 2) with an unadjusted hazard ratio for severe tubulo-interstitial damage of 5.5 (95% confidence interval (CI): 2.4–12.8, $P<0.001$) and for severe vascular lesions 4.3 (95% CI: 2.0–9.6, $P<0.001$) in reference to normal parenchyma. This was not, however, independent of the CrCl, age, and MAP at biopsy. The hazard ratios for severe tubulo-interstitial and severe vascular lesions adjusted for these clinical variables fell to 1.3 (95% CI: 0.4–4.2, $P=$NS) and 1.4 (95% CI: 0.5–4.0, $P=$NS), respectively.

The presence of the FSGS lesions produced a trend toward a lower renal survival (unadjusted hazard ratio: 1.7; 95% CI:

Table 3 | Univariate associations between histology, demographics, and clinical variables at onset

	Age at onset		MAP		Creatinine clearance	
	Mean (s.d.)	*P* (*post hoc*)[a]	Mean (s.d.)	*P* (*post hoc*)	Mean (s.d.)	*P* (*post hoc*)
Fibrosis and tubular atrophy						
None (0)	41±13	<0.001	97±12	<0.001	92±23	<0.001
Mild (1)	50±15	(0<1, 2, 3)	104±13	(0<1, 2, 3)	76±26	(0>1>2>3)
Moderate (2)	49±18		105±16		66±39	(1>3)
Severe (3)	52±14		106±13		48±30	
Vascular sclerosis						
None (0)	41±14	<0.001	100±12	0.003	90±27	<0.001
Mild (1)	51±13	(0<1, 2, 3)	102±13	(0<2, 3)	76±26	(0>1, 2>3)
Moderate (2)	54±14		106±15	(1<3)	77±26	
Severe (3)	56±15		109±15		46±25	
FSGS						
None	46±15	NS	102±14	0.10[b]	82±24	0.006
Present	47±16		105±14		69±38	

FSGS: focal and segmental glomerulosclerosis lesions; NS: not significant.
Race and proteinuria at onset were not statistically associated with histology findings. Female patients had less interstitial fibrosis and vascular sclerosis (see text). There was no statistical association between clinical variables at onset and electron microscopy stage or C3 deposition.
[a]*Post hoc* analyses were performed using the least significant difference method to determine specifically which grade was significantly different from another.
[b]Membranous patients with FSGS lesions were receiving a greater number of antihypertensive medications at presentation (*P*=0.02, Mann–Whitney test).

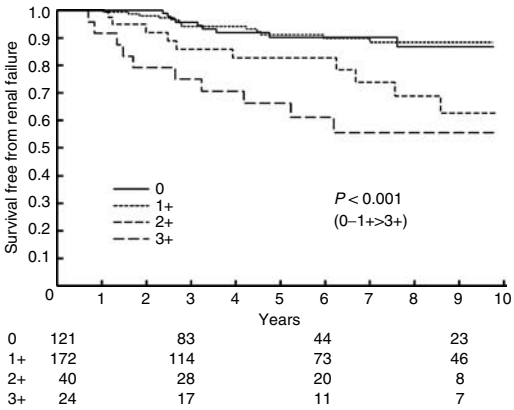

Figure 1 | Renal survival and tubular interstitial disease.

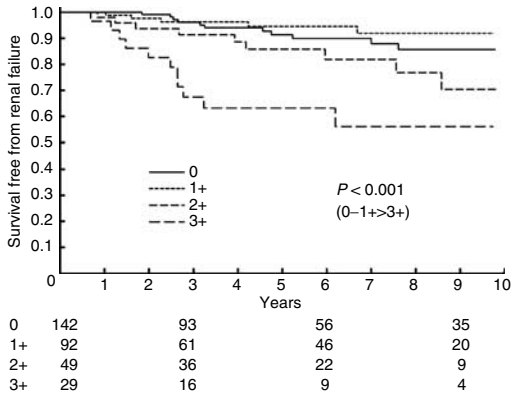

Figure 2 | Renal survival and vascular sclerosis.

0.9–3.3, $P=0.13$) but this disappeared after adjusting for the initial CrCl (Table 2).

Slope: The rate of renal function decline was not predicted by the amount of vascular or tubulo-interstitial disease or by the presence of FSGS lesions (Table 5).

