

# Incidence and Characteristics of Non-Hodgkin Lymphomas in a Multicenter Case File of Patients With Hepatitis C Virus–Related Symptomatic Mixed Cryoglobulinemias

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**Background:** Some patients with cryoglobulinemic syndrome (CS) develop frank non-Hodgkin lymphoma (NHL), but the incidence and timing of this event are still poorly defined.

**Methods:** A retrospective multicenter study was performed of hepatitis C virus–positive patients with CS observed in 11 Italian centers belonging to the Italian Group for the Study of Cryoglobulinemia.

**Results:** The inclusion criteria were satisfied by 1255 patients. During a cumulative follow-up of 8928 patient-years, 59 cases of NHL were diagnosed, for an estimated rate of 660.8 new cases per 100 000 patient-years with 224.1 new cases of aggressive NHL subtypes per 100 000 patient-years. More than 90% of the patients developing NHLs had type II cryoglobulins. The NHLs were classi-

fied as nonaggressive in 31 cases (53%), aggressive in 20 (34%), and mucosa-associated lymphoid tissue lymphomas in 6 (10%); 2 cases were unclassifiable. The median time from the diagnosis of CS to the clinical onset of NHL was 6.26 years (range, 0.81-24 years). The clinical course and response to chemotherapy in the patients with CS who had NHL were similar to those usually described in patients with NHL without CS; the course of the CS only marginally benefited from chemotherapy.

**Conclusions:** The overall risk of NHL in patients with CS is about 35 times higher than in the general population (12 times higher if nonaggressive lymphomas are excluded). The presence of CS did not significantly affect the treatment of newly diagnosed lymphomas.

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**M**IXED CRYOGLOBULINEMIAS are immunocomplex-related disorders that lead to cutaneous and systemic vasculitis, glomerulonephritis, and peripheral neuropathy.<sup>1-4</sup> The cold-precipitating immune complexes are formed by immunoglobulins and monoclonal or polyclonal IgM rheumatoid factor and respectively classified as type II and type III mixed cryoglobulinemias.<sup>5</sup>

Both cryoglobulinemic syndrome (CS) and its progression to overt lymphomas<sup>1-3</sup> were reported many years before the discovery of the association between CS and hepatitis C virus (HCV) infection, and it has more recently been suggested that there is a relationship between HCV infection and an increased risk of developing non-Hodgkin lymphoma (NHL) regardless of the presence of CS,<sup>6,7</sup> although this has not been confirmed in larger epidemiologic studies.<sup>8</sup>

Studies of relatively small case files recruited in a single center may overestimate the incidence of NHL in patients with HCV-associated CS and do not permit comparisons with the general population. Furthermore, the pathological features of the lymphoproliferation itself is puzzling because a lymphoid monoclonal infiltrate without progression to overt malignancy is usually found in the marrow and liver biopsy specimens of patients with CS,<sup>9,10</sup> and the time interval between the diagnosis of CS and the clinical evidence of lymphoma is still poorly defined. Finally, the role of interferon in reducing the incidence of lymphoma needs thorough investigation, as does the effect of chemotherapy on cryoglobulin production and CS-related symptoms.

This study was designed to answer some of these questions by retrospectively assessing the cumulative incidence and characteristics of NHL in a large series of patients with CS observed in 11 centers

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belonging to the Italian Group for the Study of Cryoglobulinemia.

## METHODS

This retrospective multicenter study included all of the patients registered at the 11 centers situated in 7 Italian regions (3 in Lombardy, 2 in Piedmont, 2 in Friuli, and 1 each in Tuscany, the Veneto, Marche, and Campania) for whom stored serum samples were available in January 1993 for the assessment of HCV status, or who could be directly screened for the virus. The centers were asked to report the date on which all of the patients included in the cohort were diagnosed as having CS, and any incident NHL cases occurring before the end of December 2002, with the date of diagnosis, histologic findings, and clinical outcome. The NHLs diagnosed before or within 6 months after the onset of CS were not considered.

