



# Cryoglobulinemia

Enrica Morra

**Mixed cryoglobulinemia (MC) type II is a disorder characterized by circulating cold-precipitable immune complexes composed of polyclonal immunoglobulin IgG and monoclonal IgM rheumatoid factor (RF). The systemic vasculitis which characterizes the disease is caused by the deposition of immune complexes on the walls of small vessels, and by the subsequent activation of the complement cascade. MC may be asymptomatic or lead to clinical manifestations characterized by a typical triad—purpura, arthralgia, and weakness—and in some cases by a more serious vasculitis with neurologic and renal involvement. In the vast majority (more than 90%) of patients, MC is**

**associated with hepatitis C virus (HCV) infection, which is considered the triggering factor of the disease. The association between cryoglobulinemia and HCV infection, and the possible evolution to B-cell lymphomas usually after long-term follow-up, suggest the role of HCV infection both in the pathogenesis of MC and in lymphomagenesis. In fact, the virus chronically stimulates the polyclonal proliferation of B cells from which a monoclonal population may emerge. This paper also reviews the treatment strategies for MC syndrome, emphasizing the issue of the eradication of HCV, and the clinical and biological activity of rituximab for selective B-cell control.**

## Cryoglobulinemia: Definition and Classification

The term cryoglobulinemia refers to the presence in the serum of single or mixed immunoglobulins (Ig), which precipitate at temperature below 37°C and redissolve at higher temperatures. This *in vitro* phenomenon can be observed in a wide spectrum of hematologic, infectious, and immunorheumatologic disorders.<sup>1,2</sup> Cryoglobulins are classified into three subgroups according to their immunochemical composition:

*Type I cryoglobulinemia* is composed of a single monoclonal Ig, usually IgM. Type I cryoglobulinemia accounts for 10% to 15% of people with cryoglobulinemia. It is mainly found in patients with lymphoproliferative disorders (immunocytoma/Waldenström macroglobulinemia, multiple myeloma). Monoclonal cryoglobulinemia is frequently asymptomatic. When present, clinical manifestations derive from hemorheologic disturbances and include acrocyanosis, Raynaud phenomena and gangrene.

*Type II and Type III cryoglobulinemia* (mixed cryoglobulinemia, MC) are immune complexes composed of polyclonal IgGs and mono- or polyclonal IgMs, respectively. The cryoprecipitating property results from the reduced solubility of the IgM-IgG immune complex. Type II mixed cryoglobulinemia accounts for 50% to 60% of patients with cryoglobulins. The monoclonal IgMs almost always contain kappa light chains and represent autoantibodies with rheumatoid factor (RF) activity. Most IgMs react with both intact IgG and the F(ab')<sub>2</sub> fragment, as well as with the F<sub>c</sub> fragment of autologous IgG. Type III mixed

cryoglobulinemia (30% to 40% of patients with cryoglobulinemia) is characterized by a combination of polyclonal immunoglobulins. Mixed cryoglobulinemia types II and III can be associated with different infectious, immunological and neoplastic diseases. Using more sensitive methods (immunoblotting, two-dimensional polyacrylamide gel electrophoresis), another serological type of MC, the oligoclonal or microheterogeneous type, has been characterized. Oligoclonal cryoglobulinemia is defined by more than two heterogeneous bands of heavy chains (oligoclonal IgMs or a mixture of polyclonal and monoclonal IgMs). The oligoclonal type seems to be an intermediate stage in the course of type III evolving to type II MC. In fact, during chronic active HCV infection MC may change its immunochemical type: type II MC is more stable over time than type III and oligoclonal.<sup>3</sup>

The clinical syndrome of MC is characterized by a systemic vasculitis involving mostly small, but sometimes larger vessels, which are damaged by the deposition of immune complexes on their walls with activation of the complement cascade.<sup>1,2,4</sup>

## Detection, isolation and immunochemical typing of cryoglobulinemia

Blood samples are stored at 37°C until complete clotting occurs and are then centrifuged. The serum is kept at 4°C for 7 days. If a cryoprecipitate is visible, the sample is centrifuged at 4°C for 15 minutes to measure a cryocrit. Cryoglobulins are purified by washing at 4°C and characterized using immunofixation electrophoresis.