Remission in proteinuria: There was no relationship seen between grade of tubulo-interstitial and vascular damage and remission in proteinuria in patients treated with dual immunosuppression, but was seen in untreated patients (Table 4). Similarly, the presence or absence of FSGS lesions made no difference in terms of response rate in treated patients (85 vs 75% respectively, $P=$NS), but in untreated patients, those with FSGS lesions were less likely to remit (44 vs 68%, $P=0.04$, χ^2). No difference in time elapsed was found from biopsy to remission or from the start of the immunosuppressive treatment to remission across the spectrum of histologic changes (data not shown).

Stage and heterogeneity of deposits by EM

The stage and heterogeneity of deposits did not statistically correlate with any clinical variables at onset (data not shown). Patients with a homogeneous pattern were more likely to achieve a compete remission during follow-up (42% compared to 20%, $P=0.01$, χ^2). However, this was offset by a 35% relapse rate in the homogeneous compared to 0% in those with a heterogeneous pattern. Neither stage nor pattern of deposits predicted rate of renal function decline or renal survival.

Complement deposition

Baseline clinical variables were similar among different grades of complement deposition, although proteinuria at onset tended to be higher in those with $\geqslant 1+$ C3 (5.6 g/day, range 0.3–26.2 g/day) compared to $<1+$ C3 (4.6 g/day, range 0.7–16.9 g/day) ($P=0.06$, Mann–Whitney *U*-test). The amount of C3 deposition did correlate with a faster rate

Table 4 | Ranking histologic findings and remissions in proteinuria (partial or complete) by treatment in nephrotic MGN patients (*n*=348)[a]

	Remission, overall (%)	*P*	Remission without immunosuppression (%)	*P*	Remission with dual immunosuppression[a] (%)	*P*
Atrophy or fibrosis						
0	79	<0.001	84	<0.001	75	NS
1+	69		59		92	
2+	50		36		57[b]	
3+	48		33		100[b]	
Vascular sclerosis						
0	71	0.06	72	0.02	69	NS
1+	71		69		75	
2+	58		40		80[b]	
3+	56		43		100[b]	

MGN: membranous nephropathy.
[a]Excludes patients who were never nephrotic. Differences between ordered categories were tested with spearman's rank order. Focal and segmental glomerulosclerosis, complement deposition, and stage of deposits did not correlate with remission.
[b]Only 10 patients with 2 or 3+ tubulo-interstitial or vascular disease received dual immunosuppression.

Table 5 | Univariate associations among severity of histologic variables and rate of progression (slope) during follow-up

	Slope[a]	*P*
Fibrosis and tubular atrophy		
0	−0.32±0.81	NS
1	−0.30±0.70	
2	−0.43±0.56	
3	−0.31±0.42	
Vascular sclerosis		
0	−0.32±0.74	NS
1	−0.34±0.66	
2	−0.20±0.53	
3	−0.34±0.58	
Segmental sclerosis		
0	−0.38±0.82	NS
1	−0.25±0.46	
Complement deposition		
0 or tr	−0.22±0.75	0.03
⩾1+	−0.46±0.76	
Stage		
1	−0.46±0.99	NS
2	−0.45±0.66	
3	−0.17±0.84	
4	−0.20±0.48	
Phase of deposits		
Homogeneous	−0.38±0.71	NS
Heterogeneous	−0.22±0.68	

tr: trace; NS: not significant.
[a]Expressed in ml/min/1.73 m^2/month.

of renal function decline (⩾1+ C3: −0.46±0.76 vs <1+ C3: −0.22±0.75 ml/min/month, $P=0.03$ (Table 5)). There was, however, no difference in renal survival by complement grade. The unadjusted hazard ratio of renal survival in those with ⩾1+ C3 in reference to those with <1+ C3 was 1.59 (95% CI: 0.68–3.68, $P>0.1$). No correlation was seen between degree of complement deposition and remissions in proteinuria or response to immunosuppressive drugs (data not shown).

Patients followed for less than 12 months

We reviewed our excluded patients based on <12 months follow-up time to assess whether this may have biased our findings. Twenty-seven MGN patients in this group had a rapid decline in renal function (>12 ml/min/1.73 m^2/year). The proportion of the patients with moderate or severe tubulo-interstitial changes was 22% and those with severe vascular change was 14%, similar to our study cohort (25 and 18%, respectively).

DISCUSSION

The long-term outcome in idiopathic MGN is highly variable. The strongest predictors of renal survival in MGN are the starting glomerular filtration rate and proteinuria.[23] Although many studies have previously demonstrated the importance of histology in predicting the risk of renal failure,[3,4,6,9–22] few have looked at its association with the rate of renal function decline or performed multivariate analysis to account for the possible association among histology, clinical, and laboratory findings at the time of biopsy.

