Predictors of renal survival in ANCA-associated vasculitis. Validation of a histopathological classification schema and review of the literature

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ABSTRACT

Objective. *In 2010 a histopathological classification of ANCA-associated glomerulonephritis was proposed to predict the outcomes at diagnosis. Our aim was to validate the proposed classification in our cohort of patients and to compare the studies already published.*

Methods. *The data of 93 patients who underwent kidney biopsy in a single Italian centre within 15 years were retrospectively collected.*

Results. *The 10-year renal and patients' survival were 60% and 81%, respectively. Biopsies were classified as 21% focal, 30% crescentic, 39% mixed and 10% sclerotic. Survival without ESRD at 5 years was 82% in focal, 37% in crescentic, 81% in mixed and 51% in sclerotic group. The Kaplan-Meier analysis highlights that renal survival was not different between sclerotic and crescentic groups (p=0.9) but both had a significantly worse prognosis than focal (p=0.04 and 0.015 respectively) and mixed groups (p=0.05 and 0.03 respectively). Focal and mixed groups had the same renal survival (p=0.7). At multivariate analysis the independent predictors of end-stage renal disease were less than 20% of normal glomeruli at kidney biopsy (p=0.022), high serum creatinine (p=0.009) and arterial hypertension at presentation (p= 0.006).*

Conclusion. *In our cohort, the proposed histological classification was not predictive of renal prognosis. The focal and the mixed classes had the same prognosis and a significantly better renal outcome than both the crescentic and the sclerotic classes. At multivariate analysis among the histological features only less than 20% of normal glomeruli defines the renal prognosis together with renal function and arterial hypertension at baseline.*

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis are heterogeneous autoimmune disorders characterised by inflammatory and necrotising alterations of the vascular wall, resulting in ischaemia of the tissue (1, 2). Renal involvement is particularly frequent in these patients and is characterised clinically, by a rapidly progressive renal failure and, at renal histology, by extracapillary necrotising glomerulonephritis with few or no immune deposits (pauci-immune) on immunofluorescence and electron microscopy. ANCA-associated vasculitides include granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), microscopic polyangiitis (MPA) and the variant limited to the kidney (kidney limited) (3-6). Renal involvement is particularly ominous because it is associated to a poor renal and patient prognosis. It has been estimated that, for GPA, the risk of death rises to a hazard ratio of 5.1 in the presence of renal insufficiency and to 8.2 in cases of dialysis dependence (7). In MPA the risk of death rises to a hazard ratio of 3.7 in patients with renal insufficiency at diagnosis (7).

The diagnostic value of kidney biopsy is well established, while its value in predicting renal and patient outcome is less clear and the results reported are controversial. In 2010 an international working group of renal pathologists proposed a histopathological classification of ANCA-associated glomerulonephritis based on four general categories of lesions: focal, crescentic, mixed and sclerotic. The classification is based only on evaluation of glomerular lesions at light microscopy (8). They performed a validation study on 100 biopsies from patients with diagnosis of ANCA-associated vasculitis included in

different randomised studies. The study shows that the phenotypical order of the above mentioned classes corresponds to the order of severity of renal function impairment at presentation, at 1 year and at 5-year follow-up. The evaluation of tubulointerstitial lesions was not included in the classification because they were not predictive of renal prognosis (8). Since the paper of Berden *et al*. (8) validation studies were performed in Japan, China, Australia, the United States, Europe, and Turkey (9-19). These studies confirmed that the focal subgroup had the best renal survival and the sclerotic subgroup the worst renal survival, while the prognostic value of crescentic and mixed subgroups varied in the different studies (20).

We have analysed retrospectively data from 93 patients who underwent renal biopsy at our centre with a diagnosis of ANCA-associated vasculitis. The purpose of the study is to compare our results with those of the above mentioned validation study (8) and with the other studies on this subject (9-19) to confirm whether the histological classification proposed in 2010 is a valuable tool to predict at diagnosis the renal outcome of this disease.

Subjects and methods

Patients

From January 1995 to December 2011, 104 patients with newly diagnosed ANCA-associated small-vessel vasculitis and renal involvement were admitted to our Renal Unit. Seven patients who did not undergo kidney biopsy, and 4 patients with inadequate renal biopsy tissue were excluded. The other 93 patients with adequate kidney biopsy tissue and with a follow-up entered this single centre retrospective observational cohort study. After hospitalisation the patients were followed in our Unit. At every subsequent visit the patients were submitted to complete clinical, biochemical and urinary evaluations; in particular C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), P- and C-ANCA, total serum protein, albumin, serum creatinine, C3 and C4 complement fractions, and 24 hours proteinuria and urinary sediment were determined.