## Epidemiology and Clinical Features of Mixed Type II Cryoglobulinemia

Since 1990 a strong association between hepatitis C virus (HCV) and MC has been demonstrated. Prior to the discov-

---

Correspondence: Enrica Morra, MD, Niguarda Ca' Granda Hospital, Piazza Ospedale Maggiore 3, Milano 20162, Italy; Phone +39 (02) 64442668, Fax +39 (02) 64442033, [ematologia@ospedaleniguarda.it](mailto:ematologia@ospedaleniguarda.it)

ery of HCV, MC without an apparent underlying disease was defined as “essential MC.” The prevalence of HCV infection in MC patients varies geographically, with high values (over 90%) in the Mediterranean area.<sup>1,4,5</sup> MC is found in 30% to 50% of patients with chronic HCV infection, with only 10% to 15% of them developing clinical symptoms of MC. A causative role of hepatitis B virus (HBV) in MC was found in less than 5% of patients.<sup>2</sup> The overall prevalence of MC is not yet defined by adequate epidemiologic studies. It is probably underestimated due to referral of patients to different specialists according to the dominant manifestation of the disease. The Italian Group for the Study of Cryoglobulinemia has proposed serological, pathological, and clinical criteria for the diagnosis of MC patients (**Table 1**).<sup>2,4</sup>

The main clinical features of MC are purpura, arthralgias, weakness (the typical clinical triad reported by the majority of patients at diagnosis), liver involvement, renal involvement, peripheral neuropathy and widespread vasculitis. The vasculitic lesions of the skin and of other organs are the consequence of vascular deposition of circulating immune complexes and complement. There is generally no relationship between the severity of vasculitic manifestations and the serum levels of cryoglobulins or complement. Patients with type II MC may develop a malignant B-cell lymphoproliferative disorder.<sup>4,6,7</sup> It usually occurs several years following diagnosis. In a series of 231 MC patients, 20 developed B-cell lymphoma after a mean time of 8.8 years of follow up.<sup>4</sup> The disease may also be complicated, although less frequently, by hepatocellular carcinoma or thyroid cancer.

In 50% of patients the MC syndrome behaves as a slowly progressive disorder. One third of patients show a moderate to severe clinical course with prognosis adversely affected by renal or liver failure. Advanced age, male sex and renal involvement are reported as the most important factors negatively affecting survival.<sup>4</sup>

### **Cutaneous symptoms**

Orthostatic purpura is the most common clinical manifestation (90% of cases). The vasculitic lesions of the skin, usually beginning in the legs, may be complicated by ulcers in 10% of patients at diagnosis. Hyperpigmentation of the skin of the legs after repeated episodes of purpura is typical. Symptoms related to Raynaud phenomenon, with or without digital gangrene, are observed in about one third of patients. Diffuse vasculitis is a rare clinical manifestation defined as widespread vasculitic involvement of the skin and of 2 or more visceral organs (kidney, gut, lung, central nervous system).

### **Rheumatologic manifestations**

MC patients commonly report polyarthralgias. Myalgias may also occur, whereas clinical signs of arthritis are relatively rare.

### **Peripheral neuropathy**

Peripheral neuropathy is defined as sensory and/or motor peripheral nerve disturbances, which are confirmed by electrophysiologic study. Vasculitis of vasa nervorum has been proposed as the pathogenetic mechanism, as well as direct autoimmune nerve damage. The incidence of neuropathy, especially involving the legs with painful paresthesias and muscle weakness, may exceed 60%. The clinical course is progressive (despite lack of progression of MC) and is not benefited by the addition of interferon to steroid treatment.<sup>8</sup> Central nervous system (CNS) involvement in patients with HCV-positive MC is rare. However, the high frequency of impaired cognitive function and the extent of magnetic resonance imaging (MRI) brain abnormalities observed in these patients suggest a specific inflammatory CNS involvement in MC.<sup>9</sup>

### **Liver involvement**

As HCV infection is detected in a large proportion of MC patients, liver damage is common in these patients. In fact, over 50% of patients show signs of mild to moderate chronic

**Table 1. Proposed criteria for the diagnosis and classification of patients with mixed cryoglobulinemia.<sup>4</sup>**

<b>Criteria</b>	<b>Serologic</b>	<b>Pathologic</b>	<b>Clinical</b>
Major	Mixed cryoglobulins Low C4	Leukocytoclastic vasculitis	Purpura
Minor	Rheumatoid factor HCV <sup>+</sup> , HBV <sup>+</sup>	Clonal B-cell infiltrates (liver and/or bone marrow)	Chronic hepatitis, MPGN, peripheral neuropathy, skin ulcers