Hepatitis C virus infection was diagnosed by means of HCV RNA determinations, and only HCV RNA-positive patients were included in the study. Stored serum samples were used to test HCV RNA in the patients with CS diagnosed before 1993, who contributed to the analysis with the entire time of observation from CS diagnosis.

The standardized diagnosis of CS required the presence of serum cryoglobulins (cryocrit concentration, >1%) for more than 6 months; at least 2 of purpura, weakness, and arthralgia; and C4 levels less than 8 mg/dL and/or positive serum rheumatoid factor. The serum samples were collected; the cryoglobulins quantified, isolated, and characterized; and the serum C4 levels and rheumatoid factor activity determined as previously described.<sup>3</sup>

As a general policy adopted by the Italian Group for the Study of Cryoglobulinemia since 1993, the patients underwent bone marrow biopsy, abdominal sonography, and standard chest radiography at the time of the diagnosis of CS. The abdominal scans and chest radiograms were repeated every other year during the follow-up, whereas the marrow was reexamined only in the case of abnormal peripheral-blood findings or as part of the staging procedure for a newly diagnosed lymphoma.

A percutaneous liver biopsy was recommended in all of the patients with abnormal alanine aminotransferase levels. The diagnosis of NHL was made on the basis of evidence of nodal or extranodal lymphoproliferative disease with pathological features that were compatible with the criteria of the Armed Forces Institute of Pathology-World Health Organization guidelines.<sup>11</sup> When bone marrow monoclonal lymphoproliferation was the only reported pathological finding, a diagnosis of NHL was arbitrarily accepted in the presence of bone marrow substitution of 30% or greater and/or the onset or worsening of peripheral-blood cytopenia (hemoglobin level, <10 g/dL; platelet count, <100 × 10<sup>3</sup>/μL; neutrophil count, <10<sup>3</sup>/μL).

All of the NHL diagnoses were centrally reviewed. The working formulation was used to classify the NHLs, with an additional subset for mucosa-associated lymphomas. To reduce data dispersion, they were further defined as nonaggressive (including lymphocytic and small-cell follicular lymphomas) or aggressive (including large-cell follicular, mixed diffuse, and large-cell centroblastic; immunoblastic; and lymphoblastic lymphomas); these 2 categories roughly correspond to the low and intermediate-high grades of the working formulation.

An improvement in CS was defined as the complete disappearance of, or substantial reduction in, purpuric manifestations, weakness, and arthralgias, accompanied by an increase in C4 levels and/or a decrease in cryocrit levels and serum rheumatoid factor titers. An improvement in liver disease was defined as alanine aminotransferase levels of less than the upper reference value or their halving at least 3 times during 6 months.

Complete lymphoma remission was defined as the total disappearance of the lymphomatous mass, and partial remission as a 75% reduction.

The statistical analyses were made with BMDP software (BMDP Statistical Software Inc, Los Angeles, Calif). The distributions of the nonparametric variables were studied with contingency tables and compared with Pearson  $\chi^2$  test; the continuous variables were compared with Gosset unpaired *t* test. The time-dependent risks were assessed by means of the life-table method and compared by Mantel test; cumulative hazard ratios were computed from the negative natural logarithm of survival function.

When applicable, multiple comparisons were corrected by the Bonferroni method.

## RESULTS

A total of 1255 HCV-positive patients with CS were included in the study. During the total follow-up period of 8928 person-years, 59 patients fulfilled the criteria for an NHL diagnosis, with an estimated incidence of 660.8 new cases per 100 000 patient-years; there were 20 aggressive NHLs, an incidence of 224.1 new cases per 100 000 patient-years.