All patients received induction therapy. Eighty-three patients (89.3%) were treated with one methylprednisolone pulse (0.5–1g according to body weight) for three consecutive days followed with oral prednisone 0.5–1 mg/kg/day for 1–2 months then progressively reduced. The other 10 patients (10.7%) were treated with oral prednisone 1–2 mg/kg/day for 1–2 months than gradually reduced. In 82 patients (82%) oral cyclophosphamide 1.5–2 mg/kg/day was added to steroids and continued for a median of 7.5 months (25th and 75th percentile 5–12.7 months). In the other 11 patients azathioprine 2 mg/kg/day was added to prednisone. In addition, 12 patients (12.9%), received a course of plasma exchange. As maintenance immunosuppressive therapy, patients received azathioprine.

Definitions

- Anti MPO and PR3 antibodies were determined by ELISA (Wieslab immunoenzimatic kit).
- Urinary sediment was evaluated with phase contrast microscopy: the number of erythrocytes/high power field (HPF) was evaluated
- Glomerular filtration rate was calculated according to Cockcroft-Gault formula.
- Arterial hypertension was defined as blood pressure >140/90 mmHg in two subsequent measures.
- Patients were classified according to the 2012 Chapel Hill Consensus Conference on the nomenclature of systemic vasculitis (6).
- A minimum of 10 glomeruli was considered adequate for a biopsy to be included. Renal biopsies were evaluated by an experienced renal histopathologist who was blinded to outcome. Renal biopsies were classified according to Berden *et al*. (8) into 4 subgroups: Sclerotic group: those with ≥50% sclerotic glomeruli, Focal group: those with ≥50% normal glomeruli, Crescentic: group those ≥50% glomeruli with cellular crescents; all the other biopsies were classified into Mixed group: <50% normal, <50% crescentic, <50% globally sclerotic glomeruli. In addition, circumferential cellular crescents, glomerular segmental necrosis, vascular fibrinoid necrosis, interstitial inflammation (absent, moderate: <50% of the renal tissue, severe ≥50% of renal tissue) and tubulointerstitial fibrosis were evaluated. According to the Banff score (21) tubulointerstitial fibrosis was classified as:1 mild= <25% , 2 moderate = 25–50%, 3=severe >50%.

The primary end point of the study was the development of end-stage renal disease (ESRD) at 5 years defined as the chronic need of renal replacement therapy (RRT).

Statistical analysis

The statistical package S-Plus was used to analyse sample data. Mean and standard deviation, together with median and interquantile (IQ) range (25^{o}–75^{o} percentile) were used as descriptive statistics. For continuous variables, the non-parametric Wilcoxon test was used for assessing any difference between the two groups of patients. Cross-tabulated data were analysed by Chi-square test, or by Fisher's exact test when the expected cell count was less than five. Survival curves were drawn using the Kaplan-Meier estimate and compared using the log-rank test. The association of the histological classification (as a categorical variable) and other clinical and histological features with the development of death censored end-stage renal disease (ESRD) was evaluated with a multivariate COX regression analysis. Relative risk (RR) and their 95% confidence interval (CI) for the covariates were derived as the antilogarithm of the regression coefficients.

Results

Of the 93 patients 49 were male and 44 female, the mean age was 58.8±16.3 years. The mean time between the first systemic manifestations of vasculitis and renal manifestations was 4.4±12.7 months. The most frequent extra-renal manifestations at presentation were fever (51 patients), malaise (41 patients), arthralgia (36 patients), anorexia (33 patients), lung involvement (24 patients), upper respiratory tract involvement (25 patients), myalgia (18 pa-

Table I. Clinical, histological and therapeutical characteristics at presentation of all patients and of patients who developed and of those who did not develop end-stage renal disease.

