Clinical and Morphologic Spectrum of Renal Involvement in Patients With Mixed Cryoglobulinemia Without Evidence of Hepatitis C Virus Infection

Marie Matignon, MD, Patrice Cacoub, MD, PhD, Magali Colombat, MD, David Saadoun, MD, PhD, Isabelle Brocheriou, MD, PhD, Béatrice Mougenot, MD, Françoise Roudot-Thoraval, MD, PhD, Philippe Vanhille, MD, Olivier Moranne, MD, PhD, Eric Hachulla, MD, PhD, Pierre-Yves Hatron, MD, PhD, Jean-Paul Fermand, MD, PhD, Fadi Fakhouri, MD, Pierre Ronco, MD, PhD, Emmanuelle Plaisier, MD, PhD, and Philippe Grimbert, MD, PhD**

Abstract: Hepatitis C virus (HCV) infection represents, by far, the major cause of mixed cryoglobulinemia (MC). The renal disease associated with this pathological condition is now well described. By contrast, renal involvement in patients with MC not associated with HCV has been only poorly described, and few cases have been reported. We analyzed the demographic, clinical, and laboratory features and outcome in patients presenting with renal disease associated with MC not related to HCV infection. Records of 20 patients with MC and renal disease, with no evidence of HCV by serology and polymerase chain reaction analysis, were retrospectively analyzed. Renal biopsies and extensive searches for lymphoproliferative disorder were performed in all patients at presentation. MC was related to primary Sjögren Syndrome (pSS) in 9 patients, and to non-Hodgkin lymphoma in 1 patient, while MC was classified as essential in the remaining 10 cases. Renal involvement was characterized by microscopic hematuria in all patients, nephrotic range proteinuria in 75% of patients, hypertension in 80% of patients, and renal failure in 85% of patients (mean glomerular filtration rate, 46 mL/min per 1.73 m^2). Membranoproliferative glomerulonephritis with subendothelial deposits was observed in all kidney specimens. Skin vasculitis was the main extrarenal manifestation. In all patients, cryoglobulinemia was classified as type II MC, characterized by monoclonal IgMκ and polyclonal IgG. Most patients (17/20) were treated with steroids or immunosuppressive agents, or both. Initial renal remission was observed in 94% of patients. However, renal relapse occurred in most patients, with 10% reaching end-stage renal disease. Three patients with essential MC developed B-cell lymphoma 36–48 months after the diagnosis of MC. Unexpectedly, B-cell lymphoma induced by Epstein-Barr virus infection occurred in only 1 of the 9 pSS patients. Forty percent of patients died as a result of extrarenal causes.

Renal disease associated with MC unrelated to HCV is characterized by the high prevalence of pSS (45%), the finding of CD20+ B-lymphocyte nodular infiltrates in the kidney interstitium, and a high incidence of overt B-cell lymphoma during follow-up. These findings emphasize the need for repetitive clinical evaluation in those patients.

(*Medicine* 2009;88: 341–348)

Abbreviations: GFR = glomerular filtration rate, HCV = hepatitis C virus, HIV-1 = human immunodeficiency virus-1, MC = mixed cryoglobulinemia, pSS = primary Sjögren syndrome.

INTRODUCTION

Cryoglobulins are proteins, mostly immunoglobulins that precipitate in the cold.[25] According to the Brouet et al[7] classification, based on the purification and immunochemical analysis of cryoprecipitable immunoglobulins, there are 3 types of cryoglobulins: type I cryoglobulins are composed of isolated monoclonal immunoglobulins (typically IgM) and are frequently associated with myeloma or Waldenstrom macroglobulinemia; type II and III are immunocomplexes formed by monoclonal (type II) or polyclonal (type III) IgM with rheumatoid factor activity plus the corresponding antigen (usually polyclonal IgG). "Mixed cryoglobulinemia" (MC) is a term used to designate type II and type III cryoglobulins.[7] MC has been observed in various lymphoproliferative disorders, and in infectious and systemic autoimmune diseases.[7] Cryoglobulinemia not associated with those disorders has been defined as "essential" cryoglobulinemia.

In the early 1990s, advances in serologic diagnosis of hepatitis C infection revealed a strong association between chronic hepatitis C virus (HCV) infection and essential MC.[1] The prevalence of HCV infection in cases of essential MC varies geographically from 40% to 92% of cases, being close to 90% of cases in the Mediterranean basin.[9,15,39] Renal involvement occurs in 20%–40% of MC cases associated with HCV infection, being more frequent when type II MC is detected together with IgMκ rheumatoid factor in the serum.[12,14,30] It is a major cause of mortality and morbidity.[1,9,15,39] However, in northern European countries, lower prevalence rates of HCV infection have been reported, and most patients with MC do not have chronic HCV infection.[11,32]

The clinical and morphologic spectrum of renal MC has been described almost exclusively in the context of HCV-related MC.[3,5,30,35] The renal manifestations may range from isolated proteinuria to overt nephritic or nephrotic syndrome with a variable progression toward chronic renal insufficiency.[5,30,35] The

From Nephrology and Transplantation Department (MM, PG), Henri Mondor Hospital, AP-HP, Institut Francilien de Recherche en Néphrologie et Transplantation (IFRNT), and Paris XII University, Créteil; Internal Medicine Department (PC, DS), Pitié Salpêtrière Hospital, AP-HP, Paris, and CNRS UMR 7087, Université Pierre et Marie Curie, Paris VI; Pathology Department (MC, BM), Tenon Hospital, AP-HP, Paris; Pathology Department (IB), Pitié Salpêtrière Hospital, AP-HP, Paris; Public Health and Biostatistics Department (FRT), Henri Mondor Hospital, AP-HP and Paris XII University, Créteil; Nephrology Department (PV), Valenciennes Hospital, Valenciennes; Nephrology Unit (OM), Nice University Hospital, Nice; Internal Medicine Department (EH, PYH), CHRU, Lille; Hematology Department (JPF), Saint-Louis Hospital, AP-HP, Paris; Nephrology and Dialysis Department (FF), Necker Hospital, AP-HP, Paris; and Nephrology and Dialysis Department (PR, EP), Tenon Hospital, AP-HP, Paris, France.

