

Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study

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The existence of an association between infection with hepatitis C virus (HCV) and B-cell non-Hodgkin lymphoma (B-NHL) remains controversial, largely because previous studies were based on prevalent case series or comparisons with less than optimal control groups. This hospital-based case-control study was conducted from January 1998 through February 2001 to evaluate the association between HCV infection and B-NHL of different types. Cases were consecutive patients with a new diag-

nosis of B-NHL; controls were patients from other departments of the same hospitals. Both groups were interviewed using a standardized questionnaire. The prevalence of HCV infection was calculated by histologic type of B-NHL and clinical behavior (indolent or aggressive). Adjusted odds ratio (OR) and HCV-attributable risk (AR) were estimated. HCV prevalence was 17.5% among the 400 lymphoma patients and 5.6% among the 396 controls. The OR of B-NHL (patients vs controls), adjusted by age, sex, level of

education, and place of birth, was 3.1 (95% confidence interval [CI], 1.8-5.2); an OR indicative of positive association was found for indolent and aggressive B-NHL. The estimated AR was 4.6%. This study confirms an association between HCV and B-NHL. In Italy, 1 of 20 instances of B-NHL may be attributable to HCV infection and may, thus, benefit from antiviral treatment. (*Blood*. 2003; 102:996-999)

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Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver diseases, including liver cirrhosis and hepatocellular carcinoma, and it has been found to be associated with extrahepatic disorders such as autoimmune diseases and mixed cryoglobulinemia, a lymphoproliferative disorder that sometimes evolves into B-cell non-Hodgkin lymphoma (B-NHL).^{1,2} Moreover, HCV-RNA has been found in peripheral blood and bone marrow mononuclear cells, and the persistence of the virus in these cells can chronically stimulate B lymphocytes.^{1,2} These findings led to the hypothesis that HCV may play a role in lymphomagenesis, and several studies have investigated the potential association between HCV infection and B-NHL, although the results of these studies are conflicting. A positive association has been found in studies conducted in countries in which the prevalence of HCV infection is relatively high—Italy,³⁻¹³ the United States,¹⁴ and Japan¹⁵—but not in others.¹⁶⁻²² However, these studies had several limitations. Specifically, all but one¹³ also included prevalent cases in addition to incident cases; most of the studies did not use an appropriate control group and did not control for possible confounding factors. Regarding the studies that did not find a positive association, some were small studies or were conducted in populations in whom HCV infection is rare.^{17,18,20}

The objective of the present study was to evaluate the potential association between B-NHL and HCV infection. To overcome these limitations, a multicenter case-control study using only newly diagnosed B-NHL (ie, incident cases) was conducted.

Patients, materials, and methods

Patients and controls

The study population consisted of patients 15 years and older admitted to 10 hospitals in different cities located throughout Italy (Bari, Bergamo, Montefiascone, Napoli, Palermo, Reggio Calabria, Roma [2 hospitals], San Giovanni Rotondo, and Sassari) from January 1998 through February 2001. Patients had been consecutively admitted to the hematologic wards of study hospitals with newly diagnosed B-NHL and had not received anticancer treatment. All participating hematologic departments were part of the Italian Cooperative Group for the Study of Haematological Diseases in Adults (GIMEMA). For all but 20 of the patients, B-NHL diagnosis was based on the results of lymph node biopsy that allowed the specific type of B-NHL to be identified. For the remaining 20 patients, the diagnosis (unspecified B-NHL) was based on bone marrow biopsy. B-NHL stage at diagnosis was determined for all patients. Type and stage of B-NHL were defined according to the Revised European American Lymphoma (REAL)/World Health Organization (WHO) classifications.^{23,24} Patients were subdivided into groups based on the specific type of B-NHL; for the purposes of this analysis, the patients with Burkitt and Burkitt-like lymphomas were included in a single group, as were the patients with lymphoplasmacytic lymphoma and Waldenstr m macroglobulinemia.

The control group consisted of patients in other departments of the same hospitals, specifically the departments of dentistry, dermatology, general surgery, gynecology, internal medicine, ophthalmology, orthopedics, otorhinolaryngology, and traumatology. As for the patients, only control patients with a newly diagnosed disease were included. Eligible control patients

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were identified once a week, throughout the study duration, and were consecutively enrolled by the local investigator in one or more of the departments listed on the title page; the number of controls enrolled was equal to the number of patients and had a disease unrelated to HCV. Written informed consent was obtained from all study participants.

Data collection

In each hospital, patients and controls were interviewed by the same physician using a standardized questionnaire that consisted of 3 sections—sociodemographic characteristics, medical history, and history of behavioral and environmental exposure, including occupational exposure. Patients and controls were interviewed within 1 week of hospital admission.