	All patients 93 patients	ESRD 33 patients	No ESRD 60 patients	*p*-value*
Follow-up months	62.7 ± 62.9	33.15 ± 48.0	78.3 ± 64.9	0.0001
Female/male	44 /49	13/20	31/29	0.08
Age at diagnosis of vasculitis	58.4 ± 16.5	63 ± 9.1	57 ± 17.4	0.057
MPO positivity	46.3%	41.4%	49.1%	0.7
PR3 positivity	39.0%	44.8%	35.8%	
ANCA negativity	14.6%	13.8%	5.1%	
MPA	36.5%	30%	40%	0.5
GPA	41.2%	39%	43%	
Kidney limited	10.7%	30%	17%	
Arterial hypertension	52 (55.9%)	72.7%	46.7%	0.001
Serum creatinine mg/dl	5.6 ± 4.4	8.3 ± 5.1	4.1 ± 2.9	0.00001
Serum creatinine ≥1.2mg/dl	87 patients			
GFR at diagnosis ml/min	23.2 ± 30.3	10.5 ± 9.3	30.2 + 35.3	0.001
Proteinuria g/day	1.9 ± 3.2	3.2 ± 5.05	1.3 ± 1.6	0.03
Nephrotic syndrome	12 patients			
Haemoglobin g/dl	9.6 ± 1.7	9.4 ± 1.4	9.8 ± 1.8	0.2
Haemoglobin <12.5 mg/dl	89 patients			
Eosinophils %	3.2 ± 4.9	3.0 ± 3.5	3.2 ± 5.5	0.6
Erythrocyte sedimetation rate	84.6 ± 33.3	92.05 ± 31.3	81.6 ± 33.9	0.8
C-reactive protein mg/dl	9.3 ± 9.2	12.8 ± 10.4	7.8 ± 8.3	0.01
C3 mg/dl	110.5 ± 29.5	100.9 ± 28.2	115.5 ± 29.4	0.03
C4 mg/dl	31.1 ± 9.9	32.95 ± 10.0	30.3 ± 9.9	0.39
Serum albumine g/dl	3.2 ± 0.7	3.25 ± 0.7	3.25 ± 0.7	0.4
Number of urinary erythrocytes / HPF	63.5 ± 38.1	65.95 ± 38.3	57.1 ± 37.9	0.26
Histological features				
Normal glomeruli n. (%)	8 ± 12 (27.7%)	3.4 ± 4.9 (14%)	7.5 ± 5.8 (53%)	0.004
Sclerotic glomeruli n. (%)	4.4 ± 4.8 (19.3%)	5.3 ± 6.4 (22.7%)	4 ± 3.7 (17.5%)	0.17
Total crescents n. (%)	(46 ± 5.7)	(53 ± 28.8)	(34.4 ± 27.8)	0.0007
Cellular crescents %	35 ± 31	47.3 ± 31.8	28 ± 27.9	0.0007
Circumferential celluar crescents %	21 ± 29.7	24.8 ± 25.1	12.5 ± 22.1	0.001
Vascular necrosis	18%	21.1%	17%	0.6
Moderate / severe interstitial inflammation	55.9%	75%	45%	0.02
Moderate / severe tubulointerstitial fibrosis	10.7%	21%	5%	0.04

*p-value are calculated with Wilcoxon test. If not differently specified numbers are expressed as Mean ± SD. ESRD: end-stage renal disease; GFR: glomerular filtration rate; MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; HPF: high power field.

tients), skin involvement (14 patients), central nervous system involvement (7 patients) and ocular involvement (5 patients).

The clinical and biochemical characteristics at presentation are reported in Table I.

Thirty-nine percent of patients were PR3 positive, 46.3% MPO-positive, 14.6% ANCA-negative, All but 2 patients presented with impairment of renal function with a mean serum creatinine of 5.6±4.4 mg/dl and a mean glomerular filtration rate of 23.2±30.3 ml/min. The mean proteinuria was 1.9±3.2g/24h and was in nephrotic range in 12 patients (13%); at urinary sediment the mean number of erythrocytes was 63.5±38.1/HPF.

Renal and patient outcome:

After a mean follow-up of 62.7±62.9 months 36 patients (38.7%) had normal renal function (mean serum creatinine 1.1±0.2mg/dl; proteinuria 0.5+0.7g/24h), 10 patients (11%) had chronic renal insufficiency (mean serum creatinine 3.0±1.5mg/dl), 33 (35.5%) are on chronic dialysis and 14 patients (15%) died; 6 with normal renal function, and 8 with chronic renal insufficiency. Twelve other patients died in median 12.3 months (2–44) after starting dialysis.

The pure kidney survival rate (without ESRD) was 83% at 1 year, 66% at 5 years, and 60% at 10 years. Patient survival was 92% at 1 year, 84% at 5 years and 81% at 10 years (Fig. 1).

Histopathological classification of renal biopsy

The mean number of glomeruli was 22.1±11.8 (median 20).

Twenty biopsies (21%) were classified as Focal, 28 (30%) as Crescentic, 36 (39%) as Mixed and 9 (10%) as Sclerotic. Table II reports the clinical characteristics at renal biopsy of the 4 groups and Table IV the GFR of the different groups at basal at 1 and at 5 years and the clinical status of the 4 groups at last observation. Crescentic and sclerotic groups had similar clinical presentation and the worst clinical presentation in terms of renal dysfunction, in comparison to the other two groups. No differences among the 4 groups emerged in type of ANCA, and in steroids or immunosuppressive therapy. In Table III the histopathological characteristics of the 4 classes are reported. In crescentic and in sclerotic classes there was the lowest number of normal glomeruli (12.2% and 7.8% respectively) and the highest frequency of interstitial inflammation. In comparison to the other groups, in the crescentic group we observed a higher percentage of glomeruli with fibrinoid necrosis, and more frequent fibrinoid necrosis of arterioles. In the sclerotic group there was the highest frequency of moderate/severe tubulointerstitial fibrosis.