*Emmanuelle Plaisier and Philippe Grimbert contributed equally to this work.

Received December 19, 2008, and in revised form June 26, 2009.
Accepted for publication July 17, 2009.

Reprints: Marie Matignon, MD, Nephrology and Transplantation Department, Henri Mondor Hospital, AP-HP, Institut Francilien de Recherche en Néphrologie et Transplantation (IFRNT) and Paris XII University, Créteil, France (e-mail: marie.matignon@hmn.aphp.fr).

ISSN: 0025-7974
DOI: 10.1097/MD.0b013e3181c1750f

histologic pattern, called "cryoglobulinemic glomerulonephritis," is well characterized. Other pathological findings have been reported, however, including focal and mesangioproliferative glomerulonephritis, membranous glomerulonephritis, and isolated cases of thrombotic microangiopathy.[14,19,21]

By contrast, renal involvement in patients with MC not associated with HCV has been only poorly described, with only a few cases reported.[5,32] This issue is important, as the proportion of patients with renal involvement in the context of type II MC is high mostly in regions where the prevalence of HCV infection is relatively low.[11,24,32,36]

In the current study we report the first large multicenter study, to our knowledge, on the clinical and pathological presentation and outcome in patients with documented renal MC without evidence of HCV infection. Results show a high prevalence of primary Sjögren syndrome (pSS), the presence of lymphoid nodules in several kidney biopsies, and a good initial response to steroid and/or immunosuppressants, but with a high relapse ratio, and a relatively high incidence of lymphoma during outcome.

PATIENTS AND METHODS

This retrospective study was conducted in 9 French hospital departments of nephrology, internal medicine, hematology, and pathology (Nephrology and Dialysis, Tenon Hospital, AP-HP, Paris; Internal Medicine and Nephrology, Pitié-Salpêtrière Hospital, AP-HP, Paris; Hematology, Saint-Louis Hospital, AP-HP, Paris; Nephrology, and Transplantation, Henri Mondor Hospital, AP-HP, Paris; Nephrology and Dialysis, Necker-Enfants Malades Hospital, Paris; Internal Medicine, and Nephrology B, CHRU, Lille; and Nephrology, Valenciennes Hospital, Valenciennes). Inclusion criteria included the diagnosis of MC with biopsy-proven renal disease and the absence of documented HCV infection (negative anti-HCV antibodies and HCV polymerase chain reaction).

Clinical renal disease was defined using the following criteria: hypertension, blood pressure >140/90 mm Hg; renal failure, glomerular filtration rate (GFR) <60 mL/min per 1.73 m^2 (GFR was estimated by Modification of Diet in Renal Disease formula [MDRD][23]); nephrotic range proteinuria, >3 g/d or >300 mg/mmol creatininuria; and hematuria, >10,000 blood cells/mL. MC extrarenal manifestations were recorded for each patient as described previously.[15] Renal remission was defined as >50% urinary protein reduction and disappearance of hematuria. Extrarenal remission was defined as disappearance of clinical symptoms.

In all cases, the diagnosis of renal disease was confirmed by a kidney biopsy. Histologic specimens were reviewed by 2 investigators blind to clinical features. Lesions were evaluated for the percentage of mesangial sclerosis; endocapillary proliferation; mesangial proliferation and double contours; the percentage of glomeruli with global, segmental, and extracapillary proliferation; and the percentage of tubular atrophy and interstitial fibrosis. Histologic specimens were also evaluated for the presence or absence of arteriosclerotic lesions and necrotizing arteritis. Immunofluorescence analysis included immunostaining using polyclonal antibodies to IgA, IgG, IgM, C3, C1q, kappa, and lambda. If lymphoid nodules were present, immunophenotyping was performed on paraffin-embedded contiguous sections. Immunophenotyping was performed using a Ventana Benchmark automated immunostainer with commercial primary antibodies directed against CD20 (Dako, Denmark, clone L26, 1/500), CD3 (Thermo Scientific, UK, clone SP7, 1/100), and CD23 (Novocastra, UK, clone 1B12, 1/40). Electron microscopy was performed on kidney biopsies of 2 patients, as previously described.[26]

In patients with cryoglobulinemia identified as previously described,[10] the presence of MC was suspected based on rheumatoid factor activity, as detected by latex and Rose-Waaler tests. Immunochemical typing of MC was performed using electrophoresis and immunoelectrophoresis. MC was classified according to the Brouet et al classification.[7] Connective tissue disease was diagnosed based on standard criteria: pSS,[41] systemic lupus erythematosus,[20] rheumatoid arthritis,[4] antiphospholipid syndrome,[42] Behçet disease,[13] and mixed connective tissue diseases.[2] Hepatitis B virus surface antigen was analyzed by enzyme-linked immunosorbent assay (ELISA). ELISA using human immunodeficiency virus-1 (HIV-1) p24 coating was used to assay anti-HIV-1 p24 antibodies. For confirmation, Western blots were used to detect proteins. Other infections were diagnosed using standard methods. Non-Hodgkin lymphoma was diagnosed based on evidence of bone marrow, nodal, or extranodal lymphoproliferative disease with pathological features compatible with the World Health Organization classification of neoplastic diseases.[18] Finally, essential MC was considered in cases in which no infectious, autoimmune, or hematologic diseases were found.

