

Clinical Spectrum, Treatment, and Outcome of Patients with Type II Mixed Cryoglobulinemia without Evidence of Hepatitis C Infection

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ABSTRACT. Objective. The clinical spectrum, etiologies, and best therapeutic approaches of type II mixed cryoglobulinemia (MC) not associated with hepatitis C virus (HCV) infection have been poorly described to date. We studied the clinical presentation and outcome of patients with type II MC with no evidence of HCV.

Methods. This was a multicenter retrospective study on the clinical presentation and outcome of patients with type II MC without evidence of HCV infection. Only patients with symptomatic MC were included.

Results. Thirty-three patients were included (median followup 67.2 mo). Extensive investigations for associated diseases were performed at presentation. MC was related to an autoimmune disease in 14 patients, to a lymphoid malignancy in 4 patients, and to an infectious disease in 2 patients, while MC was classified as essential (primary) in 13. Essential MC tended to be more severe than secondary disease with, in particular, more frequent renal and peripheral nerve involvement. Most patients were treated with steroid with or without immunosuppressive agents, mainly cyclophosphamide. These treatments were unable to induce sustained remission. One patient was successfully treated with lenalidomide. Seven patients with nonmalignant MC were treated with rituximab; 2 had a sustained complete remission, 3 improved greatly but relapsed within 5 months, and 2 experienced a disease flare.

Conclusion. An important proportion of non HCV-related type II MC remains essential. Efforts should be made to find other etiologies than HCV, because treatments with steroid and immunosuppressants are not satisfactory, especially in severe forms. In these situations anti-CD20 therapy may present the best option but should be used with caution. New agents such as lenalidomide remain to be evaluated. (J Rheumatol First Release Jan 15 2011; doi:10.3899/jrheum.100898)

Key Indexing Terms:

CRYOGLOBULINEMIA VASCULITIS RITUXIMAB RHEUMATOID FACTOR

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Cryoglobulins are proteins that undergo precipitation upon refrigeration of serum and redissolve on warming. They are typically composed of immunoglobulins and complement components. Cryoglobulins are commonly classified according to Brouet, *et al*¹. Type I are composed of isolated monoclonal immunoglobulins (usually IgM or IgG) and are frequently associated with myeloma and Waldenström macroglobulinemia. Type II and type III, designated "mixed cryoglobulinemia" (MC), are immunocomplexes formed of a monoclonal (type II) or polyclonal (type III) rheumatoid factors (RF) and polyclonal IgG. Type II and type III MC are commonly associated with clinical situations that generate large amounts of IgG and immune complexes, including chronic autoimmune diseases such as systemic lupus erythematosus (SLE) or Sjögren's syndrome, or infections such as hepatitis C virus (HCV) or HIV infections². Lymphoproliferative disorders are also associated, but more rarely. MC not associated with those disorders has been defined as essential (primary) MC.

Before the early 1990s, most of type II MC were essential. In this period it was established that most of these essential MC were associated with chronic HCV infection^{3,4,5,6,7,8}. However, this association has been reported mainly in countries with high prevalence of HCV. In northern France and northern European countries where lower prevalence rates of HCV infection have been reported, many patients with type II MC may not have HCV infection⁹.

The clinical spectrum and treatment of type II MC associated with HCV infection have been extensively studied. Most patients benefit from antiviral therapy combining interferon and ribavirin^{10,11,12}. In situations where antiviral therapy is contraindicated, not tolerated, or ineffective, B cell depletion using rituximab seems to represent an effective alternative^{13,14,15}.

By contrast, the clinical features, etiologies, and above all the best treatments of type II MC not associated with HCV infection have been poorly described. We conducted a multicenter study on the clinical presentation and outcome of 33 patients with type II MC without evidence of HCV infection.