The cumulative incidence of NHL varied from 2.1% to 11.4% in the different centers. Fifty (93%) of the 54 patients for whom the results of immunochemical cryoprecipitate testing were available had type II cryoglobulins. The overall median time between the diagnoses of CS and NHL was 6.26 years (range, 0.81-24 years); in the aggressive lymphoma subset, it was 3.5 years (range, 0.5-23 years). Forty-three patients showed histologic evidence of chronic active hepatitis or cirrhosis at the time of NHL diagnosis. The NHLs were classified as nonaggressive in 31 cases, aggressive in 20, and mucosa-associated lymphoid tissue (MALT) lymphomas in 6; 2 cases were unclassifiable (**Table 1**). Among nonaggressive lymphomas, 16 had bone-marrow infiltration as the only reported pathological finding; considering the 20 aggressive and 15 nonaggressive NHL cases not restricted to the bone marrow, the overall incidence of overt lymphomas was 329.0 per 100 000 patient-years.

After the diagnosis of NHL, the patients were followed up for a median of 5 years (range, 1-24 years); their actuarial median survival estimated by the life-table method was 10.61 years. The 2 patients with histologically unclassifiable cases were still alive after 2 and 16 years of follow-up; 24 of the other patients died (8 of NHL-related causes, 3 of liver disease, and 13 of causes unrelated to CS or NHL).

Twenty-five patients received standard courses of alfa-2b interferon treatment before developing NHL; this treatment did not influence the time between the diagnoses of CS and NHL, which was 4.8 years for the untreated patients and 4.0 years for the treated patients ( $P = .38$ ).

One patient achieved complete remission after spleen irradiation alone and was still in remission after 14 years; 39 received standard chemotherapy (CHOP [cyclophosphamide, doxorubicin, vincristine sulfate, and prednisone], MACOP [methotrexate, doxorubicin, cyclophosphamide, vincristine, and prednisone], CVP [cyclophosphamide, vincristine, and prednisone]).

**Table 1. Overall Characteristics of Observed NHLs**

Characteristic	Nonaggressive	Aggressive	MALT	P Value
Observed NHL, No.*	31	20	6	
Age, median (range), y	64 (46-78)	63.5 (54-79)	68.5 (55-86)	.51†
Sex, No. M/F	9/22	6/14	1/5	.49‡
Type of cryoglobulins, No.§				>.99‡
II	26	18	6	
III	3	1	0	
Liver histologic findings, No.				.97‡
Absence of liver lesions	9	5	0	
Chronic active hepatitis	14	10	3	
Cirrhosis	8	5	3	
Previous interferon therapy, No.				.08‡
Yes	14	5	4	
No	15	13	1	
Time from CS to NHL, median (range), y	4 (0.5-18)	3.5 (0.5-23)	6 (2-24)	.87†
Chemotherapy, No.				.02‡
Yes	16	19	3	
No	14#	1	3	
Response to chemotherapy, No.				.13‡
Complete remission	1	5	0	
Partial remission	10	8	2	
No remission	3	5	1	
Survival, No.				.47‡
Dead	11	10	3	
Alive	20	10	3	
Cause of death, No.				.19‡
NHL	2	4	2	
Hepatopathy	2	1	0	
Other causes	7	5	1	

Abbreviations: CS, cryoglobulinemic syndrome; MALT, mucosa-associated lymphoid tissue; NHL, non-Hodgkin lymphoma.

\*Lymphoma type was unclassifiable in 2 cases.

†Kruskal-Wallis test.

‡Yates corrected  $\chi^2$  test.

§Not available for 3 patients.

||Not available for 2 patients.

¶Not available for 5 patients.

#One patient received only radiotherapy.

Chemotherapy seemed to improve the survival of the patients with nonaggressive lymphomas, as the treated patients survived longer than those who were untreated ( $P = .04$ ). All but 1 of the patients with an aggressive lymphoma were treated. The effect of treatment was not influenced by age at lymphoma diagnosis, the presence of liver cirrhosis, or cryocrit levels.

Twenty of the 39 patients receiving chemotherapy died: the causes of death were progressive lymphoma in 6 cases, cardiovascular complications in 3, liver failure in 3, esophageal bleeding in 1, pneumonia in 1, lung cancer in 1, cerebral hemorrhage in 1, and undetermined in 4.