Figure 2 shows the Kaplan-Meier curves for the primary outcome (ESRD) of patients assigned to the 4 histological classes. Focal and Mixed classes had the same and the best renal outcome in comparison to the other classes with a renal survival, respectively, of 88% and 94% at 1 year; of 82% and 81% at 5 years; of 82 and 75% at 10 years (p=0.7). The renal outcome of Crescentic and Sclerotic groups were not different, their renal survival was respectively 66% and 68.5% at 1 year, 37% and 51% at 5 years, and 37% and 25% at 10 years (p=0.9). The outcome of the crescentic group was significantly worse than that of the focal group (p=0.015) and from that of the mixed group (p=0.03). The outcome of the sclerotic group was significantly worse than that of the focal group (p=0.04) and from that of the mixed group (p=0.05).

Table II. Clinical characteristics at presentation of the patients assigned to different glomerular classification groups.

	Focal	Crescentic	Mixed	Sclerotic	*p*-value
Number of patients	20	28	36	9	
Age; years Median	57.4±17.4 62.7	58.0±16.9 62.4	57.9±17.2 59.8	64.1±11.2 61.3	NS
MPO positivity	33.3%	40.8%	56.2%	50%	NS
PR3 positivity	40.0%	48.1%	31.2%	37.5%	NS
ANCA negativity	26.7%	11.1%	12.5%	12.5%	NS
Serum Creatinine mg/dl Median	3.4 ± 2.3 2.7	7.9 ± 4.9 7.5	4.7 ± 4.2 3.3	7.13 ± 2.9 7.8	0.0000
Serum Creatinine ≥1.2 mg/dl	17 (89.47%)	28 (100%)	33 (91.66%)	9 (100%)	NS
GFR ml/min Median	41.5±50.7 19	12.3±11.4 7	24.8±24.9 15	10.1±6.6 8	0.000
n. urinary erythrocytes/HPF Median	57.8±39.8 50	70.6±43.8 70	68.1±33.1 75	30.9±20.3 32.5	NS
Proteinuria g/24h Median	0.9±1.0 0.6	1.6±1.9 0.9	2.5±4.4 1.1	2.32±3.7 0.8	NS
Haemoglobin ll g/dl Median	10.4±1.9 10.1	9.2±1.8 9.3	9.6±1.4 9.6	9.1±1.4 8.7	NS
Erythrocyte sedimentation rate Median	75.1±40.5 76.5	99.5±26.5 90.2	79.7±31.4 82	94.8±26.7 90	NS
C-reactive protein mg/dl Median	9.7±9.1 8	13.7±9.6 12.6	5.2±7.1 2.45	11.1±12.2 6.1	NS
Arterial hypertension	50%	60%	60%	66.6%	NS
Methylprednisolone pulses	85%	89.2%	88.9%	100%	NS
Oral cyclophosphamide	85%	93%	86%	85%	NS
Azathioprine	15%	7%	14%	15%	NS

Unless otherwise specified, numbers are expressed as Mean ± SD.
GFR: glomerular filtration rate; HPF: high power field. *p*-values are calculated with Wilcoxon test. Serum Creatinine: Focal *vs*. Crescentic $p=0.0001$, Crescentic *vs*. Mixed $p=0.007$; GFR: Focal *vs*. Crescentic $p=0.005$, Crescentic *vs*. Mixed $p=0.017$; Haemoglobin: Focal *vs*. Crescentic $p=0.013$; Erythrocyte sedimentation rate: Focal *vs*. Crescentic $p=0.01$, Crescentic *vs*. Mixed $p=0.01$; C reactive protein: Crescentic *vs*. Mixed $p=0.0001$.

Predictors of renal outcome (Table I)

At univariate analysis, in comparison to patients who did not develop ESRD, those who developed ESRD had, at presentation, higher serum creatinine ($p=0.00001$), and higher C-reactive protein ($p=0.01$), higher proteinuria ($p=0.03$), lower glomerular filtration rate ($p=0.001$), and more frequent arterial hypertension ($p=0.001$). Among the histological features, patients who developed ESRD had less than 20% of normal glomeruli ($p=0.00007$), a higher precentage of glomeruli with crescents ($p=0.0007$) and with cellular crescents ($p=0.0007$), and with circumferential crescents ($p=0.001$), and more frequent moderate-severe interstitial infiltration ($p=0.02$), and more frequent severe tubulointerstitial fibrosis ($p=0.04$). The histological classification was not predictive of ESRD at univariate analysis. At multivariate analysis, taking into account only the histological features, less than 20% of normal glomeruli ($p=0.011$, RR: 3.38, 95% CI: 1.32–8.65), circumferential crescents ($p=0.026$, RR:1.02, CI 1.002–1.03), and tubulointerstitial fibrosis ($p=0.01$, RR: 1.88, 95% CI: 1.16–3.04) were independent predictors of ESRD.