MATERIALS AND METHODS

Patients. This retrospective study was conducted in 8 French departments of internal medicine located in the northeast part of the country. Inclusion criteria included diagnosis of type II MC and the absence of documented HCV infection (negative serology and viral load by polymerase chain reaction). Only patients with symptomatic disease were included; symptoms attributable to MC included cryosymptoms (mainly Raynaud phenomenon and cold-induced skin necrosis) and signs of MC vasculitis. Patients were included only if data on treatment and followup were available. For each patient, the following data were collected: sex, age at diagnosis, outcome, possible cause (infections, connective tissue disease, hematologic disorder), and clinical manifestations (if not explainable by another cause): cutaneous involvement (Raynaud, purpura, ulcers), rheumatologic involvement (arthralgias, arthritis), neurologic involvement (peripheral neuropathy including polyneuropathy and mononeuritis multiplex, central nervous system evidence of vasculitis), gastrointestinal (GI) involvement (blood loss from GI tract), renal involvement (proteinuria > 0.5 g/24 h and hematuria > 10,000 red blood cells/ml), and renal insufficiency (glomerular filtration rate < 60 ml/min per 1.73 m²). We defined MC as severe when there was at least one extracutaneous significant organ involvement: renal involvement required proteinuria > 1 g/24 h and/or renal insufficiency; peripheral neuropathy was required to be clinically significant (Patients 1, 2, 6, and 10 had mononeuropathy multiplex, the others combined sensorimotor disease).

Laboratory studies. Rheumatoid factor activity was detected by latex and Waaler-Rose tests or by ELISA. Immunochemical typing of MC was performed by electrophoresis and immunoelectrophoresis or immunofixation. Cryoglobulins were classified according to the Brouet, *et al* criteria¹. Antinuclear antibodies were determined by indirect immunofluorescence on Hep2 cells.

Determination of associated diseases. Underlying disorders investigated were lymphoproliferative diseases, defined autoimmune disease, and infections. Autoimmune diseases were diagnosed using standard criteria. The diagnosis of lymphoproliferative disease was made on the basis of nodal, extranodal, or bone marrow infiltration with pathological features compatible with the World Health Organization classification of neoplastic disease¹⁶. Infections were diagnosed using standard methods. Considering that patients with essential MC may have had an unrecognized chronic infection

or inflammatory disease we checked for chronic unexplained elevated C-reactive protein (CRP).

Treatment efficacy. Since several patients had more than one treatment, the responses were analyzed by comparing the variables before and after each therapeutic procedure or at the end of followup if the treatment was continuing at that time. The first-line therapy for severe forms of MC often includes plasmapheresis, which is only suspensive. To interpret the responses to drug therapies correctly, they were analyzed only at periods when patients were not undergoing plasmapheresis. In addition, each treatment sequence had to be long enough to be evaluated: at least 1 month for steroids and 2 months for immunosuppressive drugs. There is no activity scoring system for MC and no consensus recommendations. Clinical response was defined by the clinician in charge of the patient by analyzing the courses of the main clinical signs attributable to MC. We defined complete response as disappearance of all clinical manifestations of active disease (MC vasculitis and cryosymptoms) and disappearance of serum cryoglobulin; in particular, cutaneous involvement (absence of purpura and ulcers), peripheral neuropathy (clinical or electrophysiological improvement), and renal involvement (normalization of glomerular filtration, no proteinuria and hematuria). A partial response was defined by incomplete improvement in the baseline clinical manifestations and by persistence of serum cryoglobulins. All other patients were classified as nonresponders. Relapse was defined by the reappearance (or for patients with partial response, the worsening) of clinical manifestations of active disease. Severe adverse events (secondary events that led to hospitalization and/or interruption of immunosuppressant) were recorded.

Statistical analysis. Quantitative data (excepting followup) are presented as means (± 1 standard deviation) and compared using unpaired t test. Qualitative data are presented as percentages and were compared using Fisher's exact test. A value of p < 0.05 was considered significant.

RESULTS

Based on our criteria, 33 patients were studied. In all cases, typing revealed type II MC including a monoclonal immunoglobulin associated with polyclonal IgG. The monoclonal immunoglobulin was IgM_κ in 26 cases, IgM_λ in 4 cases, and IgG_κ in 3 cases.

Etiology of MC. As shown in Table 1 no associated disease could be identified in 13 patients (39%); they were classi-

Table 1. Clinical conditions associated with cryoglobulinemia. For each patient an associated disease was searched for; in particular all patients had a whole-body scan, a bone marrow biopsy, and extensive microbiological screening (including for cryptic infections such as endocarditis or dental infection). No patient was infected by HIV.