One of the 20 patients who did not receive chemotherapy was a 79-year-old woman with a poor performance status affected by a large-cell centroblastic lymphoma with a high disease burden (nodes, bone marrow, liver, and spleen), who died of spleen rupture 6 months after diagnosis. Of the remaining 19 patients, 3 had gastric MALT lymphomas, 1 had a lymphocytic lymphoma with single-node involvement and died of sepsis, and 15 had exclusively bone marrow involvement (11 lymphocytic, 3 small-cell follicular, and 1 unclassifiable lymphoma). Three of these patients died during the observation period (1 because of NHL progression).

The CS worsened after chemotherapy in 16 patients who experienced more frequent episodes of purpura. On the contrary, chemotherapy was associated with an improvement in CS-related symptoms in 19 patients, although serum cryoglobulins disappeared in only 6 cases. Liver disease progressed in 17 patients, 5 of whom died of liver failure; 7 patients showed a lasting improvement in liver function indexes without any histologic evidence of progressive liver disease (**Table 2**).

#### COMMENT

Our results confirm that the overall incidence of NHL, as well as the incidence of aggressive histotypes, is higher in patients with HCV-related CS than in the general population, and there is an excess of MALT and low-grade lymphoma in these patients. They also suggest that interferon treatment does not have a major influence on the risk of developing NHL.

The incidence of NHL reported by the participating centers varied considerably and was higher in the centers that contributed fewer patients. The overall cumulative incidence was 4.7% and that of aggressive histotypes was 1.6%,

**Table 2. Course of Cryoglobulinemia, Cryoglobulinemia Syndrome, and Liver Disease After Chemotherapy in 39 Patients**

Type of Lymphoma	Cryoglobulinemia, No.		Cryoglobulinemia Syndrome, No.			Liver Involvement, No.		
	Persistent	Disappeared	Worsened	Stable	Improved	Worsened	Stable	Improved
Aggressive NHL (n = 18)	15	3	10	2	6	8	6	4
Nonaggressive NHL (n = 17)	16	1	4	2	11	9	6	2
MALT lymphoma (n = 3)	2	1	2	0	1	0	2	1
Unclassified NHL (n = 1)	0	1	0	0	1	0	1	0

Abbreviations: MALT, mucosa-associated lymphoid tissue; NHL, non-Hodgkin lymphoma.

which is lower than the 7% to 11% reported in other published Italian series.<sup>4,9,12</sup> This difference may be explained by the smaller size of these studies and, perhaps, their less restrictive inclusion criteria. Nevertheless, in comparison with the annual incidence of 18.6 new cases of NHL per 100 000 inhabitants<sup>13</sup> reported by the Registro Tumori della Lombardia–provincia di Varese, the incidence of NHL in patients with CS is more than 35.5 times the expected figure and remains 12 times higher even if only the aggressive histotypes are considered.

Only 9% of our cases had high-grade large-cell or lymphoblastic lymphomas, which is similar to the 7% reported by La Civita et al<sup>12</sup> but clearly different from findings in the general population, in which 60% of NHLs are “aggressive” (ie, large-cell follicular, mixed diffuse, and large-cell centroblastic, immunoblastic, and lymphoblastic lymphomas).<sup>14</sup>

Our data are also markedly different from those of studies investigating the prevalence of HCV infection in patients with lymphomas: Pioltelli et al<sup>8</sup> reported that 66.7% of 48 HCV-positive NHLs observed in Lombardy, Italy, were “aggressive”; De Vita et al<sup>15</sup> found mainly high-grade large B-cell lymphomas in HCV-infected patients; and Ferri et al<sup>16</sup> reported that two thirds of 34 B-cell NHLs in HCV-infected patients were intermediate- or high-grade lymphomas. Findings similar to our own have been reported only when the clinical picture corresponded to that of CS (with kidney or liver involvement and circulating cryoglobulins), as in 17 of 35 HCV-positive patients with prevalently low-grade lymphoproliferation (immunocytomas) described by Silvestri et al.<sup>17</sup>

We included “low-grade” lymphomas with exclusively bone marrow involvement in an attempt to address the question of how to interpret them. In some respects, our findings support the view that they should be considered examples of CS-associated monoclonal lymphoproliferation (probably the pathological counterpart of serologic findings) rather than true lymphomas, although the possibility that they represent a transitional form leading to overt lymphoma cannot be excluded.