“Definite” Mixed Cryoglobulinemia Syndrome

1) serum mixed cryoglobulins (± low C4) + purpura + leukocytoclastic vasculitis

2) serum mixed cryoglobulins (± low C4) + 2 minor clinical symptoms + 2 minor serological/pathologic findings

“Essential” or “secondary” Mixed Cryoglobulinemia:

Absence or presence of well-known disorders (infectious, immunologic, or neoplastic)

Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; HCV<sup>+</sup>, HBV<sup>+</sup>, markers of HCV or HBV infection (anti-HCV ± HCV RNA; HBV DNA or HBsAg); MPGN, membranoproliferative glomerulonephritis

hepatitis at diagnosis. Liver involvement is characterized by abnormally increased liver enzymes with or without echographic or histological features of chronic hepatitis, cirrhosis, or hepatocellular carcinoma. Late evolution into overt cirrhosis occurs in 25% of cases. A highly statistically significant association between cirrhosis and cryoglobulinemia has been observed. Cryoglobulins are a prognostic indicator for increased risk of cirrhosis in patients with chronic hepatitis C.<sup>10</sup>

### **Renal involvement**

Renal involvement is clinically evident in 20% to 30% of patients with MC and is a major cause of morbidity. Cryoglobulinemic nephropathy is now considered a distinct clinicopathologic entity. The distinctive features are: proteinuria, increased serum creatinine, progressive renal failure, and membranoproliferative glomerulonephritis (MPGN) on renal biopsy. MPGN is the predominant type of glomerulonephritis caused by HCV infection in the presence or absence of cryoglobulinemia.<sup>11</sup>

### **Sicca syndrome**

Sicca syndrome is quite frequent at diagnosis of MC patients (30%). In most instances the typical serologic and histopathologic findings of Sjögren syndrome are lacking. Sicca syndrome is more frequently reported in type II than in type III cryoglobulinemia.

### **B-cell lymphoproliferative disorders**

Lymphoproliferative malignancies are the most prevalent neoplasms occurring in MC during the course of the disease. In fact, 5% to 10% of MC patients develop symptoms and histologic features of non-Hodgkin lymphoma (NHL).<sup>4,6,7</sup> According to the REAL/WHO classification, the most frequent histologic subtypes observed are: lymphoplasmacytic (29%), diffuse large B-cell (27%), follicular (16%), marginal zone (10%) and mantle cell (7%) lymphomas.<sup>6</sup> These lymphomas are characterized by a high prevalence of extranodal involvement, especially liver and major salivary glands. HCV-infected cells in these organs may act as a reservoir stimulating B-cell proliferation.<sup>6</sup> In our series of 75 patients with HCV-related MC followed for a median of 62 months (12-163), 5 patients (6.6%) showed malignant evolution: 4 to lymphoplasmacytic lymphoma and 1 to chronic lymphocytic leukemia.<sup>7</sup>

Another rare clinical manifestation of MC is the hyperviscosity syndrome, caused by high levels of cryoglobulins.

### **The Role of HCV in the Pathogenesis of Mixed Cryoglobulinemia**

HCV infection affects crucial mechanisms of the immune system predisposing to autoimmune and lymphoproliferative disorders. HCV type II cryoglobulinemia is the major extrahepatic manifestation of HCV infection. The others include: membranoproliferative glomerulonephritis, sicca syndrome, rheumatoid arthritis, inflammatory arthritis, pe-

ripheral neuropathy and NHL. In fact, the virus is able to escape immune elimination, leading to chronic infection, accumulation of circulating immune complexes, and autoimmune phenomena. Furthermore, HCV stimulates the production of monoclonal RF.<sup>11</sup> The production of IgM RF molecules is the most important event for the emergence of cryoprecipitable immune complexes. RFs in normal conditions are molecules involved in the presentation of antigen to T cells within an immune complex. A postulated hypothesis for the pathogenesis of HCV-related MC is that HCV initially stimulates the production of IgM without RF activity; the persistent antigenic stimulation may induce somatic mutations causing the acquisition of RF activity. HCV shows a cellular affinity for lymphocytes. Interaction between the virus and lymphocytes may occur through the viral envelope protein E2, which binds human CD81 expressed on both hepatocytes and lymphocytes. The interaction between HCV and lymphocytes directly affects B-cell function and may induce polyclonal activation and expansion of peripheral CD5<sup>+</sup> cells.<sup>12</sup> These cells are considered the major source of IgM RF in type III cryoglobulinemia. However, monoclonal RF may be the result of the evolution from polyclonal to oligoclonal to monoclonal RF, or may develop *de novo*.<sup>11,13</sup> Following the initial activation, the emergence of a single dominant clone producing a monoclonal IgM RF may be an evolutionary step towards type II mixed cryoglobulinemia. In the proposed model, the immune complexes contain HCV core proteins plus specific anticore IgG, which are bound to IgM with activity of RF. This large complex binds specifically to the endothelial cells through the C1q receptor.<sup>1</sup>