Statistical Analysis

Quantitative data are presented as means (1 standard deviation) or medians (extreme values) in cases of asymmetric distribution and were compared using the nonparametric Mann-Whitney test. Qualitative data are presented as percentages. Categorical data were compared by the chi-square test or the Fisher exact test when appropriate. A value of $p < 0.05$ was considered significant. Patient survival was calculated using the Kaplan-Meier method.

RESULTS

Based on our inclusion criteria, we identified 20 patients with biopsy-proven renal disease and MC without HCV infection, diagnosed between 1980 and 2006.

MC Etiology and Demographic Data

In all cases, MC typing revealed type II cryoglobulinemia, including monoclonal IgMκ associated with polyclonal IgG. Patients were classified according to MC etiology: 9 patients had MC associated with pSS, 1 patient had B-cell lymphoma, and 10 patients had MC classified as essential (Table 1). None had an infectious disease or an autoimmune connective tissue disease. All patients were white. Most patients were women (the female to male ratio was 3:1), particularly in the pSS group ($p = 0.03$), which was all women. The mean age at diagnosis of renal disease associated with MC was 60 ± 12 years (range, 32–79 yr). No differences in age were identified between the 2 groups considering patients with pSS or not.

Renal Symptoms at Presentation

As shown in Table 1, the most frequent presenting renal syndrome was nephrotic proteinuria (85% of cases) with microscopic hematuria (100% of cases) and renal insufficiency (85% of cases). Hypertension was present in 80% of cases. The mean GFR at diagnosis was 46 ± 18 mL/min per 1.73 m^2 (range, 23–91), and the median interval between renal manifestations and the first extrarenal MC-related symptoms was 12 months (range, 0–300 mo). In 10 patients including only 2 patients in the pSS group, the onset of renal disease was concomitant with extrarenal manifestations associated with MC. We observed no significant differences in renal presenting syndrome between patients with pSS and those without. However, pSS patients tended to have less hypertension than patients without pSS (67% vs. 91%, respectively), less renal failure (78% vs. 91%)

TABLE 1. Clinical and Laboratory Renal Features at Presentation

Patient	Age (yr)	Sex	Hypertension	Nephrotic Range Proteinuria	Microscopic Hematuria	GFR (mL/min per 1.73 m^2)	C3 (mg/dL) (40–135)	C4 (mg/dL) (20–75)	Interval Between Renal Manifestations and First MC Symptoms (mo)	MC Etiology
1	76	F	+	+	+	31	59	1	300	Essential
2	48	F	+	+	+	28	81	1	24	Essential
3	51	F	+	−	+	48	40	2	0	Essential
4	68	M	+	+	+	30	65	19	0	Essential
5	79	F	+	+	+	29	40	2	0	Essential
6	69	M	+	−	+	39	74	8	36	Essential
7	53	M	+	+	+	23	29	6	0	Essential
8	48	F	−	−	+	52	35	2	0	Essential
9	67	F	+	+	+	59	132	0	0	Essential
10	40	M	+	+	+	83	60	6	0	Essential
11	72	M	+	+	+	33	50	1	0	B-cell lymphoma
12	63	F	+	+	+	41	70	2	60	pSS
13	72	F	+	+	+	52	48	1	0	pSS
14	55	F	+	+	+	33	52	2	166	pSS
15	62	F	+	+	+	33	97	4	60	pSS
16	55	F	−	+	+	51	69	12	72	pSS
17	64	F	−	+	+	55	58	5	0	pSS
18	59	F	+	−	+	91	80	0	60	pSS
19	63	F	−	−	+	41	49	44	60	pSS
20	32	F	+	+	+	68	70	2	60	pSS

with a mean GFR at diagnosis of 52 ± 18 vs. 33 mL/min per 1.73 m^2 (range, 23–83) and a longer median interval between renal involvement and the first symptoms of MC: 60 (range, 60–166 mo) vs. 0 (range, 0−300 mo) months.

Morphologic Analysis of Renal Biopsy Specimens

Eighteen biopsies were available for review. The biopsies contained a median of 12 (range, 3–60) glomeruli. Type I membranoproliferative glomerulonephritis was the only histologic pattern observed. It was characterized by mild to moderate mesangial hypercellularity, moderate mesangial matrix expansion, and few to several double contours of the glomerular capillary walls. The glomeruli were infiltrated by a large number of monocytes and few polymorphonuclear cells. "Protein thrombi" were detected in 82% of patients (14/17). Superimposed focal segmental glomerulosclerosis and crescentic extracapillary proliferation involving less than 5% of the glomeruli were found in 5 and 2 patients, respectively. Immunofluorescence demonstrated subendothelial and intracapillary glomerular deposits of IgG, IgM and C3, and kappa and lambda light chains. C1q and IgA were also found in 33% (6/18) and in 22% (4/18) of patients, respectively.

Interstitial fibrosis ranged from 5% to 30% (mean, 7%) and tubular atrophy from 0% to 30% (mean, 5%). Inflammation of the interstitium, composed of mononuclear cells, affected 0%–30% of the parenchyma (mean, 10%). In addition to a mild to moderate leukocyte infiltration of the interstitium, we identified lymphoid nodules in 7 patients (3 with pSS: Patients 12, 14, 15; and 4 without pSS: Patients 2, 4, 10, 11). The nodules consisted of small normal-appearing CD20+ B lymphocytes (in 80% of cases) associated with reactive CD3+ T lymphocytes (in 20% of cases). Two nodules were positive for CD23, the follicular dendritic cell marker.

Necrotizing arteritis was observed in 4 patients (22% of cases: Patients 5, 10, 19, 20) and arteriosclerosis in 13 patients (72% of cases).

Electron microscopy analysis of kidney biopsies was performed in 2 patients: Patients 11 and 14. Capillary thickening due to double contours and subendothelial dense deposits were observed. Granular, amorphous, or organized microtubular deposits were seen in the capillary wall and in intracapillary thrombi. Monocytes-macrophages containing electron-dense material were also frequently found in the capillary lumen (Figure 1).