As MGN histology is usually assessed only once, and most commonly soon after the appearance of symptoms, analyzing its predictive value must also account for any subsequent intervention that could alter the natural history of the disease. The most common and accepted interventions include blood pressure control, the use of ACEi and/or ARB class drugs, and immunosuppressive treatment. We therefore included these three factors in our analyses. Immunosuppressive therapy was divided in those who received no vs dual immunosuppression because the most recent and best evidence demonstrating the efficacy of such treatment comes from this approach.[24–27]

We have confirmed that the severity of tubulo-interstitial and vascular damage found on light microscopy does predict renal survival but, of equal or perhaps of more importance,

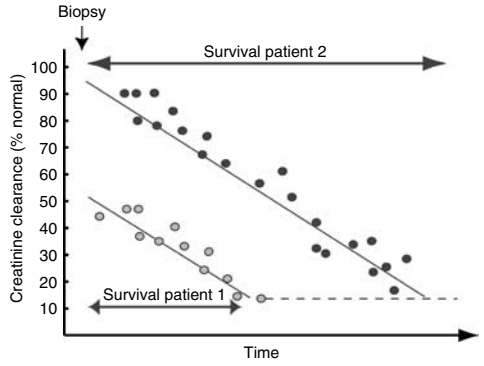

Figure 3 | An illustration of the relationship between light microscopy findings, CrCl at time of biopsy, rate of renal function decline, and renal survival in MGN. Patient 1 with significant indicators of chronic tissue injury by light microscopy has a lower CrCl at time of biopsy (*Y* axis) and a reduced renal survival time (*X* axis) in comparison to patient 2 with no chronic changes, a higher starting CrCl, and longer survival time despite a similar rate of renal function decline.

we found that they do not predict the rate of disease progression.[3,4,6,9–16] The association between the interstitial and vascular damage and renal survival was accounted for by a lower starting CrCl and not by a more rapid deterioration in renal function (Table 5 and Figure 3). These results strengthen the results from our earlier study, which demonstrated that elderly patients were more likely to progress to renal failure because of a lower starting CrCl and not because of the severity of their disease, as they had an almost identical rate of progression as the younger cohort as measured by the slope of their CrCl.[28]

We acknowledge that the error inherent to estimations of CrCl (instead of direct measurement) may increase the standard deviations of slopes and lower our statistical power. Nevertheless, the estimates of CrCl at onset were sufficiently precise to show clinically meaningful correlation with histology. In addition, the estimates of slope have previously allowed us to clearly discriminate patients long-term outcome in those with or without a remission in proteinuria.[23]

Further support for the independence of these lesions from the disease activity is the response to immunosuppression: in patients treated with dual immunosuppression, the severity of vascular and tubulo-interstitial lesions had no effect on remission rate. This indicates that remissions in proteinuria, valid surrogates of a favorable outcome,[23] are possible even in patients with severe degrees of these lesions. Although the lower rate of spontaneous remission in patients with tubulo-interstitial, vascular, or FSGS lesions was interesting, it does not, by itself, justify immunosuppressive therapy.

It could be argued that the variation in the degree of renal scarring may reflect the impact of chronicity of the membranous disease *per se*, but the lack of association between these changes and the post-biopsy progression rate, that is, slope, suggests that this is unlikely unless the natural history has been modified by subsequent therapy. We did not find this to be the case but found that the degree of tubulo-interstitial and vascular scarring only correlated with sex, age, and blood pressure at presentation. These factors have very recently been shown to be closely linked to renal function in 'normal' patients being assessed for living kidney donation with no specific renal disease.[29] This supports our contention that age and prior blood pressure level are the basis for the majority of the chronic injury observed at the time of biopsy.

