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HEPATITIS C VIRUS INFECTION AMONG CRYOGLOBULINEMIC AND NON-CRYOGLOBULINEMIC B-CELL NON-HODGKIN'S LYMPHOMAS

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ABSTRACT

Background and Objective. Since hepatitis C virus (HCV) infection has been associated with different histotypes of B-cell non-Hodgkin's lymphoma (NHL), with or without concomitant production of cryoglobulins (cryoIg), we have investigated the prevalence of the infection among NHL with the aim of defining its relationship with the histotype and with the production of cryoIg.

Methods. Four-hundred and seventy unselected, consecutive patients with a diagnosis of B-cell NHL were investigated. Anti-HCV antibodies (Ab) and cryoIg were sought in all while HCV RNA and rheumatoid factor were detected on HCV-Ab positive samples.

Results. Overall, the prevalence of HCV infection was 8.9% (42/470). It was 95.4% (#21) among the 22 patients with, and 4.6% (#21) among the 448 without production of cryoIg. The most common histotype among the HCV-positive, cryoIg-producing cases, was the immunocytoma (16/21, 76%). Among the HCV-positive, non cryoIg-producing cases, the marginal zone and the follicle center lymphomas were the commonest.

Interpretation and conclusions. Close association between HCV infection and cryoIg production, already described in mixed cryoglobulinemia, is confirmed also among B-cell NHL. Nevertheless, 50% of HCV-related lymphomas are non-cryoIg producers. Low-grade lymphomas (in particular the immunocytoma) are the most frequent HCV-related lymphomas. Since new therapeutic strategies might be necessary if the virus is detected, screening for cryoIg and for HCV-Ab among B-cell NHL at diagnosis is mandatory.
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Key words: hepatitis C virus, non-Hodgkin's lymphomas, cryoglobulins

Even though conclusive proof has not yet been provided, epidemiological data strongly favor the hypothesis that the hepatitis C virus (HCV) is the causative agent of the so-called essential mixed cryoglobulinemia (MC).[1-4]

The large number of B-cell clonal disorders described in terminal stages fo HCV-positive chronic liver diseases,[5] the fact that MC can be complicated by a B-cell non-Hodgkin's lymphoma (NHL),[6,7] and that MC is currently considered an expression of a low-grade NHL,[8-10] led to the hypothesis that HCV might be involved in the pathogenesis of B-cell NHL. After the first observation by Ferri *et al.*,[11] other reports have focused on the prevalence of HCV infection among NHL, raising more questions than answers on the real impact of the infection on the pathogenesis of such an heterogenous group of disorders, since HCV was associated with different subtypes of B-cell NHL.[12-17]

On the basis of its prevalence among 537 patients with lymphoproliferative disorders and 180 with MGUS (monoclonal gammopathy of unknown significance), we previously excluded the involvement of HCV infection in the pathogenesis of Hodgkin's disease, T-cell NHL, multiple myeloma and MGUS. We also identified a subgroup of B-cell NHL, the immunocytoma, all of which were associated with the production of cryoglobulins (cryoIg) (in particular type II cryoIg), as the probable pathogenetic target of HCV.[18,19]

Since others[11-17] have reported on possible HCV involvement among NHL not associated with the production of cryoIg, we have focused our attention on the relationship between HCV infection, histology, and cryoIg production, among B-cell NHL.

Materials and Methods

Four-hundred and seventy unselected, consecutive patients affected by B-cell NHL, seen from January 1991 at the Division of Hematology of the Udine University Hospital, were involved in the study. All patients were Italian-born and HIV negative. Diagnosis of NHL was made through a morphologic evaluation of pathologic specimens on which immunophenotypic analysis was also performed in all cases. The NHL were classified

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according to the *Revised European-American Classification of Lymphoid Neoplasms* (REAL).[20]

At diagnosis, hematological parameters, routine blood chemistry and urianalysis were performed in all the patients using standard methods. Cryoglobulins and anti-HCV antibodies (Ab) were sought at diagnosis in all patients after January 1993; for patients diagnosed before, they were sought during follow-up. Rheumatoid factor (RF) was measured by rate nephelometry in all HCV-positive patients. CryoIg isolation was performed as described elsewhere.[21]

Screening for anti-HCV Ab was carried out with a second-generation enzyme immunoadsorbent assay (EIA II) (Ortho Diagnostic System, Raritan, NJ, USA). Positive samples were retested in duplicate and confirmed by a second-generation recombinant immunoblot assay (RIBA II) (Ortho), according to the manufacturer's instructions.

On HCV-Ab positive samples, detection of HCV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as previously described.[18] Briefly, frozen (–80°C) serum aliquots (1 mL) were thawed and RNA was extracted, after ultracentrifugation following the guanidinium thiocyanate-phenol-chloroform method. The HCV-RNA detection was based on incorporation of digoxigenin during amplification and detection of amplified product using a biotin-labeled capture probe and anti-digoxigenin HRP conjugate.