### **HCV and Lymphomagenesis**

The frequent association of MC with HCV infection, systemic autoimmune disorders (autoimmune hepatitis, sicca syndrome, glomerulonephritis, thyroiditis), as well as the possible evolution of all of these into a B-cell malignancy, suggests a close link between viral infection, autoimmunity, and lymphoproliferation.<sup>2,6,13,14</sup> HCV may initiate a multistep process of lymphomagenesis but additional factors (genetic, epigenetic, environmental, immunological) are probably necessary for malignant clonal expansion. HCV infection may exert an inhibitory effect on B-cell apoptosis through bcl-2 overexpression. In fact, a prevalence of bcl-2 rearrangement and of t(14;18) translocation has been found in patients with HCV-related MC with or without B-cell lymphomas.<sup>15</sup> The regression of established lymphoma observed in patients with HCV-driven MC after antiviral treatment likely reflects the dependence of the disease process on stimulation by the virus.<sup>16</sup>

### **Management of Mixed Cryoglobulinemia**

The targets of treatment in HCV-related mixed cryoglobulinemia are the HCV infection and the autoimmune disorder. Given the documented association with HCV and its role in the pathogenesis of MC and in lymphomagenesis,

the treatment should be directed at interrupting the pathogenic sequence leading to the vasculitis-related damage. As a consequence, sustained HCV clearance should be attempted in patients with chronic HCV infection and type II MC to ameliorate symptoms and to prevent the complications of chronic liver disease and the evolution toward malignant lymphoproliferative disorders.

While no initial treatment is required in asymptomatic MC, in symptomatic disease the therapeutic approach should be adapted to the individual patient according to the intensity of clinical symptoms (**Table 2**). In patients with mild to moderate cryoglobulinemic symptoms (purpura, arthralgias, peripheral sensory neuropathy) first-line immunosuppression usually consists of low-dose steroids. Patients with severe manifestations of MC (cryoglobulinemic nephropathy, skin ulcers, sensory motor neuropathy, widespread vasculitis) should promptly receive high-dose steroids with or without cyclophosphamide. Removal of circulating immune complexes by plasma exchange may be useful, particularly in active cryoglobulinemic nephropathy.<sup>2,17</sup>

Because of the etiologic role of HCV in most MC patients, HCV eradication is the main target of treatment. By analogy with the management of chronic HCV hepatitis, interferon (IFN)- $\alpha$ , either alone or with ribavirin, represents standard treatment for HCV-associated MC. Several reports have shown that IFN produces significant clinical improvement in 40% to 70% of MC patients and that its efficacy is closely associated with inhibition of HCV replication. In responsive patients, reduction of HCV-RNA usually heralds the decline of the cryocrit. In a recent study by Mazzaro et al on 18 patients with HCV-related MC treated with pegylated IFN plus ribavirin for 48 weeks, HCV-RNA became undetectable in 15 patients (83%) and most had a clinical improvement. Virological, clinical and biochemical response was sustained in 44% of patients.<sup>18</sup> These authors also set criteria to define the type of response (complete, partial, no response) of MC patients to the antiviral approach in terms of: 1) viral response, i.e., the effect on HCV-RNA; 2) biochemical response; i.e., the effect on liver function tests; 3) immune response; i.e., the effect on RF

and cryocrit levels; 4) clinical response; i.e., the effect on the clinical manifestations of the disease. The use of IFN in patients with MC is limited by its transient efficacy and by the peculiar side effects of this agent (depression, thyroiditis, and neuropathy). In patients with clinically evident peripheral neuropathy, nephropathy, or skin ulcers, IFN may aggravate these manifestations.<sup>1,2</sup>