Associated Disease	N = 33 (%)
Autoimmune disease	14 (42.4)
Primary Sjögren's syndrome	7
Systemic lupus	4
Rheumatoid arthritis	2
Celiac disease	1
Infectious disease	2 (6.1)
Chronic hepatitis B	1
Parvovirus B19	1
Hematologic malignancy	4 (12.1)
Angioimmunoblastic T cell lymphoma	1
Mucosa-associated lymphoma tissue lymphoma	1
Non-Hodgkin B cell lymphoma	2
No identified disease: "essential"	
mixed cryoglobulinemia	13 (39.4)

fied as having true “essential” (primary) type II MC. Fourteen patients had an autoimmune disease, the most frequent being primary Sjögren’s syndrome. Two patients had an infectious disease; 1 had chronic hepatitis B and the second developed MC during a severe infection with parvovirus B19. Four patients had a hematologic malignancy.

Demographic data and clinical features. The comparative characteristics of patients with secondary and essential MC are presented in Table 2. No differences in age at diagnosis were identified between the 2 groups. The female to male ratio was slightly higher in the “secondary” group due to the high frequencies of Sjögren’s syndrome and SLE. Cutaneous features were the most frequent clinical manifestations: purpuric rash of the lower limbs was the most common symptom in both groups (close to 90%). Interestingly, peripheral neuropathy and renal involvement were significantly more frequent in the essential than in the secondary groups ($p = 0.001$ and 0.026 , respectively). Indeed, patients with true essential MC tended to have more severe disease than patients with secondary MC (Tables 2 and 3), although cryoglobulin concentrations did not differ significantly (2.8 ± 3.1 g/l and 2.5 ± 2.9 g/l, respectively). The 7 patients with renal insufficiency had renal biopsy results showing diffuse membranoproliferative glomerulonephritis¹⁷ in all cases.

Tests for RF were positive in 29 patients. They were negative in the 3 patients with an IgG because common tests do not detect RF of this isotype. C4 levels were low in 100% of cases. Antinuclear antibodies were present at low level (1/320) in only one patient with essential MC. Only one out

of the 13 patients had a chronic unexplained inflammatory syndrome. This patient underwent extensive microbiological screening as well as a bone marrow biopsy, which was uninformative. No patient was infected with HIV.

Treatment and outcome. MC vasculitis associated with lymphoma usually improves with treatment of the malignancy. This was the case of our 4 patients. The patient with T cell lymphoma was treated with CHOP (combination of cyclophosphamide, doxorubicin, vincristine, prednisone), but died rapidly from sepsis. The responses to therapies of patients with nonmalignant MC are presented in Table 4 and in Table 5 for those who were treated with rituximab. Significant side effects are summarized in Table 6. The median followup was 67.2 months (range 3–240 mo). Two patients were untreated: one indolent form associated with Sjögren’s syndrome because of spontaneous remission, one with limited purpura who was lost after 3 months’ followup.

Most patients were treated with corticosteroid as first-line therapy. A complete response was never observed. A significant improvement was observed in most patients, but all relapsed during corticosteroid tapering. In 21 patients, immunosuppressants were added to corticosteroid either after early relapse (11 cases) or immediately in severe cases. Cyclophosphamide (CYC) was used 17 times. A complete response was never observed and early relapse was the rule. Mycophenolate mofetil (MMF) and azathioprine (AZA) were not more successful. Methotrexate was used in a patient with rheumatoid arthritis, who had a complete remission. The patient with chronic hepatitis B was treated

Table 2. Characteristics of patients according to type of “essential” or “secondary” mixed cryoglobulinemia (MC). Data are numbers (%) or mean \pm SD. Comparison between variables assessed by Fisher exact test. Comparison of means (age at diagnosis and cryoglobulin level) assessed by unpaired t test.

Characteristics	Essential Type II MC, N = 13	Secondary Type II MC N = 20	p
Age at diagnosis, yrs	62.8 \pm 12.2	57.3 \pm 13	0.84
Female/male	6/7	15/5	0.14
Severe disease*	10 (77)	6 (30)	0.013
Clinical manifestations			
Raynaud phenomenon	5 (38.5)	5 (25)	0.46
Purpura	12 (92.3)	16 (80)	0.62
Skin ulcers	5 (38.5)	2 (10)	0.083
Arthralgia	5 (38.5)	11 (55)	0.48
Arthritis	1 (7.7)	2 (10)	0.99
Renal involvement	8 (61.5)	4 (20)	0.026
Renal insufficiency	4 (30.7)	3 (15)	0.39
Peripheral neuropathy	11 (84.6)	4 (20)	0.001
Laboratory features			
Cryoglobulin level, g/l	2.8 \pm 3.1	2.5 \pm 2.9	0.77
Low C4 complement level	13 (100)	20 (100)	NA
Rheumatoid factor activity	13 (100)	16 (80)	0.13
Antinuclear antibodies	1 (7.7)	10 (50)	0.022
Chronic elevated CRP	1 (7.7)	9 (45)	0.049

* Clinically overt involvement of at least one extracutaneous organ (see text). CRP: C-reactive protein; NA: not applicable.