Extrarenal Clinical and Laboratory Features

Extrarenal clinical and laboratory characteristics of patients at diagnosis of renal disease are shown in Table 2. Cutaneous features were the most frequent extrarenal clinical manifestation; purpuric rash involving the lower extremities was the most common symptom (in 80% of cases). Arthralgia and peripheral nervous system involvement were less common (in only 35% and 30% of cases, respectively). Only 1 patient had renal involvement without extrarenal signs. Patients with pSS had nervous system involvement more frequently than patients without pSS (44% vs. 18%, respectively), and cutaneous manifestations less frequently (67% vs. 91%, respectively), although these differences did not reach statistical difference.

Tests for rheumatoid factors were positive in 17 patients. Hypocomplementemia was present in 100% of cases. C4 levels were low in 19 of 20 patients— virtually undetectable in 12 of them—while C3 was normal or near normal in 18 of 20 patients. Among the 12 patients with virtually undetectable levels of C4, study for C4 deficiency was performed in 6 patients (Patients 6, 7, 8, 9, 16, 17) and was negative for all patients. Moreover, 3/17 (17%) patients normalized complement level

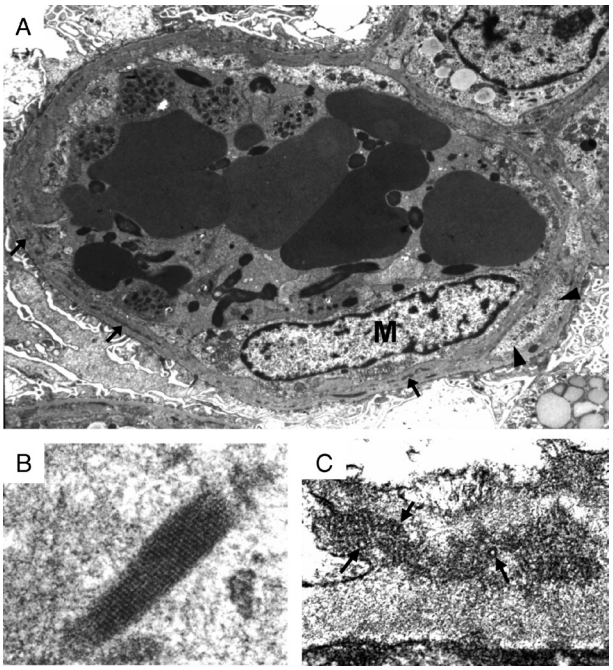

FIGURE 1. Ultrastructural aspect of renal biopsies. **A.** Irregular thickening of the capillary wall with dense deposits (arrows) and double contours (arrowheads). Macrophages (M) containing electron-dense material are filling the capillary lumen (Patient 14) (original magnification × 25,000). **B.** Intracapillary organized thrombi with a ''gridlike'' crystalline appearance on transversal section (Patient 14) (original magnification × 50,000). **C.** Subendothelial deposits with organized microtubular and annular (arrows) aspects (Patient 11) (original magnification × 50,000).

after treatment. ANA titers were positive in 13 patients. Autoantibodies were significantly more frequent in pSS patients.

Treatment and Outcome

Treatment and outcome for individual patients are depicted in Table 3. The median follow-up was 48 months (range, 3–264 mo). Three patients were untreated, 1 because of early death (Patient 14) and the other 2 due to spontaneous remission (Patients 4 and 6). Most patients were given steroids (n = 15) as first-line therapy, alone (n = 5) or associated with alkylating agents (n = 10), mainly cyclophosphamide (n = 9) or azathioprine (n = 1). Plasmapheresis was associated with steroids and cyclophosphamide in 1 patient and was the only treatment for another (Patient 19). One patient with B-cell lymphoma received chloraminophene alone (Patient 11). Initial remission of the nephropathy was observed in 16 of 17 patients, and remission of extrarenal symptoms occurred in 13 patients. Despite clinical remission, the C4 complement fraction was always low and cryoglobulinemia disappeared in only 4 cases (20% of patients).

Relapses were observed in 12 patients. They included 7 renal relapses associated or not with extrarenal relapses and 5 isolated extrarenal relapses. The median number of relapses was 1 (range, 0–3) and the median time to relapse was 18 months (range, 1–60 mo). All relapses were treated with steroids (n = 10), associated with cyclophosphamide (n = 3), plasmapheresis (n = 3), or azathioprine (n = 3). Two patients received interferon alpha or anti-CD20 as third-line therapy. Monoclonal antibody anti-CD20 was used to treat B lymphoma concomitant with an MC relapse (purpura, Patient 9).

The median GFR at the end of follow-up was 53 (± 25) mL/min per 1.73 m^2. Doubling of serum creatinine levels or end-stage renal disease occurred in 5 patients (3 patients with pSS and 2 patients without pSS). Proteinuria reduction was observed in 70% of patients. Complement levels normalized in only 17% of all patients, and MC disappeared in 20% of all patients.

One patient from the pSS group and 3 from the non-pSS group developed B-cell lymphoma after a median follow-up of 78 months (range, 36–184 mo). Histopathological evaluation revealed nodal marginal B-cell lymphoma in Patients 2 and 9 and lymphoplasmacytoid lymphoma in Patient 10, all from the non-pSS group; and Epstein-Barr virus-associated large B-cell lymphoma in Patient 20 from the pSS group. The lymphoma was discovered concomitantly with renal or extrarenal MC relapse in Patients 2 and 9. All patients displayed hematologic remission at the end of follow-up.