HCV antibody and viral assays

Serum samples were collected from patients and controls at hospital admission and were sent to the Laboratory of Virology at the Istituto Superiore di Sanità (National Health Institute of Italy, Rome), where they were stored at -80°C until testing. Testing for HCV was performed in one batch (ie, once all the samples had been collected) and in the same laboratory. HCV antibodies were detected using an enzyme immunoassay (EIA-3, Ortho HCV 3rd generation; Ortho Diagnostic Systems, Raritan, NJ). HCV immunoreactivity was confirmed with a third-generation immunoblot assay (Riba-3; Chiron, Emeryville, CA; Ortho Diagnostic Systems). HCV-RNA was also determined in all patients (to make sure that HCV⁻ findings were not accounted for immunosuppression in those with B-NHL) and in anti-HCV⁺ controls. HCV-RNA was also measured in a randomly chosen 10% sample of anti-HCV⁻ controls (Cobas Amplicor 2.0; Roche Diagnostic Systems, Branchburg, NJ). Genotyping was performed using the Innogenetics Line Probe Assay (Innogenetics, Zwijndrecht, Belgium).

For the purposes of this analysis, patients were considered HCV⁺ if they had antibodies to HCV or if HCV-RNA was detected. All patients with anti-HCV⁺ lymphoma were tested for the presence of anti-HIV using the Abbott HIV1/2 gO EIA (Abbott Laboratories, Abbott Park, IL).

Data analysis

Crude and adjusted odds ratio (OR) and corresponding 95% confidence interval (CI) were computed by means of unconditional multiple logistic regression including age (both as a categorical variable, in 10-year groups, and as a continuous variable), sex, level of education, and place of birth. Furthermore, to determine whether the inclusion of patients and controls with histories of blood transfusion, intravenous drug use, previous chronic illnesses, or surgical intervention could have biased the OR estimates, we determined the distribution of these factors among patients and controls and adjusted for them when distribution varied between the 2 groups. Attributable risk was computed using the method described by Bruzzi et al.²⁵

Results

The study population consisted of 400 persons diagnosed with B-NHL (patients) and 396 controls. The hospital departments in

Table 2. HCV prevalence by type of B-NHL

| Type of B-NHL | No. patients | No. HCV ⁺ | % HCV ⁺ |
|--------------------------------|--------------|----------------------|--------------------|
| Diffuse large B-cell lymphoma | 205 | 39 | 19.0 |
| Burkitt/Burkitt-like lymphoma | 10 | 2 | 20.0 |
| Mantle cell lymphoma | 15 | 2 | 13.3 |
| Small lymphocytic lymphoma* | 18 | 2 | 11.1 |
| Lymphoplasmacytic lymphoma† | 13 | 4 | 30.8 |
| Follicular lymphoma | 79 | 11 | 13.9 |
| MALT§ lymphoma | 25 | 3 | 12.0 |
| Marginal zone B-cell lymphoma‡ | 15 | 4 | 26.6 |
| Unspecified B-cell lymphoma | 20 | 3 | 15.0 |
| Total | 400 | 70 | 17.5 |

*Excludes B-cell chronic lymphocytic leukemia and prolymphocytic leukemia.

†Includes Waldenström macroglobulinemia.

‡Four nodal, 11 splenic.

§Mucose-associated lymphoid tissue.

which the controls were recruited were as follows: internal medicine (26.3%), ophthalmology (24.5%), general surgery (13.9%), dermatology (11.9%), orthopedics (7.1%), and other (16.4%). The most frequent diagnoses were retinal detachment and cataract (19%), nephrolithiasis (14%), thrombophlebitis, cardiomyopathy, hypertension, or hypercholesterolemia (13%), atopic dermatitis or urticarial eruption (7%), and trauma (5%).

Table 1 shows the prevalence of HCV infection by sex and age for patients and controls. Of the 400 patients, 70 were considered HCV⁺; specifically, 69 of these patients were positive for HCV antibodies, of whom 10 were negative for HCV-RNA; a single patient was negative for HCV antibodies yet positive for HCV-RNA. Of the 396 controls, 22 were considered HCV⁺, all of whom were positive for HCV antibodies and 6 of whom were HCV-RNA⁻. Overall prevalence among the patients was higher than that among the controls (17.5% for patients compared to 5.6% for controls). In each sex and age group, prevalence was always significantly higher in patients than in controls. As expected, prevalence increased with age for patients and controls. One patient with HCV⁺ lymphoma was anti-HIV1⁺ and anti-HIV2⁺ and was excluded from the analysis.