Including clinical and histological features, at multivarite analysis, serum creatinine ($p=0.009$ Relative risk 1.11 for every mg of serum creatinine, 95% CI: 1.03–1.21), presence of arterial hypertension (p= 0.006 RR 5.54, 95% CI 1.6–18.8) and less than 20% of normal glomeruli ($p=0.022$ RR 3.05, 95% CI 1.17–7.93) emerged as the independent predictors of development of ESRD.

Discussion

ANCA-associated glomerulonephritis is a rare but severe disease that can lead to ESRD and death in a considerable number of patients (22), and prompt diagnosis and institution of immunosuppressive therapy has been shown to improve patient outcome (23). The diagnostic value of kidney biopsy is well established, while its value in predicting renal and patient outcome is less clear and the results reported are conflicting.

We retrospectively analysed the outcome of our single centre experience on 93 patients with ANCA-associated renal vasculitis submitted to kidney biopsy. This cohort was followed for a mean period of 5 years and treated with an homogeneous schedule based mainly on steroids and cyclophosphamide. The survival rate without ESRD was 83% at 1 year, 66% at 5 years, and 60% at 10 years while patient survival rate was 92% at 1 year, 84% at 5 years and 81% at 10 years.

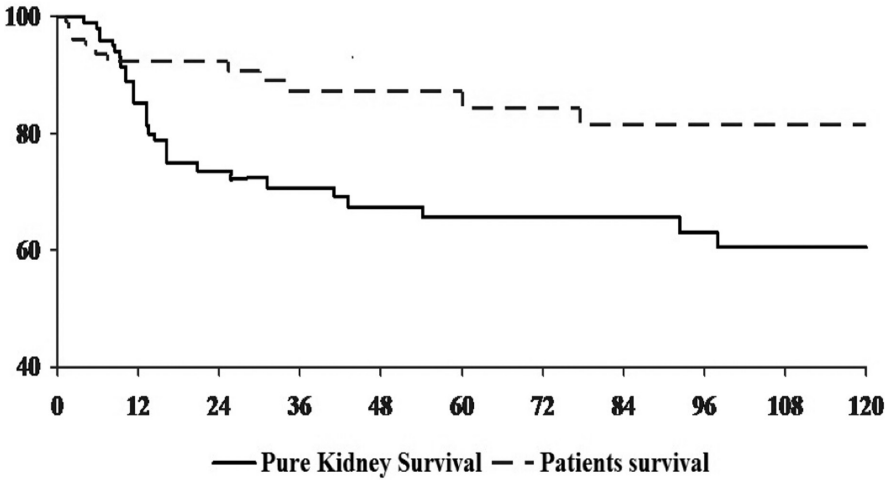

Fig. 1. Kaplan-Meier estimates of patient (dashed line) and of survival without end-stage renal disease probability censored for death (solid line) in ANCA-associated renal small-vessel vasculitis.

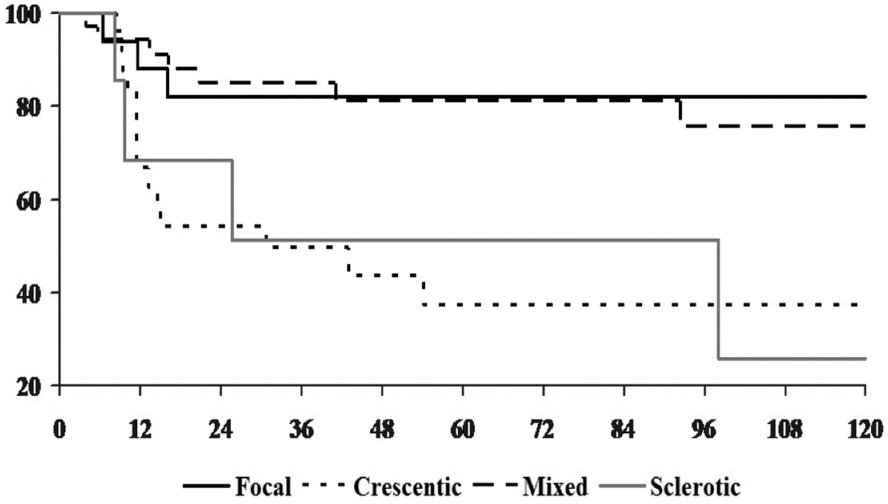

Fig. 2. Kaplan-Meier estimates of survival without end-stage renal disease of the crescentic, mixed and sclerotic groups. p=0.00001, Focal *vs*. crescentic p=0.0015, Focal *vs*. sclerotic p=0.04, Focal *vs*. mixed p=0.07, Crescentic *vs*. mixed p=0.03, Crescentic *vs*. sclerotic p=0.9, Mixed *vs*. sclerotic p=0.05.