Eight of the 20 patients (40%) died during follow-up including 5 patients in the pSS group (56%) and 3 patients in the non-pSS group (27%). Causes of death were cerebral hemorrhage in 3 cases, gastrointestinal bleed in 1, infectious disease in 1, diffuse vasculitis in 1 case, and were undetermined in 2 cases. The median interval between the diagnosis of renal disease and death was 51 months (range, 3–264 mo).

DISCUSSION

Renal involvement in patients with MC and HCV infection-related MC has been well documented in large series of patients. In contrast, there is little information, derived only from case

TABLE 2. Clinical, Laboratory, and Immunologic Features

	All Patients (n = 20) % (No.)	pSS Group (n = 9) % (No.)	Non-pSS Group (n = 11) % (No.)	p Value
Clinical features				
Cutaneous	80% (16)	67% (6)	91% (10)	0.28
Articular	35% (7)	33% (3)	36% (4)	1
Peripheral nerve	30% (6)	44% (4)	18% (2)	0.34
Liver	30% (6)	22% (2)	36% (4)	0.64
Lung	15% (3)	11% (1)	18% (2)	1
Gastrointestinal	15% (3)	11% (1)	18% (2)	1
Lymphadenopathy	30% (6)	22% (2)	36% (4)	0.64
Laboratory features				
Anemia	75% (15)	78% (7)	73% (8)	1
Thrombocytopenia	10% (2)	11% (1)	9% (1)	1
Lymphopenia	40% (8)	44% (4)	36% (4)	1
Immunologic features				
Rheumatoid factor	85% (17)	89% (8)	82% (9)	1
ANA	65% (13)	100% (9)	36% (4)	0.0047
Ro/SS-A antibody	35% (7)	67% (6)	9% (1)	0.01
La/SS-B antibody	10% (2)	11% (1)	9% (1)	1
U1-RNP antibody	15% (3)	22% (2)	9% (1)	0.56
Monoclonal gammopathy	85% (17)	89% (8)	82% (9)	1
Hypogammaglobulinemia	80% (16)	78% (7)	82% (9)	1
Low C3	30% (6)	22% (2)	36% (4)	0.38
Low C4	95% (19)	89% (8)	100% (11)	0.69

Abbreviations: ANA = antinuclear antibodies, U1-RNP = antiribonucleoprotein antibodies.

TABLE 3. Treatment and Outcome

Patient	Age (yr)	Etiology	Plasma-pheresis	Steroids	Cyclophosphamide	Fludarabine	Chlorami-nophene	Azathioprine	IFN-α	Anti-CD20	Relapse (no.)	GFR at Diagnosis (mL/min per 1.73 m^2)	At End of Follow-Up: GFR (mL/min per 1.73 m^2)	At End of Follow-Up: Proteinuria (g/d)	Death
1	76	Essential	−	+	+	−	−	−	−	−	0	31	21	1.5	+
2	48	Essential	−	+	+	+	−	−	−	−	2	28	62	2.5	−
3	51	Essential	−	+	+	−	−	−	+	−	3	48	62	0.5	−
4	68	Essential	−	−	−	−	−	−	−	−	1	30	25	1.5	+
5	79	Essential	−	+	−	−	−	−	−	−	2	29	33	0	−
6	69	Essential	−	−	−	−	−	−	−	−	0	39	68	0	−
7	53	Essential	−	+	−	−	−	−	−	−	0	23	71	0	−
8	48	Essential	+	+	+	−	−	+	−	+	2	52	69	0	−
9	67	Essential	−	+	+	−	−	−	−	−	2	59	48	NA	+
10	40	Essential	−	+	+	+	−	−	−	−	1	83	87	0	−
11	72	B-cell lymphoma	−	−	−	−	+	−	−	−	0	33	68	1	−
12	63	pSS	−	+	−	−	+	+	−	−	2	41	17	NA	−
13	72	pSS	+	+	+	−	−	−	−	−	2	52	7	NA	+
14	55	pSS	−	−	−	−	−	−	−	−	0	33	NA	NA	+
15	62	pSS	−	+	+	−	−	−	−	−	0	33	85	1.5	−
16	55	pSS	−	+	+	−	−	−	−	−	0	51	75	0	−
17	64	pSS	+	+	+	−	−	−	−	−	1	55	74	NA	+
18	59	pSS	+	+	+	−	−	−	−	−	3	91	39	0	+
19	63	pSS	+	−	−	−	−	−	−	−	0	41	41	1.4	+
20	32	pSS	−	+	+	−	−	+	−	−	3	68	73	3	−

Abbreviations: IFN = interferon, NA = not available.

reports and small series, on the incidence and characteristics of kidney disease in the patients without HCV infection.[5,11,32] Therefore, we carried out a French multicenter retrospective study of 20 patients with MC-related kidney involvement but without HCV infection, to our knowledge representing the largest series available so far. Our aim was to define the clinical and pathological spectrum of the renal disease comparatively with HCV-related MC, to identify the etiology of MC, and to analyze the response to therapy and outcome. Main results are the high prevalence of pSS, the finding of lymphoid nodules in the biopsy, and the high percentage of lymphoma in patients without pSS during follow-up.