The dichotomy between severity of tissue injury associated with age, hypertension, and the presenting CrCl but not with the subsequent rate of renal function decline or severity of presenting proteinuria, was of major interest to us. Although other studies have found a correlation between tissue scarring and proteinuria at biopsy, the strength of this association was weak ($r^2 \sim 10\%$).[30] Our interpretation is that pre-existing factors in the patient are primarily responsible for these lesions and they in turn result in a lower presenting CrCl. The severity of the membranous disease in contrast would appear to be best reflected by the severity of proteinuria and subsequent rate of deterioration in renal function, that is, slope of CrCl.[23] Whether the time and quality of life gained related to prolonged renal survival time with a remission is worth the risk of such therapy in a patient with underlying scarring and already impaired renal function is a separate question and cannot be answered by this study. This interpretation is further supported by recent studies showing that immunosuppressive treatment can be effective in reducing proteinuria and stabilizing glomerular filtration rate even in MGN patients with significant impairment of renal function.[31,32]

Other points relating renal histologic changes and outcome are also noteworthy. The lesions of focal and segmental glomerulosclerosis in MGN have been linked to a worse prognosis.[15,18–21] Although we also found a trend toward a reduced renal survival in MGN patients with added FSGS lesions, this group had a lower initial CrCl, largely explaining their lower renal survival. Importantly, this group was not different in factors we associate more severe disease with, that is, presenting proteinuria and/or progression rate (Tables 3 and 5).

The predictive value of the stage of the deposits on electron microscopy (EM) varies, with some positive[3,6,9,11,33–35] and some negative.[10,36–39] A recent retrospective study of 105 patients further suggested that a heterogeneous pattern was an independent predictor of end-stage renal disease.[22] We looked at both stage and heterogeneity of deposits and found that neither was predictive of rate of decline or of renal survival. We did find however that dividing the patients into homogeneous and heterogeneous deposits can be difficult, particularly with regard to separating the patient into pure stage 2 or 3 vs a 2–3 overlap.

Perhaps of most interest, the only histology finding predicting rate of renal function deterioration was the amount of complement deposition. Certainly, there is strong support from animal MGN models that complement activation is a prerequisite for the development of tissue injury

and subsequent proteinuria.[34–36] Our data would also fit with earlier human studies suggesting that the membrane attack complex, the end product of complement activation, is increased in patients with the most severe and progressive variants of MGN. Although our results do support a link between quantitative complement deposition and progression, we are cautious in drawing too definitive a conclusion from this association considering the limited number of patients, the semiquantitative and unverified grading system, and the variations in reagent specificity used to estimate complement deposition over the last three decades. An additional concern about this finding is the lack of association with our other outcome, renal survival.

Finally, we reviewed the pathology of those who had a rapid progression but were excluded because of <12 months observation. Our concern was if this group had a higher proportion with severe tubulo-interstitial and vascular disease, this would introduce a bias in our overall results by excluding the patients with severe tubular interstitial and/or vascular changes and rapid progression from our analysis. We found no difference in the percentage with severe changes between this group and our overall cohort, further strengthening our opinion that the grade of these features does not reflect membranous disease severity.

In conclusion, in adult MGN patients, certain histology findings by light microscopy do predict the likelihood of renal failure but they are more closely linked to pre-existing and often nonmodifiable factors such as age and sex rather than to proteinuria and rate of deterioration, our current best indicators of membranous disease severity. The introduction of the concept of separating pre-existing changes from those that reflect MGN disease severity should lead to a new perspective in assessing the clinical pathological correlates and treatment timing in this disorder.

MATERIALS AND METHODS

All idiopathic adult MGN patients from the Toronto Glomerulonephritis Registry were considered. This database began in 1974 and includes all biopsy-proven cases of glomerulonephritis from the Toronto area. Patient information at onset is compiled using a standard form and registrars perform a periodic prospective assessment of the patient's clinical status, medication, and laboratory results. This study focuses on MGN patients older than 16 years at presentation with at least 12 months follow-up.

Parameters collected

Demographics included age, sex, race, and body mass index at presentation. Clinical and laboratory data collected included both initial and follow-up information on systolic and diastolic blood pressure, weight, serum creatinine, and 24 h urine protein and creatinine. Also recorded was exposure to immunosuppressive agents and antihypertensive medications including the ACEi and ARB drug classes.