Our aim was to validate in our cohort the prognostic power of the histological classification proposed in 2010 by a group of international pathologists (8) in predicting the development of ESRD and to revise the other studies on this subject. This classification suggests classifying patients with ANCA-associated renal vasculitis based on the predominance at renal biopsy of normal glomeruli, cellular crescents or global sclerotic glomeruli. Four histological classes were identified: focal, crescentic, mixed and sclerotic class. The study shows that the phenotypical order of the above mentioned classes corresponds to the order of severity of renal function impairment at presentation, and at 1 year and at 5 years follow up. Five studies (11-13, 17, 19, 20) reported the 5 year renal survival of the four classes (Fig. 3). Only in one of these studies (17) did the order of the survival of the four classes correspond to that reported by Berden *et al*. (8) with a 5-year renal survival of 98% for the focal class, 86% for the crescentic class, 82% for mixed class and 61% for the sclerotic class. The other 4 studies (11-13, 19) confirmed the good outcome of the focal class and three studies (11, 12, 19) the poor outcome of the sclerotic class. However, the outcome of the crescentic and the mixed classes was different from that of the original classification, the mixed class having a better survival than that of the crescentic class.

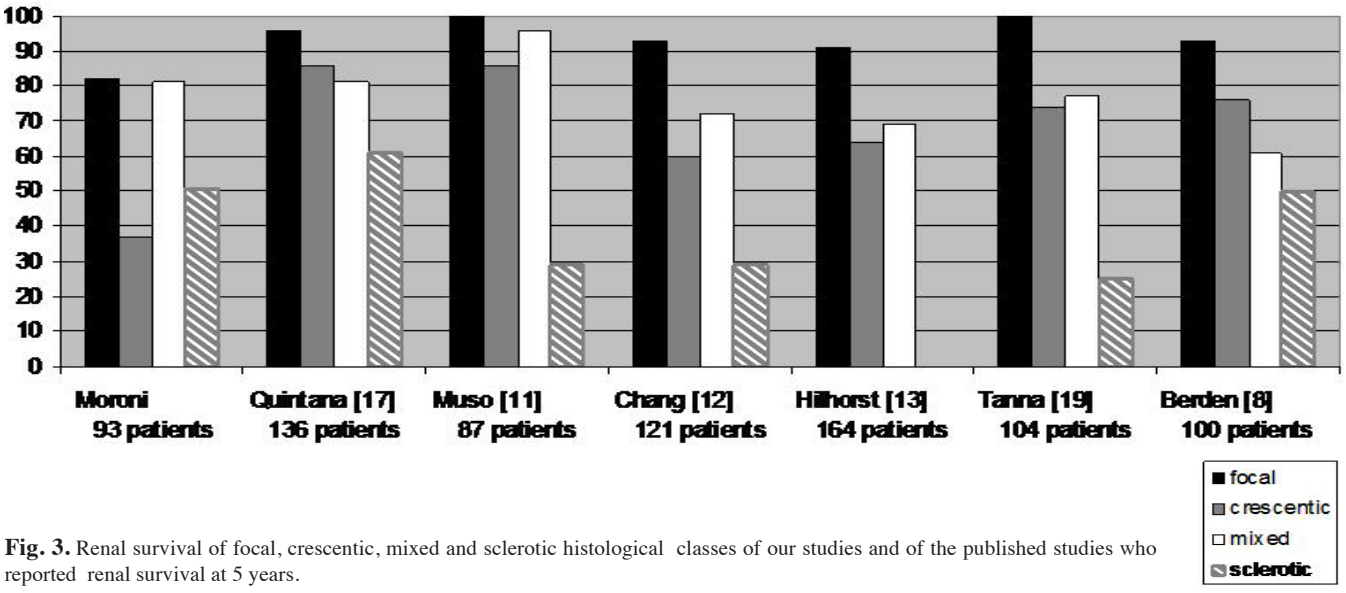

Fig. 3. Renal survival of focal, crescentic, mixed and sclerotic histological classes of our studies and of the published studies who reported renal survival at 5 years.

Table III. Histological features at kidney biopsy of the patients assigned to different glomerular classification groups.

