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Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis

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Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. We report the clinical outcome of 105 essential mixed cryoglobulinemia (EMC) patients with renal involvement collected throughout 25 years in three renal Units of Milan. The median follow-up was 72 months since renal biopsy and 131 months since the clinical onset of EMC. Patient survival was 49% at 10 years after renal biopsy. Forty-two patients died primarily from cardiovascular and liver disease or infection, whereas 15 patients developed chronic renal failure. Two patients had a complete remission of the disease while 15 had a remission only of renal signs. Thirty-one patients are alive with persistent renal and extrarenal manifestations. Anti-HCV antibodies were retrospectively detected in 34 patients and were present in 85% of them. This variable was not included in the statistical evaluation. At multivariate analysis, age older than 50 years, purpura, splenomegaly, cryocrit levels higher than 10%, C3 plasma levels lower than 54 mg/dl, and serum creatinine higher than 1.5 mg/dl were independent risk factors for death or dialysis. In conclusion, several factors may influence the outcome of patients with EMC nephritis. Markers of disease activity and an impaired renal function can herald a bad prognosis. It should be stressed, however, that only a minority of patients eventually develop renal failure, probably because in the most severe cases patients die earlier.

After the initial description by Meltzer and Franklin [1], several papers have reviewed the clinical features of the so-called essential mixed cryoglobulinemia (EMC) [2–4], a disease caused by the formation of high molecular weight aggregates of two immunoglobulins: usually a polyclonal IgG and a monoclonal IgM with antiglobulin activity. The etiology of EMC is unknown. Recently it has been shown that most of patients with EMC are carriers of hepatitis C virus which could represent the triggering agent leading to the abnormal immune response [5–7].

Clinically, EMC is characterized by the triad of purpura, arthralgias and weakness. The clinical course of patients with cryoglobulinemia is variable: some patients have an indolent course while others develop vasculitic lesions in the lung, nervous system and in the bowel. Of particular importance is the development of a renal disease, since nephritis represents a hallmark of a severe prognosis [8]. However, differences in the severity of renal involvement, in the duration of follow-up and in the type of treatment, as well as the small number of patients enrolled in different series, have not permitted a definition of the long-term prognosis and the prognostic predictors of patients with EMC nephritis.

In this paper we report the long-term outcome of 105 patients with EMC nephritis who were followed by three renal units working in Milan.

Methods

The medical histories of all patients with EMC who were hospitalized at the Nephrology Divisions of Ospedale Maggiore, Ospedale S. Carlo and Ospedale Ca' Granda of Milan, between 1966 and 1990 were reviewed. Patients were eligible for the study if they had a diagnosis of EMC nephritis made according to the following criteria: (a) circulating cryoglobulins, (b) rheumatoid factor activity in their serum, (c) history of recurrent episodes of purpura, (d) urinary protein excretion greater than 0.5 g per day and/or urinary sediment showing erythrocytes and casts, (e) renal biopsy showing a pattern of cryoglobulinemic nephritis, (f) a minimum follow-up of one year after diagnosis unless the patient died or progressed to renal failure. Out of 140 patients given a diagnosis of EMC, 35 were excluded because of a too short follow-up or lack of renal biopsy; 105 patients fulfilled the above inclusion criteria and were selected for this study. The cryoglobulin typing showed: type I cryoglobulinemia in 4 patients, type II in 60, type III in 18. In 23 patients the light chains of both M and G immunoglobulins were undetermined.

Proteinuria was defined as an urinary protein excretion greater than 0.5 g per day. Microscopic hematuria was defined as more than 1 plus detected with multistix. Nephritic syndrome was defined as a combination of microscopic hematuria, proteinuria, hypertension and a rapid increase in plasma creatinine over 1.5 mg/dl. Nephrotic syndrome was defined as an urinary protein excretion greater than 3.5 g per day. Chronic renal failure was defined as a plasma creatinine over 4.0 mg/dl for at least six consecutive months.