Table 3. Severe forms of mixed cryoglobulinemia. Severe form indicates at least one significant extracutaneous organ involvement.

Patient	Main Clinical Features at Baseline	Etiology
1	Purpura+++, nephritis, RI, neuropathy, GI bleeding	Essential
2	Purpura+++, skin ulcers, neuropathy	Essential
3	Purpura+++, neuropathy, GI bleeding	Essential
4	Purpura++, neuropathy, nephritis	Essential
5	Purpura+++, arthralgias, neuropathy, nephritis, RI	Essential
6	Purpura++, arthralgias, neuropathy, nephritis, RI, GI bleeding	Essential
7	Purpura++, nephritis	Essential
8	Purpura+++, RI, skin ulcers, nephritis, arthralgias, neuropathy	Essential
9	Purpura++, skin ulcers, nephritis, GI bleeding, seizures*	Essential
10	Purpura+++, arthralgias, neuropathy	Essential
11	Nephritis, RI, CNS*	Sjögren syndrome
12	Purpura++, nephritis, RI, arthralgias	Sjögren syndrome
13	Purpura++, skin ulcers, neuropathy	Rheumatoid arthritis
14	Purpura++, nephritis, RI, arthritis	Parvovirus B19
15	Purpura+, skin ulcers, arthritis, neuropathy	B cell lymphoma
16	Purpura++, neuropathy, arthralgias	T cell lymphoma

* MRI suggesting cerebral vasculitis. Purpura: + = limited and fluctuating involvement of lower limbs; ++ = persistent and diffuse involvement of lower limbs; +++ = diffuse involvement of lower limbs plus trunk and/or upper limbs. GI: gastrointestinal; RI: renal insufficiency; CNS: central nervous system.

Table 4. Responses to therapies of patients with nonmalignant type II mixed cryoglobulinemia.

Treatment	No. of Therapeutic Sequences (patients)	No Response	Partial Response	Complete Response	Early Relapse or Dependence	Late Relapse	Arrest for Secondary Effects
CS alone	14 (12)	1	11	0	11	0	2
CS + AZA	2 (2)	1	1	0	1	0	1
CS + CYC	17 (13)	2	12	0	11	0	4
CS + MMF	5 (5)	1	2	0	2	0	3
CS + MTX	1	0	0	1	0	0	0
Interferon- α	2	2	0	0	0	0	0

CS: corticosteroid; AZA: azathioprine; CYC: cyclophosphamide (500–750 mg/m² intravenous monthly); MMF: mycophenolate mofetil; MTX: methotrexate. Late relapse was defined as occurring at least 3 months after treatment had been stopped. Early relapse was defined as occurring less than 3 months after treatment had been stopped. Dependence was defined as relapse occurring during treatment tapering.

with lamivudine, with no significant effect on the cryoglobulinemia, although HBV DNA became undetectable in the serum. Seven patients with severe forms were treated with rituximab (Table 5). A complete response was obtained in 2 patients; clinical remission lasted at least 6 months. Due to the severity of the initial presentation, they were given one infusion (375 mg/m²) every 6 months as maintenance therapy. They remain in complete remission with 2 and 5 years' followup, respectively. Three patients improved greatly but relapsed before 6 months. Patient 14 was treated with lenalidomide (15 mg every other day from Days 1 to 21: 2 courses and 3 additional weeks with 5 mg daily). After the last therapeutic course the patient was in complete remission; the parvovirus plasma load also became undetectable. In the following months, he presented a full recovery of renal function and had not relapsed at the last visit, at 1-year followup.

Eighteen severe adverse events occurred in 8 patients (27.6% of patients with nonmalignant MC; Table 6). They were dominated by severe sepsis (11 cases, all bacterial when documented). CYC plus steroid regimens were particularly marred by side effects (8 events including 1 death). CYC was stopped due to secondary effects in 4 patients; one of them died from septic shock (this was the only death in the patients without lymphoid malignancy).