	Focal	Crescentic	Mixed	Sclerotic	*p*-value
Number of patients	20	28	36	9	
Number of glomeruli	20.6±8.2	20.8 ± 11.5	24.3 ± 13.5	20.8 ± 13.4	NS
Normal glomeruli n. %	13.0±5.6 63±12.8	2.9 ± 3.0 12.2 ± 11.8	5.7 ± 4.9 24.9 ± 14.7	1.9 ± 2.0 7.8 ± 7.6	0.0000
Less than 20% normal glomeruli n. patients (%)	0	20 (71%)	13 (36%)	8 (88.8%)	0.0000
Crescentic glomeruli n. %	2.8±3.4 13.7±14.0	15.3 ± 8.7 75.6 ± 17.3	5.4 ± 5.2 20.3 ± 13.5	3.0 ± 2.3 13.5 ± 9.7	0.0000
Circumferential cellular n. crescents %	2.2±4.5	42.7 ± 27.6	7.3 ± 9.7	7.6 + 9.4	0.0002
Glomeruli with segmental necrosis %	9±9.8	34.4 ± 27.9	15.8 ± 17.2	6 ± 8.9	0.006
Globally sclerotic glomeruli n. %	2.6±2.2 12.3±8.9	2.6 ± 3 11.6 ± 8.9	5.1 ± 4.7 19.7 ± 13.2	11.8 ± 8 58 ± 9.5	0.002
Vascular fibrinoid necrosis n. (%)	3 (15%)	9 (32%)	6 (16.6%)	0	0.1
Moderate/severe interstitial inflammation n. (%)	7 (35%)	20 (71%)	18 (50%)	8 (88%)	0.01
Moderate/severe tubulo interstitial fibrosis n. (%)	5 (25%)	6 (21.4%)	14 (38.8%)	8 (88%)	0.002

Unless otherwise specified, numbers are expressed as Mean ± SD. *p*-values are calculated with Wilcoxon test. Glomeruli with segmental necrosis: Focal *vs*. Crescentic *p*=0.001, Crescentic *vs*. Mixed *p*=0.006, Crescentic *vs*. Sclerotic *p*=0.004; Vascular fibrinoid necrosis: Crescentic *vs*. Mixed *p*=0.06; Interstitial inflammation: Focal *vs*. Crescentic *p*=0.027, Focal *vs*. sclerotic, *p*=0.02.

Table IV. Outcome at 1 and at 5 years of glomerular filtration rate and clinical status at last observation of the patients assigned to different glomerular classification groups.

	Focal	Crescentic	Mixed	Sclerotic
Follow-up, months Median	65.9±66.9 41.5	39.4±45.1 20.6	80.3±69-1 51	54.3±60.6 25.7
GFR ml/min basal Median	41.5±50.7 19	12.3±11.4 7	24.8±24.9 15	10.1±6.6 8
GFR ml/min, at 1year Median	67.6±52.1 61.9	27.1±19.5 32.4	49.2±27.8 42	14.2±11.2 20.2
GFR ml/min, at 5 years Median	65.8±60.4 48.7	33.3±10.1 35	59.7±32.3 43.9	11.6±8.5 13.3
Renal outcome at last observation				
Number of patients (%)				
- Normal renal function	14 (70%)	6 (21.4%)	22 (61,1%)	0
- Chronic Kidney Disease	3 (15%)	7 (25%)	5 (13.9%)	3 (33.3%)
- End-stage renal disease	3 (15%)	15 (53.6%)	9 (25%)	6 (66.7%)
- Deaths	6 (30%)	3 (10.7%)	4 (11.1%)	1(11.1%)

Unless otherwise specified, numbers are expressed as Mean ± SD.
GFR: glomerular filtration rate.

The difference in survival between the mixed and the crescentic classes was minimal in the study of Tanna *et al*. (19) (5-year renal survival 77% and 74%, respectively), and in that of Hilhorst (13) (69% and 64%, respectively). In the study of Chang *et al*. (12) the 5-year survival of the mixed class was 72% and that of the crescentic 60%, and in the study of Muso *et al*. (11) they were 96% *versus* 86%, respectively.

According to Berden *et al*. (8) and with the other above mentioned studies (11-13, 17, 19), our focal subgroup had a good 5-year renal survival with the lowest percentage of patients who developed ESRD, better preservation of renal function and the best GFR at diagnosis, and at 1 and at 5 years in comparison to the sclerotic class in which we observed a poor renal outcome at 5 years. In the sclerotic subgroup there was the highest percentage of patients who developed ESRD, the lowest rate of renal recovery and the worst GFR at basal, and at 1 and 5 years. Instead, the outcome of crescentic and mixed subgroups of our cohort did not agree with that of Berden *et al*. (8) either. The renal survival at 5 years was worse in our crescentic subgroup in comparison to that of the mixed subgroup. Moreover, the Kaplan-Meier curves estimating survival without development of ESRD, did not show significant differences between the focal and the mixed group or between the crescentic and the sclerotic group. Consequently, survival without ESRD was significantly better for the focal and the mixed groups in comparison to that of the crescentic and the sclerotic groups.