We reviewed the main series of MC-related kidney disease as yet reported in order to delineate potential singularities of non-HCV MC (Table 4). In 1995, Tarantino et al[35] retrospectively described the clinical outcome of 105 patients with essential MC and renal involvement. Anti-HCV antibodies were retrospectively searched for in 34 patients, 85% of whom had HCV infection.[35] Although, anti-HCV antibodies were searched for in only 30% of the patients (34 of 105 patients), the study suggests that HCV is a major cause of MC-related kidney disease. In 2002, Beddhu et al[5] reported 17 cases of renal MC. Eleven of these 17 patients had MC associated with HCV, and 6 had MC without HCV. There was no difference in renal presentation between the 2 patient groups, although the outcome—defined as doubling of creatinine, normalization of complement, and disappearance of cryoglobulin—tended to be better in non-HCV MC. In a 2007 retrospective study, Roccatello et al[30] reported clinical, serologic, and morphologic data from 146 patients with cryoglobulinemic glomerulonephritis, 88% of whom had HCV-associated MC. There were no findings specific to patients without HCV in that series.[30]

In the current study, MC was related to pSS in 45% of patients, while MC was classified as essential in 50% of cases and was related to hematologic disease in only 1 patient. Our results differ from those of a previously published study,[32] in which non-HCV-related type II MC was related to connective tissue diseases (mostly systemic lupus erythematosus) in 33% of patients, hematologic diseases (mainly non-Hodgkin lymphoma) in 31%, and infectious diseases in 9%. However, kidney involvement was not a criterion of inclusion in the latter study.

The leading renal manifestations presented by our patients were nephrotic range proteinuria with microscopic hematuria and renal insufficiency. In HCV-associated MC, the presence of renal syndrome appears to be less serious, as nephrotic proteinuria occurs in only 25% of cases and renal insufficiency in only 40% of cases.[14,30,35]

All patients exhibited membranoproliferative glomerulonephritis, which is also the main nephropathy observed in patients with HCV infection (see Table 4). However, an unexpected finding was the presence of interstitial lymphocytic nodule infiltration in 7 patients (3 from the pSS group and 4 with essential MC). Interstitial lymphocytic infiltration has been reported previously in patients with HCV-related MC, but without nodular organization.[5,14,30] The nodules were mainly composed of B-lymphocytes (80%). In addition, follicular dendritic T cells (CD23+) were present in 2 patients. Although the origin of these B-cell nodules is unclear, they may result from the occurrence of lymphoid neogenesis and/or ectopic germinal center formation, as previously described in salivary glands from patients with pSS and in renal transplant humoral rejection.[33,38] Ultrastructural analysis available in 2 patients showed usual aspects of cryoglobulinemic glomerulonephritis, with some deposits exhibiting a typical microtubular organization.

TABLE 4. Pattern of Renal Involvement in Patients With MC With or Without HCV Infection, Previous and Present Reports

	Beddhu 2002[5] (n = 11)	Roccatello 2007[30] (n = 146)	Beddhu 2002[5] (n = 6)	Present Report (n = 20)
HCV-positive patients	11/11 (100%)	128/146 (88%)	0 (0%)	0 (0%)
Demographics				
Mean age at diagnosis (yr)	43.1 ± 1.2	152.2 ± 13	54.7 ± 3.2	60 ± 12
Women	18%	56.8%	66%	60%
Clinical renal disease				
Hypertension	75%	55%	NA	80%
Renal failure	72%	58%	66%	85%
Microscopic hematuria	100%	88%	100%	100%
Nephrotic range proteinuria	45%	21%	33%	75%
GFR (mL/min per 1.73 m^2)	NA	NA	NA	46 (18)
Interval between renal involvement and first MC symptoms (mo)	NA	31.2 ± 52.8	NA	12 (range, 0–300)
Type II cryoglobulin	54%	69%	33%	100%
Histologic renal disease				
MPGN	63%	87%	>50%	100%
Evolution				
Relapse (median number, range)	NA	1 (1–4)	NA	1 (0–3)
GFR (mL/min per 1.73 m^2)	NA	NA	NA	53 ± 25
Disappearance of urinary abnormalities	45%	NA	66%	70%
Creatinine doubled	45%	NA	0%	20%
Normalization of complement levels	18%	NA	50%	15%
Disappearance of cryoglobulin	18%	NA	50%	40%
Death	NA	21%	NA	40%

Abbreviations: MPGN = membranoproliferative glomerulonephritis, NA = not available.

Type II MC was identified in 100% of patients and was invariably formed by polyclonal IgG and monoclonal IgMκ. In HCV infection, the prevalence of type II cryoglobulins, with predominant IgMκ rheumatoid factor, was significantly greater in patients with nephrotic syndrome than in patients without renal involvement (74% vs. 27%, respectively).[12,30] This suggests that clonal restriction is likely to be a determinant factor of renal involvement. Similarly, in patients with pSS, membranoproliferative glomerulonephritis seems to occur exclusively in those with type II MC and the IgMκ component, again suggesting monoclonal B-cell activation.[17] In the absence of clinical or histopathological features of lymphoproliferative disorders, bone marrow B-cell clonal expansion was shown to be associated with nephritis in MC syndrome.[27] These data, which tend to relate the occurrence of nephritis to that of B-cell clonal proliferation (whose IgMκ is a marker), have important therapeutic implications because they favor the use of anti-B cell therapy in patients with MC-related kidney disease with or without HCV infection.[8] They are corroborated by the efficacy of anti-CD20 monoclonal antibody treatment for severe MC manifestations.[37]

Most of our patients presented with a typical pattern of low or undetectable C4 and normal or relatively normal C3 serum levels. Familial cryoglobulinemia and C4 deficiency were previously described in 1 family.[6] We did not observe C4 deficiency in the tested patients in our series; none of the patients exhibited a familial history of MC.

Spontaneous remission has been described previously in patients with HCV infection, in less than 10% of patients.[14] In this study, spontaneous remission occurred in 2 patients. In MC patients with HCV, remission of renal disease has been observed despite the persistence of MC and complement activation in many patients.[14,35] Similarly, in the current series, disappearance of MC and normalization of complement levels occurred in 40% and 15%, respectively. These data suggest that in addition to cryoglobulin deposition, downstream events including monocyte infiltration and release of inflammatory mediators play a role in full-blown expression of the disease.