Renal biopsies were reviewed and the diagnosis of EMC was based upon the already established histological criteria [9, 10]. The severity of the pathological involvement was scored by means of ordinal scale. A score of 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) was assigned to each of the following morphological

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features: intracapillary proliferation, epithelial proliferation, leukocyte and monocyte exudation, subendothelial deposits, intraluminal thrombi, glomerulosclerosis, interstitial inflammation, tubular atrophy and/or interstitial fibrosis, fibrinoid necrosis of arterial wall, arterial intimal fibrosis and sclerosis. The percentage of hyaline glomeruli was graded as 0 (absent), 1 (≤10%), 2 (>11%). An activity index was calculated on the basis of the sum of nine items, that is, intracapillary proliferation, epithelial proliferation, subendothelial deposits, leukocyte and monocyte exudation, intraluminal thrombi, interstitial inflammation and fibrinoid necrosis of the arterial wall. A chronicity index was calculated by summing the scores of four items: percentage of sclerosed glomeruli, glomerular sclerosis, tubular atrophy and/or interstitial fibrosis, arterial intimal fibrosis and sclerosis. The renal biopsies were also processed by immunofluorescence. The intensity of immunoreactants (IgA, IgM, IgG, C1q, C3 and C4) were graded as 0, 1, 2, 3.

After November 1989, an ELISA method was available to check the presence of anti-HCV antibodies in patients' sera. For this purpose, the following materials were utilized: 8 fresh serum samples, 15 serum samples frozen at −80°C, purified cryoprecipitate components stored at −80°C (33 samples). Thirty-four patients were tested. Frozen serum samples and purified cryoprecipitate components were collected since 1980. The detection of anti-HCV antibodies was simultaneously evaluated by second generation ELISA (Ortho Diagnostic System, Raritan, NJ, USA) and by recombinant immunoblot assay (RIBA 2, Ortho).

Purified cryoglobulin fractions were obtained by eluting the cryoprecipitate dissolved in 0.1 M acetic acid through a Sephadex G-200 column (Pharmacia Fine Chemicals, Upsala, Sweden) with acetic acid 0.1 M solution, pH 3. Fractions were collected automatically and optic densities were read in a Nikon 860 spectrophotometer at 280 nm. Fractions were then extensively dialyzed against phosphate buffered solution at pH 7.2 and concentrated with Aquacide IIA (Calciobiochem, Behring, La Jolla, CA, USA). Two peaks were obtained and analyzed by immunofixation for the presence of IgA, IgG, IgM, kappa and lambda light chains using monospecific antibodies.

Potential predictors for outcome measured at the time of diagnosis which was concomitant with renal biopsy, included clinical variables (sex, age, purpura, fever, arthralgias, hepatosplenomegaly, neurological involvement, hypertension defined as resting diastolic pressure above 95 mm Hg for more than 2 consecutive measurements), laboratory variables [cryocrit (1 ≤ 10%, 2 > 11%); serum creatinine (1 ≤ 1.5 mg/dl, 2 > 1.6 mg/dl); serum C3 (1 ≤ 54 mg/dl, 2 > 55 mg/dl) and C4 level (1 ≤ 20 mg/dl, 2 > 21 mg/dl); serum IgA (1 ≤ 90 mg/dl, 2 > 91 ≤ 420 mg/dl, 3 > 421 mg/dl), IgG (1 ≤ 800 mg/dl, 2 > 801 ≤ 1800 mg/dl, 3 > 1800 mg/dl), IgM (1 ≤ 60 mg/dl, 2 > 61 ≤ 250 mg/dl, 3 > 251 mg/dl) concentrations; platelets counted as more or less than 100,000/mm^3; urinary protein excretion: 1 = absent, 2 lower than 3.5 g/day, 3 > than 3.5 g/day; hematuria: 1 = absent, 2 = 2+, 3 = 3+; ALT and AST lower (= 1) or higher (= 2) than 40 UI/liter] and histological variables.

Because the nature of the study was retrospective, treatment regimens have varied largely. For statistical purposes, patients could be divided in four subgroups: 21 patients never received either steroid or cytotoxic agents, 21 patients were given steroids, 12 patients were given cytotoxic drugs alone; 61 patients were treated by a combination of steroids and cytotoxic agents. Moreover, methylprednisolone pulses (51 patients) and plasmapheresis (70 patients) were used in several occasions for renal or extrarenal flare-ups.