Antiviral treatment proved effective in splenic lymphoma with villous lymphocytes associated with HCV infection and mixed cryoglobulinemia, resulting in the clearance of HCV-RNA, remission of lymphoma, and regression of clinical manifestations of MC.<sup>16</sup> These results represent a strong argument in favor of the etiologic link between HCV infection, MC, and lymphoma. Interestingly, among B-cell NHL, splenic marginal zone lymphoma shows a particularly high incidence (35%) of HCV infection.<sup>19</sup> Three series have recently reported that antiviral treatment with IFN +/- ribavirin is effective in HCV-associated indolent and marginal zone lymphoma, most with cryoglobulinemia.<sup>20-22</sup> In these studies, where the majority of patients had splenic lymphoma with villous lymphocytes, most patients achieved a sustained response of lymphoma with regression of symptoms of MC after clearance of HCV RNA. A complete remission with IFN + ribavirin has been also reported in a patient with HCV-associated mantle cell NHL resistant to chemotherapy and rituximab.<sup>23</sup>

A selective control of the B-cell clone has been attempted in MC using the anti-CD 20 monoclonal antibody rituximab. This agent, successfully employed in B-cell lymphomas and other chronic lymphoproliferative diseases, has shown activity in other autoimmune disorders. Rituximab at the standard dose of 375 mg/m<sup>2</sup> weekly for 4 weeks was effective and well tolerated, resulting in a significant and rapid improvement of clinical signs (purpura, arthralgia, peripheral neuropathy) and a decline of cryocrit in most patients with MC, including patients resistant to IFN.<sup>24-26</sup> A remission of the underlying malignant lymphoproliferative disorder was obtained.<sup>26</sup> Rituximab proved to be safe and effective treatment for cryoglobulinemic nephropathy.<sup>27</sup> Complete clinical remission was associated with a significant reduction of RF activity and anti-HCV antibody titers. Although an increase of viremia was observed in responders, no significant variation of transaminases or deterioration of liver disease was noted.<sup>25</sup> The addition of pegylated IFN and ribavirin after anti-CD20 treatment has been proposed to eliminate HCV while maintaining the remission obtained with rituximab.<sup>26</sup> The first dose of rituximab may induce a transient rise of the cryobulin level that does not indicate resistance to treatment.<sup>28</sup>

## Conclusion

In conclusion, important advances have been obtained in the understanding of the epidemiology, etiology, and pathogenesis of mixed type II cryoglobulinemia. The recognition of the major role played by HCV infection as a trigger and maintenance factor of the disease has allowed effec-

**Table 2. Main therapeutic options for symptomatic patients with mixed, hepatitis C virus (HCV)-related, cryoglobulinemia.**

Category	Activity
Corticosteroids (low-, high-dose)	Anti-inflammatory, immunosuppressive
Cyclophosphamide	Immunosuppressive
Plasma-exchange	Removal of circulating immune complexes
Interferon + Ribavirin	Antiviral (HCV eradication)
Anti-CD20 antibody (rituximab)	Selective suppression of B-cell clone

tive antiviral treatment for this condition. The demonstration of the underlying B-cell clonal expansion in MC and the possible evolution into a B-cell lymphoma as a result of prolonged HCV stimulation represents a biological basis for innovative approaches with anti-CD20 monoclonal antibodies. Several biological aspects, however, remain to be clarified. Regarding treatment, the appropriate indication for antiviral and antilymphoma agents, as well as the best modality for their integration, need to be defined in future studies.