MALT lymphomas were more frequent in our study than in the HCV-positive NHL series of Pioltelli et al<sup>8</sup> (10% vs 6.5% of cases). Further investigations are needed to verify whether this unusual prevalence in areas in which the incidence of MALT is otherwise about 7% of all B-cell lymphomas is causally attributable to the virus.

Most of the patients with CS who developed NHL in our study and previously published series had type II cryoglobulins. This is a third peculiarity of this type of

cryoglobulinemia, which is more frequently associated with renal impairment and less frequently with severe liver disease than type III.<sup>18,19</sup>

Interferon therapy did not seem to influence the risk of lymphoma or the time of its development. Sansonno et al<sup>20</sup> observed the occurrence of B-cell NHLs in cryoglobulinemic patients whose HCV RNA cleared after interferon therapy, thus suggesting that factors other than HCV are also involved in lymphomagenesis. However, none of our patients showed a sustained viral response to interferon therapy, and none of the incident NHLs was observed in patients in whom HCV RNA had become stably negative.

The response to chemotherapy of the 39 treated patients was comparable with that observed in HCV- and CS-negative patients with NHL: 11 patients (28%) were still alive after 5 years. Chemotherapy did not significantly influence the clinical course of CS, but CS seems to have a favorable clinical trend in patients who respond well to chemotherapy.

The transition from early bone marrow and liver B-lymphocyte infiltrates to overt lymphoproliferation, which was first reported in patients with CS by Monteverde et al,<sup>9,10</sup> is still unexplained. Lymphoid aggregates in the liver are more common in patients with type II than in those with type III CS or noncryoglobulinemic chronic hepatitis and have many of the features usually considered to be markers of malignant lymphoid proliferation: Bcl-2 overexpression, monotypic IgM  $\kappa$  restriction of cryoglobulin production,<sup>21,22</sup> and a high rate of monoclonal IgM rearrangements. Many authors have attributed these characteristics to HCV infection and considered them markers of oligoclonal nonneoplastic B-cell expansion; however, they are so frequent in low-grade lymphomas (regardless of viral status) that they are usually considered to be neoplastic markers and are currently used as indicators of residual disease. In molecular terms, the lymphoid infiltrates associated with CS are similar to those of overt low-grade lymphomas, the only differences being their slower growth and clinical features. It therefore remains to be established whether HCV infection not only triggers the events leading to CS but is also a first step toward lymphomagenesis.<sup>23</sup>

Dammacco et al<sup>24</sup> identified 2 types of B-cell NHLs associated with HCV infection: the NHLs arising in cryoglobulinemic patients, which usually have a low-malignancy phenotype but may occasionally evolve into a high-malignancy phenotype; and the NHLs not associated with cryoglobulinemia, which usually do not involve the bone marrow and are frequently highly malignant from the start.

Our finding that the vast majority of patients with CS do not develop an overt malignancy during a long follow-up period suggests that a number of individual and environmental factors may be involved.

The major limitations of our study include its retrospective design and the difficulty of distinguishing CS with bone marrow infiltrates (a common finding in type II CS) from CS with bone marrow-infiltrating NHLs without any other localization. Furthermore, systemic symptoms may reduce the sensitivity and specificity of our observations. Nevertheless, the large number of patients and our efforts to standardize the data collection criteria make us confident about the reliability of our findings.

In conclusion, patients with CS show an unusually high incidence of high-grade and low-grade NHLs, as well as MALT lymphomas, in comparison with the general population. Hepatitis C virus remains highly suspected as the cause of these phenomena, but its role remains to be assessed in more detailed longitudinal studies. Despite the fact that their long-lasting chronic disease compromises the function of a number of organs, patients with CS seem to tolerate chemotherapy for NHL well.

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