Table 1. Clinical features

Signs or symptoms	At diagnosis	During the follow-up
	N (%)	
Purpura	73 (70)	98 (94)
Hepatomegaly	51 (49)	95 (91)
Arthralgias	48 (46)	85 (81)
Fever	20 (19)	63 (60)
Splenomegaly	38 (36)	60 (57)
Neuropathy	31 (22)	40 (38)
Abdominal pain	8 (8)	28 (27)

Statistical analysis

Statistical analysis has been performed combining the two end points: patient's death and chronic renal failure. The follow-up was stopped at 120 months owing to the scanty number of patients remaining at risk beyond this period, and since one cannot exclude that the final event was not correlated with the underlying disease over this period. The prognostic relevance of the baseline variables on the combined events was analyzed estimating the cumulative probability according to the Kaplan and Meier product limit method [11]. Between groups, comparisons of continuous variables were carried out by means of generalized Wilcoxon test. Multivariate statistical analysis was performed according to Cox's proportional hazards model using a backward procedure. Assumptions of Cox's model were checked by graphic method and by stratified analysis [12].

Results

The mean age of 105 patients at presentation was 52.7 ± 10.65 years, median 52, range 21 to 75. There was a slight prevalence of women (62/43). The median follow-up after diagnosis was 72 months ranging from 1 to 283 months. The median total follow-up from the first signs and symptoms was 131 months (3 to 584).

The distribution of extrarenal signs and symptoms of EMC at diagnosis and during the course of illness are shown in Table 1. Purpura was the most common symptom both at the time of diagnosis and during the follow-up. Only five patients never developed purpura, although their follow-ups were comparable to that of other patients. Thirty-four out of 105 patients were examined for anti-HCV antibodies. Anti-HCV antibodies were detected in 29 of the 34 (85%) and in the 80% of purified cryoprecipitate fractions.

The median interval between the first symptoms of EMC and renal involvement was 48 months (range 0 to 492). The onset of renal disease was concomitant to that of extrarenal manifestations of EMC in 14 patients. Proteinuria greater than 0.5 g per day and hematuria were the most common features of renal disease at diagnosis in 58 patients (55%). Nephritic or nephrotic syndrome was present in 26 and 21 patients, respectively (25% and 20%). Eighty-six patients were hypertensive (82%). Fourty-eight patients had elevated plasma creatinine at the time of renal biopsy (47%).

Renal biopsy showed a pattern of membranoproliferative glomerulonephritis in 80% of patients, diffuse mesangial proliferative

Table 2. Immunofluorescence results

Antigen (*N*)	*N* +++	*N* ++	*N* +
IgA (83)	1 (1.2)	2 (2.4)	29 (34.9)
IgG (83)	4 (4.8)	20 (24)	44 (53)
IgM (83)	15 (18)	34 (40.9)	32 (38.5)
C1q (81)	2 (2.4)	4 (4.9)	25 (30.9)
C3 (83)	3 (3.6)	16 (19.2)	51 (61.4)
C4 (76)	—	–	13 (17)

changes in 18% and focal mesangial proliferative glomerulonephritis in 2%. Crescents were observed in 11.4% of patients but involved only a small number of glomeruli. Intraluminal thrombi were the most characteristic histological feature in 26 biopsies. Vasculitis with fibrinoid necrosis and/or proliferative changes of arterial wall was observed in 34 specimens.

Immunofluorescent results are reported in Table 2. IgM was found in 98% of biopsies, IgG in 82%, IgA in 38%, C3 in 84%, while C1q and C4 antigens were detected with less frequency and a minor intensity (38% and 17%, respectively). Electron microscopy performed in 28 patients showed a picture of membranoproliferative glomerulonephritis in 25 and a mesangial proliferative in 3. Extensive osmiophilic deposits were present in all cases mainly in subendothelial position, while intraluminal thrombi, extracellular protein deposits occluding glomerular loops were found in about 70% of cases. The extent of hyaline thrombi was variable. Both subendothelial deposits and intraluminal thrombi had the unique crystalloid structure consisting of 100 to 1000 nm cylinders on longitudinal sections. On cross sections they appeared as annular bodies with a diameter of 62 to 63 nm, made up of a light center, a dense ring and a lighter peripheral coat. In the capillary lumina a variable number of monocytes was found in all specimens. The electron microscopy findings could not be utilized for statistical purpose because they were available in a minority of patients.

The cumulative 10-year probability of being alive without renal failure after diagnosis was 0.49 (95% CI 0.60 to 0.38; Fig. 1). As mentioned in the **Methods** section, for stastistical purposes only the events occurring within 10 years from the diagnosis of EMC have been taken into account; several patients died or developed chronic renal failure after that interval (Table 3). During the follow-up 42 patients died mostly of cardiovascular diseases, liver failure or infections (Table 4), and 15 progressed to chronic renal failure. In 7 patients death occurred during an exacerbation of renal and extrarenal manifestation of EMC disease. Four patients suffered from cancer: larynx carcinoma, lung carcinoma, hepatocarcinoma and multiple myeloma, respectively, after a median time of 70 months from the diagnosis of cryoglobulinemia (range 7 to 104). In all 4 patients death was strictly correlated with neoplasia. Fourty-six patients were alive after a median follow-up of 120 months. Fourteen patients were lost to follow-up after a median time of 23.5 months (12 to 110).