Tolerance of MMF was also poor. Two patients presented a disease flare during rituximab treatment. Patient 4 presented fever and worsening of purpura 24 hours after the first infusion. Patient 14 deteriorated after the third infusion, with extension of the purpuric rash, painful neuropathy affecting the forearms, worsening renal failure requiring dialysis, and rising cryoglobulin level. Rituximab was otherwise well tolerated, in particular with no infectious complications, with the possible exception of Patient 14 who

Table 5. Responses to rituximab of patients with nonmalignant type II mixed cryoglobulinemia.

Patient	Sex/Age, yrs	Etiology	Previous Therapies	Clinical Features Before Rituximab	Outcome
1	M 66	Essential	None	Purpura, nephritis, RI, neuropathy, GI bleeding	Complete response
4	F 41	Essential	CS, AZA, CB, IFN	Purpura, nephritis, neuropathy	Arrest due to secondary effect
5	F 53	Essential	CS, CYC, CB	Purpura, neuropathy, nephritis, RI, arthralgias	Complete response
8	M 58	Essential	None	Skin ulcers, purpura, neuropathy, nephritis, arthralgias	Partial response
11	M 55	Sjögren syndrome	CS, CYC MMF	Nephritis, RI, CNS	Partial response
12	F 52	Sjögren syndrome	CS	Purpura, nephritis, arthralgias	Partial response
14	M 62	Parvovirus B19	CS	Purpura, nephritis, RI, arthralgias	Arrest due to secondary effect

CB: chlorambucil; with the exception of Patient 1, the protocol was 375 mg/m² weekly for 1 month. Patient 1 was given 2 infusions of 1 g at 15-day intervals. CS: corticosteroid; AZA: azathioprine; IFN: interferon; CYC: cyclophosphamide; MMF: mycophenolate mofetil; RI: renal insufficiency; GI: gastrointestinal; CNS: central nervous system.

Table 6. Severe adverse events in patients with nonmalignant type II mixed cryoglobulinemia. All patients were also treated with steroids. Severe adverse events indicate secondary events that led to hospitalization and/or interruption of medication.

Adverse Event	Therapy (value)
Infections	
Septic shock	CYC (2)*
Bacterial septicemia	CYC (2), CS (1), MMF (2)
Pneumonia	CYC (2), MMF (1)
Pyelonephritis	RTX (1)
Other	
Hepatitis	MMF (1), AZA 91)
Hemorrhagic cystitis	CYC (1)
Disease flare	RTX (2)
Peptic ulcer hemorrhage	CS (1)
Death	CYC (1)*

* One patient died from septic shock during neutropenia secondary to cyclophosphamide (CYC) therapy. CS: corticosteroid; MMF: mycophenolate mofetil; RTX: rituximab; AZA: azathioprine.

presented a pyelonephritis (*Klebsiella pneumoniae*) 6 weeks after the last infusion. At that time he was under dialysis and high-dose steroids.

DISCUSSION

Few data on non-HCV-associated type II MC are available. Previous studies generally included fewer patients, or did not clearly make the distinction between type III and type II MC, or did not provide detailed information on treatment and outcome^{9,17,18,19,20,21}. In some studies many patients were recruited on the presence of serum cryoglobulin but were asymptomatic^{18,19,20}. In such a situation it is difficult

to be sure that polyneuropathy or renal involvement with no biopsy is indeed due to MC. Sensitive techniques can detect cryoglobulins at low levels in many apparently healthy subjects, but the significance of this remains elusive.

The etiologies and their frequencies are close to those reported in some studies^{9,18,19}, but differ substantially from those from Saadoun, *et al*, in which type II MC were related to hematologic diseases (mainly B cell lymphoma) in 31%, autoimmune diseases in 33% (mainly SLE), and infectious diseases in 9%²⁰. The higher incidence of lymphoid malignancies in this last study may explain the poorer outcome reported (14% of patients died after a mean followup of 49.4 months). In our study, with a similar mean followup, only 2 patients died (6%). In addition no patient developed B cell lymphoma during followup. Our series and others show that lymphoid malignancies associated with MC are quite diverse. It is therefore difficult to propose a unique diagnostic strategy. In the absence of an orientating symptom we propose to systematically perform a whole-body scan and a bone marrow biopsy.