Definitions

All pathology reports were independently reviewed by two of the authors, masked to the clinical data (LR, ST). They assessed the following features on light microscopy: degree of interstitial fibrosis, tubular atrophy, vascular sclerosis, and the presence of FSGS. Sixty of these patients had their light microscopy slides reviewed independently by a third author (AH), masked to the clinical data, who also graded the vascular sclerosis and the amount of interstitial fibrosis and tubular atrophy as absent (0), mild $(1+)=<25\%$, moderate $(2+)=25$–50%, or severe $(3+)=>50\%$ of parenchyma. The final score for degree of vascular sclerosis was based on the most severe disease seen in either arterioles or arteries. Arteries were graded by the highest degree of damage in an affected artery not cut tangentially. Grading of the arteries was made as follows: mild was intimal thickening less than the diameter of the lumen or the thickness of the media, moderate was intimal thickening approximately the same as the diameter of the lumen or the thickness of the media, and severe was intimal thickening significantly greater than the diameter of the lumen or the thickness of the media causing significant luminal narrowing. Arterioles were graded as hyalinosis of the most affected arterioles as mild (mural hyalinosis causing no narrowing and width of hyalinosis significantly less than the medial thickness), moderate (mural hyalinosis causing some narrowing and width of hyalinosis is transmural), and severe (transmural hyalinosis causing significant luminal narrowing). Focal glomerulosclerosis in MGN was defined as the presence of segmental sclerosis or capillary collapse with or without capillary adhesions to the capsule or segmental hyalinosis[19] and graded present or absent. A minimum of 10 glomeruli per biopsy were required before assigning a negative score to that patient. A κ-score was determined for each of the light microscopic parameters.

The amount of C3 deposition on immunofluorescence was graded semiquantitatively as absent (0), trace (tr), mild $(1+)$, moderate $(2+)$, or severe $(3+)$. Deposits were staged by EM from 1 to 4 according to the Ehrenreich and Churg[40] classification and assigned the most advanced stage if more than one stage was present. In addition, we considered the deposits as synchronous (homogeneous) when they were all at any one stage, and heterogeneous when distributed in two or more stages.[22] All the original EM photographs were also reviewed by our nephropathologist (AH), the stage reassessed, and deposits classified as homogeneous or heterogeneous.

CrCl estimates were adjusted for age, sex, and weight using the Cockcroft–Gault method. Nephrotic patients were identified by a proteinuria value $\geqslant 3.5$ g/day at any point during follow-up. A compete remission of proteinuria was defined by a single proteinuria value $\leqslant 0.3$ g/day in a previously nephrotic patient. A partial remission was defined by a proteinuria value <3.5 and >0.3 g/day plus a 50% reduction from its peak value. Subjects who had both a partial remission and a compete remission were only included in the compete remission group and time to remission was calculated from the first clinical assessment suggestive of renal disease (abnormal proteinuria or serum creatinine). Renal failure was defined by a CrCl $\leqslant 15$ ml/min or by initiation of dialysis. MAP was estimated as the diastolic pressure plus a third of the pulse pressure. For each patient, an average MAP was determined for each 6-month period of follow-up. Time-average MAP represents the average of their 6 months means. Immunosuppressive treatment is reported as intent to treat regardless of the duration of therapy. Patients are categorized as having received no, mono-, or dual immunosuppressive therapy (10). This last group was defined by a minimum of 10 mg of prednisone plus at least 1.5 mg/kg of azathioprine or cyclosporine or 1 mg/kg of cyclophosphamide or 0.15 mg/kg of chlorambucil or 1000 mg of mycophenolate mofetil. Monotherapy was defined as

exposure to any form of immunosuppressive treatment that did not satisfy the dual therapy definition (e.g. steroids alone). Therapy with ACEi or ARB is defined as any exposure to these classes of drugs.

Statistical analysis

Normally distributed variables were expressed as mean $\pm$ standard deviation and compared using Student's *t*-test, one-way analysis of variance or Pearson test. Nonparametric continuous variables were expressed as median and range and compared using either Mann–Whitney or Kruskal–Wallis test. Categorical variables were expressed in percentage and compared using χ^2 test. Correlations between ordered categories were performed using Spearman's ρ coefficient. Agreement between the reviewers of the pathology was assessed using the κ statistic.[41]

The rate of renal function decline (slope) and survival from renal failure were the two definitive outcomes. The slope was determined by fitting a straight line through the calculated CrCl values on each patient using the principle of least squares. This was plotted and visually examined in all subjects. Periods of reversible acute renal failure defined as a rapid reduction and recovery in CrCl of $\geqslant$40% within a month were censored. Multiple linear regression was used to determine independent variables predictive of slope. Only variables associated by univariate analysis were included in a multivariate model.

Survival analysis was performed to test the association between renal survival and each parameter collected. Survival times were obtained from first to last follow-up or the time to renal failure. Univariate comparisons of renal survival were performed by Kaplan–Meier curves and log-rank test. A multivariate Cox proportional hazard model was constructed to determine independent variables associated with this outcome.

All *P*-values were two-tailed and values less than 0.05 were considered statistically significant. CI included 95% of predicted values. Analyses were carried out using SPSS software (version 11, SPSS Inc., Chicago, IL, USA).

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