Fifteen out of 105 patients had a complete and prolonged remission of renal symptoms, whereas systemic symptoms persisted. The remission occurred after a median period of 84 months (range 2 to 156 months) since diagnosis and lasted for a median period of 24 months (7 to 143 months). In two patients clinical manifestations disappeared, circulating cryoglobulins became undetectable, complement levels normalized and rheumatoid factor became negative 91 and 168 months after the onset of disease. In one patient remission occurred after the start of regular dialysis, in the other one signs and symptoms reversed after the patient became HIV positive.

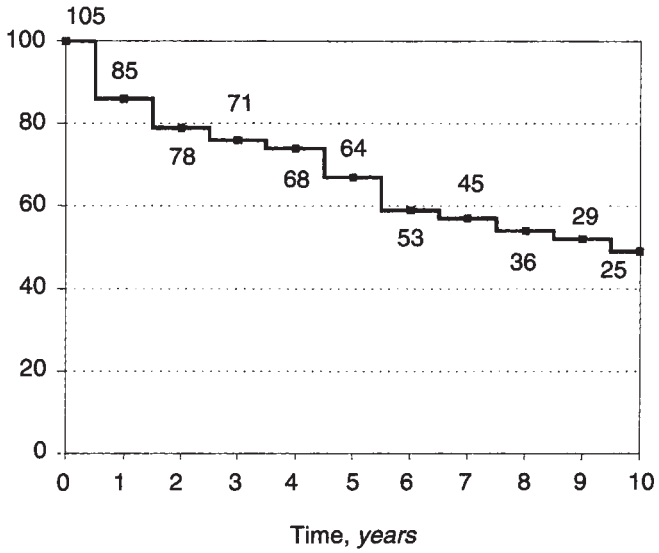

Fig. 1. *Cumulative probability of survival.*

Table 3. Patient outcome

	Death	Follow-up *months*	Renal failure	Follow-up *months*
Before 10 years	35	44.6 ± 42.67	10	90.2 ± 46.52
range		(1–150)		(36–204)
After 10 years	5		5	
After renal failure	2			

Table 4. Causes of death

	Number of patients
Cardiovascular disease	12
Infection	9
Liver failure	8
Neoplasia	4
Acute respiratory distress syndrome	1
Unknown	8
Total	42

At univariate analysis, among the 17 clinical and laboratory variables examined, age older than 50 years, presence of purpura, presence of splenomegaly, low serum levels of IgG and C3, high levels of circulating cryoglobulins, and evidence of more severe renal disease were significantly associated with death or chronic renal failure (Table 5). The most significant prognostic factor was a plasma creatinine higher than 1.5 mg/dl (Fig. 2). The fact that hypertension was not associated with the end-points was somehow unexpected and prompted us to furtherly evaluate this aspect. We therefore stratified patients according to the presence or absence

Table 5. Clinical, therapeutic and histologic predictors of death or CRF by univariate analysis

Variables	Generalized Wilcoxon (Breslow)	P
Age *>50 years*	4.98	0.0261
Purpura	6.06	0.0138
Splenomegaly	10.75	0.0010
Plasma creatinine *>1.5 mg/dl*	19.67	0.0000
IgG plasma level *<800 mg/ dl*	8.25	0.0041
C3 plasma level *<54 mg/dl*	15.13	0.0001
Cryocrit *>10%*	10.07	0.0015
Plasmapheresis	4.14	0.0418
Interstitial fibrosis	6.10	0.0135