After reverse transcription, cDNA aliquots were amplified using primers chosen from highly conserved 5' noncoding region nucleotide sequence of HCV genome. The final digoxigenin-labelled PCR product was detected by means of an automatic analyzer (ES 300; Boehringer Mannheim, Germany) using a commercially available kit (Enzymun-Test DNA Detection; Boehringer, Germany) following the manufacturer's instruction.

Statistical analysis

The prevalence of HCV infection in each group of patients was calculated dividing the number of patients infected by the number of patients analyzed. The relative risk (RR) of being infected by HCV, as compared with that of the general population in our area (prevalence of 2.87%)[22] was calculated using the χ^2 Yates corrected test or the Fisher exact test whenever appropriate. All the reported p values are two-tailed. Data analyses were performed with a SPSS/PC statistical package (SPSS, Inc. Chicago, IL, USA).

Results

Overall, out of 470 cases analyzed, 42 anti-HCV Ab-positive cases were identified with a prevalence of HCV infection of 8.9% and a relative risk of being infected of 3.12 (95% confidence interval 2.27-4.30). The risk is significantly higher than in the general population of the same area (p < 0.0001).

Diagnosis of NHL in these 42 cases was performed on lymph node biopsies in 13 cases (2 marginal zone lymphomas [MZL], 2 immunocytomas, 6 follicle center lymphomas [FCL], and 3 diffuse large cell lymphomas [DLCL]), and on different extranodal specimens in 9 cases (5 MZL, 2 FCL, 1 DLCL and 1 mantle cell lymphoma); in all the other cases diagnosis was made on bone marrow biopsies.

Table 1 shows the prevalence (95.4%) and the relative risk (33.35) of HCV infection among 22 patients with B-cell NHL and a concomitant production of cryoIg. As can be seen, not all histologic subgroups are associated with HCV infection and cryoIg production. The prevalent histologic substrate of cryoIg-producing NHL corresponds to the immunocytoma (16/21 cases, 76%).

Overall, among all immunocytomas analyzed, the prevalence of HCV infection was 25.7% (18/70), with a RR of being infected almost 9-fold (8.98) superior to that of the general population (p < 0.0001). Seventeen of these 70 patients (24.3%) were producing cryoIg (mainly type II cryoIg) and 16 of these 17 (94.1%) were HCV-positive, with a risk of infection, among cryoIg-producing immunocytomas, of 32.88 (p < 0.0001).

Table 2 shows the prevalence (4.6%) and the relative risk (1.64) of HCV infection among 448 patients with B-cell NHL not associated with the production of cryoIg.

Some histotypes, such as prolymphocytic leukemia, hairy cell leukemia, Burkitt's lymphoma and lymphoblastic lymphoma are never associated with HCV infection. Some others, such as chronic lymphocytic leukemia, immunocytoma and diffuse large cell lymphoma, have an RR of being associated with HCV overlapping that of the general population, while the subgroups of marginal zone and follicle center lymphomas have an increased RR (3.88 and 2.84 respectively), statistically significant (p = 0.009 and 0.012 respectively), of being associated with HCV.

As far as clinical and pathological characteristics

Table 1. Prevalence of HCV infection among cryoglobulinemic B-cell non-Hodgkin's lymphomas.

Histologic subgroup	*No. of patients*	*Monoclonal component*	*Anti-HCV antibodies (%)*	*RR for HCV**	*95% CI°*	*p-value*
Lymphoplasmacytoid lymphoma/immunocytoma	17	15 type II cryoIg 2 type I cryoIg	16 (94.1)	32.88	24.42-39.43	< 0.0001
Follicle center lymphoma	1	1 type II cryoIg	1 (100)	NT		
Marginal zone lymphoma	2	2 type II cryoIg	2 (100)	NT		
Mantle cell lymphoma	1	1 type III cryoIg[#]	1 (100)	NT		
Diffuse large cell lymphoma	1	1 type II cryoIg	1 (100)	NT		
Total	22	22	21 (95.4)	33.35	28.28-39.32	< 0.0001

*Abbreviations: cryoIg = cryoglobulins; N.T. = not tested, due to the low number of cases. *relative risk of being infected by HCV. °95% confidence interval. [#]type III cyroIg = polyclonal.*

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Table 2. Prevalence of HCV infection among non-cryoglobulinemic B-cell non-Hodgkin's lymphomas.

Histologic subgroup	*No. of patients*	*Monoclonal component*	*Anti-HCV antibodies (%)*	*RR for HCV**	*95% CI°*	*p-value*
Chronic lymphocytic leukemia	131	8GK; 7MK; 6 double MC	4 (3.0)	1.07	0.40-2.83	0.790
Prolymphocytic leukemia	3	1 Mλ; 1 Gλ	0	–	–	–
Hairy cell leukemia	35	0	0	–	–	–
Lymphoplasmacytoid lymphoma/immunocytoma	53	27 MK; 5 Mλ; 5 Gλ; 1 AK; 1 Aλ; 3 double MC	2 (3.8)	1.32	0.34-5.17	0.664
Follicle center lymphoma	86	1 GK; 1MK; 4 double MC	7 (8.1)	2.84	1.38-5.86	0.012
Marginal zone lymphoma	45	1Gλ	5 (11.1)	3.88	1.68-8.97	0.009
Mantle cell lymphoma	10	0	0	–	–	–
Diffuse large cell lymphoma	76	4 MK; 2 Gλ	3 (3.9)	1.38	0.45-4.22	0.481
Burkitt's lymphoma	3	0	-	–	–	–
Precursor B-lymphoblastic leukemia/lymphoma	2	0	-	–	–	–
B-cell lymphoma, unspecified	4	0	0	–	–	–
Total	448	78	21 (4.6)	1.64	1.06-2.54	0.039