The rarity of associated infectious diseases challenges a popular scenario for induction of MC, in which chronic infections that generate large amount of IgG-containing immune complexes may lead to RF B cell activation and predispose to the selective expansion of B cell clone(s) leading to type II MC. It is noteworthy that none of our patients had a history of type III MC preceding the development of type II MC. Alternatively, type II MC may often represent monoclonal gammopathy of undetermined significance, with possibly no role for an (auto)antigen, the monoclonal immunoglobulin having RF activity by chance. The pathogeny of MC associated with autoimmune diseases may

be different. They could be the consequence of the chronic B cell activation that characterizes these diseases. Thus, an interesting finding from our study is that essential MC seem to be more severe than those associated with autoimmune diseases. The significance of this remains elusive, but a similar observation was reported by Matignon, *et al*¹⁷.

The treatment of non-HCV-related MC is usually similar to that of other vasculitides, with steroid as first-line therapy and in most severe cases the use of immunosuppressants, in particular CYC. Although most patients were improved by these regimens, only one patient had a sustained complete response. In addition, these treatments, particularly those including CYC or MMF, were associated with significant side effects including severe sepsis, which was the major cause of death in longterm studies^{20,21}. Anti-CD20 therapy may represent the best option, at least in severe forms. Several uncontrolled studies have shown that rituximab is efficient in treating both HCV and non-viral type II MC by suppressing RF production^{13,14,15,22}. In our study 2 patients had a complete response and 3 greatly improved. Given the severity of the initial presentation, 2 patients with complete response were treated by one infusion every 6 months as maintenance therapy. This systematic approach may be questioned, but the fact that the 3 other patients relapsed suggests that maintenance therapy may be required. By contrast, 2 patients experienced a disease flare after rituximab infusion. Such an adverse event has been reported in patients with MC and may be related to complex formation between rituximab and RF^{23,24,25}. Consequently, Sene, *et al*²³ advised use of the 375-mg protocol and plasma exchanges before infusion of rituximab in patients with high levels of cryoglobulins. We think this approach should be extended to all patients with a severe form of MC.

Recently, data from a French registry were reported that included 23 patients with nonviral cryoglobulinemia (all types, 8 malignant) treated with rituximab²⁵. Although clinical efficacy was noted for most patients, tolerance was marked by a high level of side effects including severe infections in 6 patients, of whom 3 died. This rate is much higher than in patients with lupus or rheumatoid arthritis from that same registry, and higher than in our patients with MC. The infections occurred in a subgroup of patients with age > 70 years, type II MC, and renal failure, using high-dose steroids. The only obvious difference between these patients and ours is their older age (73 ± 5 vs 55.3 ± 8 years, respectively). In the French Autoimmunity and Rituximab (AIR) registry, relapses were also very frequent, indicating that rituximab is not curative and raising again the question of maintenance therapy. Finally, our Patient 14 was successfully treated with lenalidomide, which was also well tolerated. This agent was chosen for its effect on plasma cell neoplasia and its stimulating activity on virus-specific cytotoxic CD8⁺ T cells^{26,27}. To our knowledge this is the first report of a successful treatment of type II MC with lenalidomide.

We show that an important proportion of non-HCV-related type II MC remains the essential type. Significant efforts should be made to find etiologies other than HCV, because treatments with steroid and immunosuppressants are not satisfactory, especially in severe forms. In these situations anti-CD20 therapy may present the best option, but should be used with caution, in particular in older people with renal insufficiency. Its role in induction and/or maintenance therapy needs to be evaluated in randomized studies. New agents such as lenalidomide also need to be evaluated.