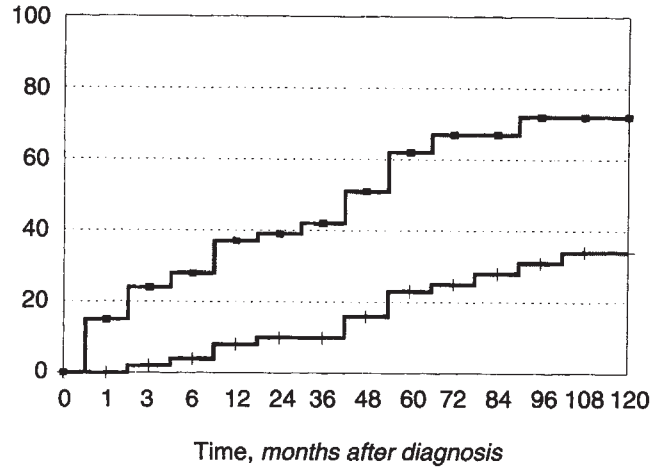

Fig. 2. *Cumulative probability of death or chronic renal failure for the classes by plasma creatinine at diagnosis.* $P = 0.000$.

of hypertension and to the response to antihypertensive treatment. Patients were divided into three groups: (a) non-hypertensive patients (19); (b) patients with a moderate hypertension (75), defined by the use of less than three antihypertensive drugs in order to keep diastolic blood pressure below 100 mm Hg; (c) patients with a refractory hypertension [11], whose diastolic blood pressure was higher than 100 mm Hg in spite of the use of three or more antihypertensive drugs. The percentage of final events in group a was 31.6%, in group b was 42.7%, and in group c was 63.6%. Although there was a trend towards a worse prognosis in severely hypertensive patients (group c), it did not reach statistical significance ($P = 0.2312$). The type of cryoglobulins was not included in the univariate analysis since it was not tested in 18 patients. Most of the remaining patients had type II cryoglobulins. Chi-square analysis did not show any difference between type II and type III (data not shown).

No firm indication about therapy could be drawn from this study. Patients were not prospectively assigned to one of the above-mentioned therapeutical schedules, but the decision of what and when therapy had to be initiated was taken by the doctor in charge of the patient. We found a correlation between plasmapheresis and the combined end-points ($P < 0.04$). However, it is likely that this correlation simply reflects our attitude to use plasmapheresis when the disease activity was quite severe or nonresponding to other treatment.

Table 6. Significant covariates from the Cox survival analysis

Variable	Relative risk	95% Confidence interval
Age *>50 years*	2.46	1.21
Vascular purpura	2.22	1.00
Splenomegaly	2.09	1.10
Cryocrit levels *>10%*	2.33	1.21
C3 plasma levels *<54 mg/dl*	2.36	1.24
Serum creatinine *≥1.5 mg/dl*	2.15	1.11

Among the histological variables, only interstitial fibrosis was associated with the combined end-points. Neither the activity ($P = 0.5252$) nor the chronicity indices ($P = 0.4464$) predicted the final outcome. The apparent discrepancy between the significance of interstitial fibrosis and the nonsignificance of chronicity index may be explained by the fact that interstial fibrosis contributed for only one fourth to the total chronicity score.

At multivariate analysis older age, vascular purpura, splenomegaly, high cryocrit levels, low C3 plasma levels, and high plasma creatinine at the time of biopsy were identified as independent prognostic indices significantly correlated with death or renal failure (Table 6).

Discussion

In this series the three major clinical features of EMC, namely purpura, arthralgias, hepatomegaly occurred very frequently during the course of the disease similarly to what reported in previous studies [1–4]. Of note, however, five patients never showed purpuric lesions, although they had a follow-up time comparable with that of the other patients. In this series 85% of tested patients had positive HCV antibodies. These data are in agreement with previous studies showing that 50% to 100% of patients with EMC were HCV positive [5, 13–15]. Abnormal liver function tests were detected in 25% of patients during the follow-up, similarly to the 27% reported by Cordonnier et al [16] and the 24% reported by Invernizzi et al [4]. Conversely Gorevic et al [8] reported an increase of liver enzymes in 70% and Bombardieri et al [17] in 84% of their patients with EMC. It is difficult to explain these discrepancies, but it is possible that patients in the series of Gorevic and Bombardieri had more severe EMC. Peripheral neurophathy was observed in 22% of our patients. In previous smaller series this complication was found to be rare [1, 18], but when electromyography was routinely made an abnormal peripheral nerve conducibility was found in 68% to 82% of patients with EMC [19, 20]. In some reports pulmonary symptoms were observed only occasionally [17, 21] and death due to acute respiratory distress was a rare event [16, 22, 23]. However, when pulmonary function was routinely investigated [24], functional abnormalities referred to the immunological aggression to lung interstitium were observed in 61% of patients. Abdominal colicky pain affected only few patients in this series. It is generally caused by intestinal vasculitis initiated by the deposition of circulating cryoglobulins, even if the occurrence of intestinal infarct is confined to isolated cases [25]. Lymphoadenopathy, Sjögren's syndrome, thrombocytopenia, central nervous system involvement and Raynaud's phenomenon were rarely observed in our experience as well as in other series [26, 27].