*Abbreviations: MC = monoclonal component. *relative risk of being infected by HCV. °95% confidence interval.*

at presentation of the 42 HCV-positive patients, 13 of them (all cryoIg producers) presented with purpura, 12 with hepatomegaly, 17 with splenomegaly and 16 with adenopathy.

Chronic hepatitis was present in 16 patients, 12 with and 4 without cryoIg production. In 8 patients this was bioptically documented, in the other 8 diagnosis was based on persistent increase of transaminase levels. In 5 cases, all cryoIg producers, a membranoproliferative glomerulonephritis was also bioptically documented. Rheumatoid factor (normal values < 40 UI/mL) was negative in all the non-cryoIg producing cases and in the only patient producing a type I cryoIg, while it was positive in all the other cryoIg producing cases (median value 372, range 43-5600 UI/mL). Finally, it must be underlined that 8/42 patients had a previous history of blood products transfusion, and that in 31 of the 39 (79.5%) patients where it was sought, the viral genome was found in the serum.

Discussion

Our previous observations led us to identify a subgroup of B-cell NHL, the immunocytoma, as the probable pathogenetic target of HCV.[18,19] Since most of the patients with HCV infection and NHL were found to produce cryoIg, and since HCV is known to infect peripheral blood mononuclear cells[23-25] and to induce a clonal Ig-gene rearrangement of B-lymphocytes,[26,27] we concluded that the HCV was the putative ethiologic agent of the lymphoproliferative disorders producing cryoIg.

Recent reports seem to confirm our previous findings, since Mazzaro *et al.*[17] detected the majority of HCV-Ab positive B-cell HNL cases among immunocytomas, most of which produced cryoIg, while Mangia *et al.*[28] found a prevalence of HCV infection quite similar to that of the control population in patients with cryoIg-negative monoclonal gammopathies. On the other hand, other studies have reported a high prevalence of HCV infection among non-cryoglobulinemic B-cell NHL. In a preliminary report, Santini *et al.*[29] detected HCV RNA in all six cases with Waldenström macroblobulinemia; in only 4 of the 17 HCV-positive patients (34% of the total analyzed) reported by Ferri *et al.*[11] cryoIg were detected, while Luppi *et al.*[14] reported a prevalence of 42% of HCV infection among 69 non-cryoIg producing B-cell NHL, and in particular a 50% prevalence among 16 lymphomas of MALT.[14,16] Finally, Cavanna *et al.*[12] and Pioltelli *et al.*[15] found prevalence of HCV infection of 25% (among 150 cases) and 21% (among 126 cases), respectively, but unfortunately, in these last 2 studies, no data on the concomitant production of cryoIg was reported.

The differences in the prevalence of infection and cryoIg production observed among different studies might reflect a different selection of patients among institutions with a different clinical address. In fact one should expect a greater number of cryoIg-producing cases among the NHL complicating the course of mixed cryoglobulinemia, than among the cases referred to a Hematology Division for a *de novo* NHL. This is not our experience, since we found that 50% of our HCV-positive patients with B-cell NHL were cryoIg-producers. Moreover, it must be underlined that the prevalence of HCV infection was only slightly (though significantly) higher (4.6%) than in the general population, among the NHL not associated with the production of cryoIg, while it was very significantly higher

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(95.4%) among the cases associated with cryoIg production.

A tentative explanation of this phenomenon (the production of cryoIg among HCV-positive patients) was recently proposed by Mondelli *et al*.[30] Among polyclonal virus-specific cryoIg in cryoprecipitates of HCV-infected patients, Mondelli *et al*. found monoclonal bands of IgG3, which is known to be a potent cryoIg inducer. This suggests that specific immunoglobulin isotypes involved in viral antigen binding may be relevant to the production of cryoIg and that HCV acts as an exogenous stimulus, triggering the B-cell clonal expansion that eventually leads to the develpment of an NHL.

Our epidemiological data on the production of cryoIg among NHL seem indirectly to validate this hypothesis. We also confirm, on a larger sample, that the immunocytoma is the most frequently represented histotype among HCV-positive B-cell NHL.

Two main clinical applications arise from these observations; first of all, the requirement for routine screening for the presence of cryoIg and for evidence of HCV infection all the patients with B-cell NHL at diagnosis; second, the development of new strategies for the treatment of HCV-related hematological malignancies.

Taking as an example the regression of gastric MALTomas after *Helicobacter pylori* eradication,[31] it also seem likely that the elimination of HCV would result in the cure of the associated lymphoma. This hypothesis needs to be tested in a prospective study.

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