As previously reported[31] and observed in the current study, response to chloraminophene and fludarabine therapy was optimal in 3 patients (including relapse concomitant with B-cell lymphoma in 2 of them). Previous studies have suggested that rituximab was at least as effective as cyclophosphamide in treating membranoproliferative glomerulonephritis in MC patients with HCV infection, by blocking cryoglobulin production.[8,16,22,28,29,34,37,43] Only 1 of our patients received rituximab, because most patients were seen before availability of the monoclonal antibody. Rituximab was used as first-line treatment in this setting. Whether rituximab would have an equivalent efficacy in patients without HCV infection remains to be determined, but the use of this therapy appears to be a reasonable strategy for these patients.

As previously reported[14,30] in patients with HCV, the overall patient survival rate was almost 60%, and the causes of death were mainly related to extrarenal complications. In the current series, 4 patients developed B-cell lymphoma during follow-up. Unexpectedly, this complication was observed in 3 patients with essential MC and only 1 with pSS-related MC. The high rate of lymphoma is in keeping with a 2006 report[32] indicating that MC patients without HCV had a 4-fold greater risk of developing B-cell non-Hodgkin lymphoma. By contrast, a lower incidence of B-cell lymphoma has been reported in patients with MC related to HCV, after a mean follow-up of 8.8 ± 8.1 years.[15] These results strongly suggest that essential MC may indicate a pre-B-cell lymphoma state, and thus should be supervised.[27,40]

In conclusion, we report what is to our knowledge the first large series of patients with renal involvement related to MC without HCV infection. The occurrence of overt B-cell lymphoma during follow-up emphasizes the need for repetitive clinical evaluation of patients. Although the response to immunosuppressive therapy was favorable in most cases, prospective controlled randomized studies are required to establish evidence-based guidelines for treating MC-related glomerulopathies unrelated to HCV. Because of the presence of CD20+ B-cell lymphoid nodules in the kidney interstitium and the efficacy of anti-CD20 monoclonal antibody in HCV-related MC, it is reasonable to anticipate that anti-CD20 antibody would be a first-line option in these patients.

ACKNOWLEDGMENT

We thank M. C. Verpont (Electron Microscopy Platform, Federative Institute of Research 65 and INSERM UMR S702, Tenon Hospital) for technical assistance.

REFERENCES

1. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med*. 1992;327:1490–1495.
2. Alarcon-Segovia D, Amigo MC, Reyes PA. Connective tissue disease features after thallium poisoning. *J Rheumatol*. 1989;16:171–174.
3. Alric L, Plaisier E, Thebault S, Peron JM, Rostaing L, Pourrat J, Ronco P, Piette JC, Cacoub P. Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. *Am J Kidney Dis*. 2004;43:617–623.
4. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*. 1988;31:315–324.
5. Beddhu S, Bastacky S, Johnson JP. The clinical and morphologic spectrum of renal cryoglobulinemia. *Medicine (Baltimore)*. 2002;81:398–409.
6. Berliner S, Weinberger A, Zamir R, Hazaz B, Pinkhas J. Familial cryoglobulinemia and C4 deficiency. *Scand J Rheumatol*. 1984;13:151–154.
7. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med*. 1974;57:775–788.
8. Cacoub P, Delluc A, Saadoun D, Landau DA, Sene D. Anti-CD20 monoclonal antibody (rituximab) treatment for cryoglobulinemic vasculitis: where do we stand? *Ann Rheum Dis*. 2008;67:283–287.
9. Cacoub P, Fabiani FL, Musset L, Perrin M, Frangeul L, Leger JM, Huraux JM, Piette JC, Godeau P. Mixed cryoglobulinemia and hepatitis C virus. *Am J Med*. 1994;96:124–132.
10. Cacoub P, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, Yamamoto AM, Camproux AC, Hausfater P, Musset L, Veyssier P, Raguin G, Piette JC. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. *Medicine (Baltimore)*. 2000;79:47–56.
11. Cohen Tervaert JW, Van Paassen P, Damoiseaux J. Type II cryoglobulinemia is not associated with hepatitis C infection: the Dutch experience. *Ann N Y Acad Sci*. 2007;1107:251–258.
12. Cordonnier D, Vialtel P, Renversez JC, Chenais F, Favre M, Tournoud A, Barioz C, Bayle F, Dechelette E, Denis MC, Couderc P. Renal diseases in 18 patients with mixed type II IgM-IgG cryoglobulinemia: monoclonal lymphoid infiltration (2 cases) and membranoproliferative

glomerulonephritis (14 cases). *Adv Nephrol Necker Hosp*. 1983;12:177–204.

13. Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease. *Lancet*. 1990;335:1078–1080.
14. D'Amico G. Renal involvement in hepatitis C infection: cryoglobulinemic glomerulonephritis. *Kidney Int*. 1998;54:650–671.
15. Ferri C, Sebastiani M, Giuggioli D, Cazzato M, Longombardo G, Antonelli A, Puccini R, Michelassi C, Zignego AL. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum*. 2004;33:355–374.
16. Ghijsels E, Lerut E, Vanrenterghem Y, Kuypers D. Anti-CD20 monoclonal antibody (rituximab) treatment for hepatitis C-negative therapy-resistant essential mixed cryoglobulinemia with renal and cardiac failure. *Am J Kidney Dis*. 2004;43:e34–e38.
17. Goules A, Masouridi S, Tzioufas AG, Ioannidis JP, Skopouli FN, Moutsopoulos HM. Clinically significant and biopsy-documented renal involvement in primary Sjogren syndrome. *Medicine (Baltimore)*. 2000;79:241–249.
18. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting—Airlie House, Virginia, November 1997. *J Clin Oncol*. 1999;17:3835–3849.
19. Herzenberg AM, Telford JJ, De Luca LG, Holden JK, Magil AB. Thrombotic microangiopathy associated with cryoglobulinemic membranoproliferative glomerulonephritis and hepatitis C. *Am J Kidney Dis*. 1998;31:521–526.
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1997;40:1725.
21. Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, Couser WG, Corey L, Wener MH, Alpers CE, Willson R. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med*. 1993;328:465–470.
22. Koukoulaki M, Abeygunasekara SC, Smith KG, Jayne DR. Remission of refractory hepatitis C-negative cryoglobulinaemic vasculitis after rituximab and infliximab. *Nephrol Dial Transplant*. 2005;20:213–216.
23. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130:461–470.
24. Mascia MT, Ferrari D, Campioli D, Sandri G, Mussini C, Ferri C. Non HCV-related mixed cryoglobulinemia. *Dig Liver Dis*. 2007; 39(Suppl 1):S61–S64.
25. Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia—a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med*. 1966;40:837–856.
26. Messiaen T, Deret S, Mougenot B, Bridoux F, Dequiedt P, Dion JJ, Makdassi R, Meeus F, Pourrat J, Touchard G, Vanhille P, Zaoui P, Aucouturier P, Ronco PM. Adult Fanconi syndrome secondary to light chain gammopathy. Clinicopathologic heterogeneity and unusual features in 11 patients. *Medicine (Baltimore)*. 2000;79:135–154.
27. Quartuccio L, Fabris M, Salvin S, Isola M, Soldano F, Falleti E, Beltrami CA, De Re V, De Vita S. Bone marrow B-cell clonal expansion in type II mixed cryoglobulinaemia: association with nephritis. *Rheumatology (Oxford)*. 2007;46:1657–1661.
28. Quartuccio L, Soardo G, Romano G, Zaja F, Scott CA, De Marchi G, Fabris M, Ferraccioli G, De Vita S. Rituximab treatment for glomerulonephritis in HCV-associated mixed cryoglobulinaemia: efficacy and safety in the absence of steroids. *Rheumatology (Oxford)*. 2006;45:842–846.
29. Roccatello D, Baldovino S, Rossi D, Mansouri M, Naretto C, Gennaro M, Cavallo R, Alpa M, Costanzo P, Giachino O, Mazzucco G, Sena LM. Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. *Nephrol Dial Transplant*. 2004;19:3054–3061.
30. Roccatello D, Fornasieri A, Giachino O, Rossi D, Beltrame A, Banfi G, Confalonieri R, Tarantino A, Pasquali S, Amoroso A, Savoldi S, Colombo V, Manno C, Ponzetto A, Moriconi L, Pani A, Rustichelli R, Di Belgiojoso GB, Comotti C, Quarenghi MI. Multicenter study on hepatitis C virus-related cryoglobulinemic glomerulonephritis. *Am J Kidney Dis*. 2007;49:69–82.
31. Rosenstock JL, Stern L, Sherman WH, Appel GB, Radhakrishnan J. Fludarabine treatment of cryoglobulinemic glomerulonephritis. *Am J Kidney Dis*. 2002;40:644–648.
32. Saadoun D, Sellam J, Ghillani-Dalbin P, Crecel R, Piette JC, Cacoub P. Increased risks of lymphoma and death among patients with non-hepatitis C virus-related mixed cryoglobulinemia. *Arch Intern Med*. 2006;166:2101–2108.
33. Salomonsson S, Jonsson MV, Skarstein K, Brokstad KA, Hjelmstrom P, Wahren-Herlenius M, Jonsson R. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjogren's syndrome. *Arthritis Rheum*. 2003;48:3187–3201.
34. Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. *Blood*. 2003;101:3818–3826.
35. Tarantino A, Campise M, Banfi G, Confalonieri R, Bucci A, Montoli A, Colasanti G, Damilano I, D'Amico G, Minetti L, Ponticelli C. Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. *Kidney Int*. 1995;47:618–623.
36. Tedeschi A, Barate C, Minola E, Morra E. Cryoglobulinemia. *Blood Rev.* 2007;21:183–200.
37. Terrier B, Saadoun D, Sene D, Sellam J, Perard L, Coppere B, Karras A, Blanc F, Buchler M, Plaisier E, Ghillani P, Rosenzwajg M, Cacoub P. Efficacy and tolerability of rituximab with or without PEGylated interferon alfa-2b plus ribavirin in severe hepatitis C virus-related vasculitis: a long-term followup study of thirty-two patients. *Arthritis Rheum*. 2009;60:2531–2540.
38. Thaunat O, Field AC, Dai J, Louedec L, Patey N, Bloch MF, Mandet C, Belair MF, Bruneval P, Meilhac O, Bellon B, Joly E, Michel JB, Nicoletti A. Lymphoid neogenesis in chronic rejection: evidence for a local humoral alloimmune response. *Proc Natl Acad Sci U S A*. 2005;102:14723–14728.
39. Trejo O, Ramos-Casals M, Garcia-Carrasco M, Yague J, Jimenez S, de la Red G, Cervera R, Font J, Ingelmo M. Cryoglobulinemia: study of etiologic factors and clinical and immunologic features in 443 patients from a single center. *Medicine (Baltimore)*. 2001;80:252–262.
40. Vallat L, Benhamou Y, Gutierrez M, Ghillani P, Hercher C, Thibault V, Charlotte F, Piette JC, Poynard T, Merle-Beral H, Davi F, Cacoub P. Clonal B cell populations in the blood and liver of patients with chronic hepatitis C virus infection. *Arthritis Rheum*. 2004;50:3668–3678.
41. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002;61:554–558.
42. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC, Brey R, Derksen R, Harris EN, Hughes GR, Triplett DA, Khamashta MA. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum*. 1999;42:1309–1311.
43. Zaja F, De Vita S, Mazzaro C, Sacco S, Damiani D, De Marchi G, Michelutti A, Baccarani M, Fanin R, Ferraccioli G. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood*. 2003;101:3827–3834.