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REFERENCES

1. Brouet JC, Clauvel JP, Dannnon F, Klein M, Seligmann M. Biological and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med* 1974;57:775-88.
2. Tedeschi A, Barate C, Minola E, Morra E. Cryoglobulinemia. *Blood Rev* 2007;21:183-200.
3. Dammacco F, Sansonno D, Piccoli C, Tucci FA, Racanelli V. The cryoglobulins: an overview. *Eur J Clin Invest* 2001;31:628-38.
4. Horcajada JP, Garcia-Bengoechea M, Cilla G, Etxaniz P, Cuadrado E, Arenas JJ. Mixed cryoglobulinemia in patients with chronic hepatitis C infection: prevalence, significance and relationship with different viral genotypes. *Ann Med* 1999;31:352-8.
5. Lunel F, Musset L, Cacoub P, Frangeul L, Cresta P, Perrin M, et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C and liver damage. *Gastroenterology* 1994;106:1291-300.
6. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992;327:1490-5.
7. Pozzato G, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G, et al. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. *Blood* 1994;84:3047-53.
8. Misiani R, Bellavita P, Fenili D, Borelli G, Marchesi D, Massazza M, et al. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. *Ann Intern Med* 1992;117:573-7.
9. Cohen Tervaert JW, Van Paassen P, Damoiseaux J. Type II cryoglobulinemia is not associated with hepatitis C infection. *Ann NY Acad Sci* 2007;1107:251-8.
10. Zuckerman E, Keren D, Slobodin G, Rosner I, Rozenbaum M, Toubi E, et al. Treatment of refractory, symptomatic, hepatitis C virus related mixed cryoglobulinemia with ribavirin and interferon-alpha. *J Rheumatol* 2000;27:2172-8.
11. Cacoub P, Ratzin V, Myers RP, Ghillani P, Piette JC, Moussalli J, et al. Impact of treatment on extra hepatic manifestations in patients with chronic hepatitis C. *J Hepatol* 2002;36:812-8.
12. Alric L, Plaisier E, Thebault S, Peron JM, Rostaing L, Pourrat J, et al. Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. *Am J Kidney Dis* 2004;43:617-23.
13. 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;101:3818-26.
14. Zaja F, De Vita S, Mazzaro C, Sacco S, Damiani D, De Marchi G, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood* 2003;101:3827-34.
15. Quartuccio L, Soardo G, Romano G, Zaja F, Scott CA, De Marchi G, et al. Rituximab treatment for glomerulonephritis in

- HCV-associated mixed cryoglobulinaemia: efficacy and safety in the absence of steroids. *Rheumatology* 2006;45:842-6.
16. Harris NL, Jaffe ES, Diebold J, Glandrin G, Muller-Hermelink HK, Vardiman J, et al. 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;17:3835-49.
 17. Maignon M, Cacoub P, Colombat M, Saadoun D, Brocheriou I, Mougenot B, et al. Clinical and morphologic spectrum of renal involvement in patients with mixed cryoglobulinemia without evidence of hepatitis C virus infection. *Medicine* 2009;88:341-8.
 18. Trejo O, Ramos-Casals M, Garcia-Carrasco M, Yague J, Jimenez S, de la Red G, et al. Cryoglobulinemia: Study of etiologic factors and clinical and immunologic features in 443 patients from a single center. *Medicine* 2001;80:252-62.
 19. Mascia MT, Ferrari D, Campioli G, et al. Non HCV-related mixed cryoglobulinemia. *Dig Liver Dis* 2007;39 Suppl 1:S61-4.
 20. 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;166:2101-8.
 21. Tarantino A, Campise M, Banfi G, Confalonieri R, Bucci A, Montoli A, et al. Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. *Kidney Int* 1995;47:618-23.
 22. 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;67:283-7.
 23. Sene D, Ghillani-Dalbin P, Amoura Z, Musset L, Cacoub P. Rituximab may form a complex with IgM kappa mixed cryoglobulin and induce severe systemic reactions in patients with hepatitis C virus-induced vasculitis. *Arthritis Rheum* 2009;60:3848-55.
 24. Cohen H, Green S, Jones S, Amos N, William BD. Lack of efficacy of rituximab in a patient with essential mixed cryoglobulinemia. *Rheumatology* 2007;46:366-7.
 25. Terrier B, Launay D, Kaplanski G, Hot A, Larroche C, Cathebras P, et al. Safety and efficacy of rituximab in non-viral cryoglobulinemia vasculitis: Data from the French AIR registry. *Arthritis Care Res* 2010 Aug 25. [Epub ahead of print]
 26. Zeldis JB, Knight RD, Jacques C, Tozer A, Bizzari JP. Lenalidomide in multiple myeloma: current role and future directions. *Expert Opin Pharmacother* 2010;11:829-42.
 27. Haslett PA, Hanekom WA, Muller G, Kaplan G. Thalidomide and a thalidomide analogue drug costimulate virus-specific CD8+ T cells in vitro. *J Infect Dis* 2003;187:946-55.