As reported in other studies (9, 10, 12-18), our crescentic subgroup had a basal GFR lower than that of the focal and mixed subgroups but not different from that of the sclerotic subgroup. Our crescentic group, in addition to a high proportion of crescents, was characterised by other histological features such as segmental fibrinoid necrosis in around 1/3 of glomeruli and in some small arteries; lesions that are considered severe but reversible with therapy

(24). However, in spite of a treatment with steroids and cyclophosphamide, around half of patients did not recover renal function. In contrast, in the mixed subgroup, the recovery of renal function after therapy led to achieving a 5-year renal survival not different from that of the focal subgroup. Altogether 53% of patients in the crescentic subgroup developed ESRD in comparison to 25% of those of the mixed subgroup. The worse outcome of our crescentic subgroup could be attributed to some other histological features. As a matter of fact in this subgroup we found only 12% of normal glomeruli, the lowest percentage in comparison to that of all the other groups with the exception of that of the sclerotic group. Hilhorst *et al*. (13) in a study of 164 patients with a diagnosis of ANCA-related vasculitis, found that renal survival was significantly worse when the percentage of normal glomeruli was less than 25%. Moreover, in our cohort, at univariate analysis, we found that a percentage of normal glomeruli of less than 20% was one of the predictors of development of ESRD. This data could explain the bad renal outcome of our crescentic group in which 70% of patients had less than 20% normal glomeruli, and the good outcome of the mixed group in which only 35% of patients had less than 20% normal glomerui. In addition, more than half of the crescents present in our crescentic subgroup were circumferential. The negative prognostic power of circumferential crescents has already been reported (16, 25). Unlu *et al*. performed a multicentre study with 141 cases from Turkey aimed to validate the proposed histological classification of ANCA vasculitis (16). In this study the Cox regression model revealed a 2.6-fold increased risk for dialysis of the circumferential crescents. Another possible explanation for the worse outcome of the crescentic group could be the low number of patients treated with plasma exchange, a treatment indicated in cases of severe impairment of renal function (26).

Some discrepancies from the proposed histological classification (8) have been demonstrated by other studies in addition to those already mentioned (9, 14). Ford *et al*. (14) analysed data of 120 patients with ANCA-associated vasculitis from a single centre in Australia. They tested the reproducibility of the classification among 3 histopathologists blinded to patient's clinical outcome. Reproducibility of the classification was seen only in patients with a sclerotic pattern. Patients with sclerotic injury had an increased risk of ESRD or death compared with all the other classes. The sclerotic class, together with a degree of chronic interstitial damage and kidney function at baseline independently predicted poor outcomes. The negative renal predictive power of chronic tubulointerstital damage has been identified in some histological studies of ANCA vasculitis (17, 24, 27-29) and in many other kidney diseases. However, the addition of tubulointerstial scores to the proposed classification system did not improve the prognostic power of the model and for this reason these lesions were not included in the proposed classification (8). In our cohort, when only the histological features were taken into consideration, the multivariate analysis showed that moderate/severe tubolointerstitial fibrosis was an independent predictor of development of ESRD together with a percentage of normal glomeruli less than 20% and circumferential crescents. Including clinical and histological features, at multivarite analysis, high serum creatinine, presence of arterial hypertension and less than 20% of normal emerged as the independent predictors of development of ESRD. On the other hand, in our cohort, the proposed histological classification was not predictive of renal outcome at univariate and at multivariate analysis. The importance of serum creatinine and of the number of normal glomeruli as predictors of renal survival have been already demonstrated by many previous studies (13, 19, 28, 29-32).

The limits of our study are the low number of patients included and the retrospective nature of the analysis, the strengths are the provenience of all patients and data from the same centre, the uniformity of treatment and the fairly long follow-up.

In conclusion, we confirm the results of the proposed histological classification regarding the good 5-year renal prognosis of the focal class in comparison to the bad renal prognosis of the sclerotic class at 5 years. However, as in other studies (9, 11, 12, 14, 17), the results are controversial with regard to the intermediate classes: mixed and crescentic. Based on our data, it is not possible to differentiate the renal outcome of the crescentic from the sclerotic group and that of mixed from focal group using the classification proposed. Our results suggest that, among the histological features, the number of normal glomeruli defines the prognosis of our patients together with renal function and arterial hypertension at baseline. The role of tubulointerstial chronic lesions needs a more thorough evaluation.

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