## References

1. Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinemia, and vasculitis: immune complex relations. *Lancet Infect Dis.* 2005;5:227-236.
2. Ferri C, Zignego AL, Pileri SA. Cryoglobulins. *J Clin Pathol.* 2002;55:4-13.
3. Sene D, Ghillani-Dalbin P, Thibault V, et al. Long-term course of mixed cryoglobulinemia in patients infected with hepatitis C virus. *J Rheumatol.* 2004;31:2199-206.
4. Ferri C, Sebastiani M, Guggioli D, et al. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum.* 2004;33:355-374.
5. Dammacco F, Sansonno D, Piccoli C, Tucci FA, Racanelli V. The cryoglobulins: an overview. *Eur J Clin Invest.* 2001;31:628-638.
6. Trejo O, Ramos-Casals M, Lopez-Guillermo A, et al. Hematologic malignancies in patients with cryoglobulinemia: association with autoimmune and chronic viral diseases. *Semin Arthritis Rheum.* 2003;33:19-28.
7. Cesana C, Barbarano L, Miquelisz S, et al. Clinical characteristics and outcome of immunoglobulin M-related disorders. *Clin Lymphoma.* 2005;5:261-264.
8. Ammendola A, Sampaolo S, Ambrosone L, et al. Peripheral neuropathy in hepatitis-related mixed cryoglobulinemia: electrophysiologic follow-up study. *Muscle Nerve.* 2005;31:382-385.
9. Casato M, Saadun D, Marchetti A, et al. Central nervous system involvement in hepatitis C virus cryoglobulinemia vasculitis: a multicenter case-control study using magnetic resonance imaging and neuropsychological tests. *J Rheumatol.* 2005;32:484-488.
10. Kayali Z, Buckwold VE, Zimmermann B, Schmidt WN. Hepatitis C, cryoglobulinemia, and cirrhosis: a meta-analysis. *Hepatology.* 2002;36:978-985.
11. Agnello V, De Rosa FG. Extrahepatic disease manifestations of HCV infection: some current issues. *J Hepatol.* 2004;40:341-352.
12. Curry MP, Golden-Mason L, Doherty DG, et al. Expansion of innate CD5 pos B cells expressing high levels of CD81 in hepatitis C virus infected liver. *J Hepatol.* 2003;38:642-650.
13. Dammacco F, Sansonno D, Piccoli C, Racanelli V, D'amore FP, Lauletta G. The lymphoid system in hepatitis C virus infection: autoimmunity, mixed cryoglobulinemia, and overt B-cell malignancy. *Semin Liver Dis.* 2000;20:143-157.
14. Ramos-Casals M, De Vita S, Tzioufas AG. Hepatitis C virus, Sjögren's syndrome and B-cell lymphoma: linking infection, autoimmunity and cancer. *Autoimmunity Rev.* 2005;4:8-15.
15. Zignego AL, Ferri C, Giannelli F et al. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas. *Ann Intern Med.* 2002;137:571-580.
16. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med.* 2002;347:89-94.
17. Gertz MA. Cold agglutinin disease and cryoglobulinemia. *Clinical Lymphoma.* 2005;5:290-293.
18. Mazzaro C, Zorat F, Caizzi M, et al. Treatment with peg-interferon alfa-2b and ribavirin of hepatitis C virus-associated mixed cryoglobulinemia: a pilot study. *J Hepatol.* 2005;42:632-638.
19. Arcaini L, Paulli M, Boveri E, et al. Splenic and nodal marginal zone lymphomas are indolent disorders at high hepatitis C virus seroprevalence with distinct presenting features but similar morphologic and phenotypic profiles. *Cancer.* 2004;100:107-115.
20. Saadoun D, Suarez F, Lefrere F, et al. Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? *Blood.* 2005;105:74-76.
21. Kelaidi C, Rollot F, Park S, et al. Response to viral treatment in hepatitis C virus-associated marginal zone lymphomas. *Leukemia.* 2004;18:1711-1716.
22. Vallisa D, Bernuzzi P, Arcaini L, et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, non-Hodgkin's lymphoma: a multicenter Italian experience. *J Clin Oncol.* 2005;23:468-473.
23. Levine A, Shimodaira S, Lai MMC. Treatment of HCV-related mantle-cell lymphoma with ribavirin and pegylated Interferon alfa. *N Engl J Med.* 2003;349:2078-2079.
24. Zaja F, De Vita S, Mazzaro C, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood.* 2003;101:3827-3834.
25. Sansonno D, Valli De Re, Lauletta G et al. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon with an anti-CD20. *Blood.* 2003;101:3818-3826.
26. Lamprecht P, Lerin-lozano C, Merz H, et al. Rituximab induces remission in refractory HCV associated cryoglobulinaemic vasculitis. *Ann Rheum Dis.* 2003;62:1230-1233.
27. Roccatello D, Baldovino S, Rossi D, et al. Long term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinemic glomerulonephritis. *Nephrol Dial Transplant.* 2004;19:3054-3061.
28. Ghobrial IM, Uslan DZ, Call TG, Witzig TE, Gertz MA. Initial increase in the cryoglobulin level after rituximab therapy for type II cryoglobulinemia secondary to Waldenström Macroglobulinemia does not indicate failure of response. *Am J Hematol.* 2004;77:329-333.