All our patients had some signs of kidney disease but this datum is biased by the fact that all patients were hospitalized in three

renal units. Renal involvement was reported in 2 to 50% of patients in previous studies [2, 3, 9, 26]. It is possible that besides differences in patients selection, ethnic and racial factors as well as different criteria for diagnosis can account for these discrepancies.

The present study that collected the largest number of patients with EMC nephritis, confirms a poor prognosis for these patients. Among 105 patients followed for a median period of 131 months from the onset of the disease, 42 died and 15 developed ESRD. The other 15 patients who were in long-lasting remission of renal manifestation had recurrent episodes of systemic symptoms and persistence of laboratory parameters related to circulating cryoglobulins. The remaining patients showed an unremitting course, excluding two patients who underwent a complete disappearance of both clinical and laboratory features respectively after the discovery of HIV infection and after the beginning of dialytic treatment. Although our patients with EMC nephritis had a bad prognosis, the mortality rate was lower than that reported in other series. Death was reported in 18 of 22 patients by Gorevic et al [8], in 12 of 15 by Cordonnier et al [16], in 8 of 12 patients by Frankel et al [28] and in 12 of 21 patients by Invernizzi et al [26] notwithstanding, the follow-ups were comparable with that of our patients. In our patients death was mainly related to cardiovascular disease, while the number of patients who developed chronic renal failure was small (15 to 105). These results would indicate that most patients with EMC nephritis die before renal failure develops. It is likely that hypertension and vasculitis contributed to the high rate of cardiovascular disease. On the other hand, the poor prognosis observed in older patients suggests that factors not related to the disease *per se* can also influence the outcome of these patients.

Multivariate analysis showed that among the clinical variables tested, older age, purpura, splenomegaly, elevated plasma creatinine at presentation, high serum cryocrit and low C3 serum levels were independent predictors for death or for development of renal failure.

To our surprise, hypertension was not associated with a poor outcome. To better evaluate the role of hypertension, we stratified patients into three groups. There was a trend to a bad prognosis for patients with poorly controlled hypertension in comparison with patients with controlled hypertension or spontaneously normal blood pressure. This difference was not significant, possibly because of the small number of patients at risk.

Therapy did not influence the final oucome. This may be due either to the low effectiveness of the treatments or to the fact that corticosteroids, immunosuppressive drugs and/or plasmapheresis were given only in the most severe cases. The use of steroids and/or cytotoxic agents in EMC is still controversial, especially in the long-term. Considering whether or not a patient has to be given a therapy, one should bear in mind that the disease usually takes a long time to evolve into renal failure and it is punctuated by flare-ups. Therefore, we feel that an aggressive treatment should be reserved only for the acute flare-ups [29, 30]. The current therapeutical approach will be probably modified after the discovery that hepatitis C virus infection represents a pathogenetic factor in EMC. Agents capable to inhibit viral replication such as interferon alpha could play a major role in the treatment of the disease at least when renal disease is not too advanced [31, 32].

None of the histological variables had an independent prognostic significance even when evaluated as activity or chronicity index. This might be expected since it is unlikely that a renal biopsy made at some time could predict the long-term outcome of a disease characterized by recurrent flares of renal and extrarenal symptoms [32]. On the other hand, renal biopsy is important not only to differentiate EMC from other fibrillar diseases by electron microscopy, but also in making the therapeutic decision as it distinguishes active disease from irreversible chronic lesions in patients with renal failure.

The possibility that EMC can evolve into an hematological malignancy has been described [8, 9]. In this study four patients developed malignancies, among which two were of hematological origin. Theoretically, these cases could be accounted for either by the oncogenic effect of a prolonged immunosuppression or by the abnormal proliferation of a B cell clone producing IgM rheumatoid factor with eventual malignant transformation.

In summary, several factors may influence the outcome of patients with EMC nephritis. Markers of disease activity and an impaired renal function with or without severe proteinuria can herald a bad prognosis. In these patients death often precedes end-stage renal failure so that only a minority of patients has to be submitted eventually to regular dialysis.

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