Table 2 shows the prevalence of HCV infection among patients by type of B-NHL. The prevalence of HCV for each B-NHL type was consistently higher than the prevalence observed among controls. The highest prevalence rates were found among patients with lymphoplasmacytic and marginal zone lymphomas, both of which are considered indolent B-NHL. Among the 2 largest subgroups of B-NHL, HCV prevalence seemed more elevated among large B-cell lymphoma (19.0%), an aggressive B-NHL, than among follicular B-NHL, an indolent lymphoma. Among 10 patients with Burkitt/Burkitt-like B-NHL, the HCV prevalence was 20.0%. Both patients who were HCV⁺ had received diagnoses of Burkitt-like B-NHL.

Table 3 shows HCV prevalence among patients and controls by the degree of histologic differentiation of B-NHL (indolent or aggressive). When comparing prevalence in patients and in controls, adjusting for possible confounding factors (sex, age, level of education, and place of birth), the OR was 3.1 (95% CI, 1.8-5.2) for all types of B-NHL, 2.3 (95% CI, 1.3-4.4) for indolent B-NHL, and 3.5 (95% CI, 2.0-6.3) for aggressive B-NHL.

With regard to other possible confounding variables, 1 (0.2%) of 400 patients and 10 (2.5%) of 396 controls reported intravenous drug use, and 50 (12.5%) of 400 patients and 31 (7.8%) of 396 controls reported blood transfusion. When adjusting for these variables, the OR differed only slightly from those reported in

Table 1. HCV prevalence by sex and age among patients with B-NHL and controls

| | Patients | | | Controls | | |
|---------------|--------------|----------------------|--------------------|--------------|----------------------|--------------------|
| | No. patients | No. HCV ⁺ | % HCV ⁺ | No. controls | No. HCV ⁺ | % HCV ⁺ |
| Sex | | | | | | |
| Male | 246 | 42 | 17.1 | 205 | 15 | 7.3 |
| Female | 154 | 28 | 18.2 | 191 | 7 | 3.6 |
| Age, y | | | | | | |
| 15-35 | 34 | 2 | 5.9 | 103 | 0 | 0.0 |
| 36-55 | 129 | 16 | 12.4 | 128 | 6 | 4.7 |
| 56-75 | 187 | 41 | 21.9 | 125 | 10 | 8.0 |
| 76 and older | 50 | 11 | 22.0 | 40 | 6 | 15.0 |
| Total | 400 | 70 | 17.5 | 396 | 22 | 5.6 |

Table 3. HCV prevalence and crude and adjusted OR (patients vs controls) by degree of severity of B-NHL

| | Total | No. HCV ⁺ | % HCV ⁺ | Crude OR (95% CI) | Adjusted OR (95% CI) |
|-------------------------------|-------|----------------------|--------------------|-------------------|----------------------|
| Controls | 396 | 22 | 5.6 | 1 | 1 |
| Indolent B-NHL | 170 | 27 | 15.9 | 3.2 (1.8-5.8) | 2.3 (1.3-4.4) |
| Aggressive B-NHL | 230 | 43 | 18.7 | 3.9 (2.3-6.7) | 3.5 (2.0-6.3) |
| Patients with all types B-NHL | 400 | 70 | 17.5 | 3.6 (2.2-6.0) | 3.1 (1.8-5.2) |

OR was adjusted by age (continuous), sex, level of education, and place of birth.

Table 3 (data not shown). The proportion of patients who had a previous chronic illness or who had undergone surgery (76%) was similar to that among controls (73%); thus, we did not adjust for this variable. In our study, therefore, the fraction of B-NHL attributable to HCV infection was 4.6% (95% CI, 2.7-6.5).

Table 4 shows that the distribution of HCV genotypes was similar among HCV⁺ patients and controls. The most commonly found genotypes were 1b and 2a/2c.

Discussion

To the best of our knowledge, this is the largest case-control study on the possible association between HCV infection and B-NHL. The finding that the patients were 3.1 times more likely to have been infected than the controls suggests that an association does indeed exist. An association was observed among indolent and among aggressive B-NHL (OR, 2.3 and 3.5, respectively). In contrast to our study, some previous studies^{4,5} that included prevalent and incident disease found a particularly high HCV prevalence in indolent NHL. These studies, however, could have been affected by a prevalence bias if, for example, patients with aggressive lymphoma infected by HCV had poorer survival.

With regard to an association between HCV infection and specific types of B-NHL, the number of patients of each type of B-NHL was too small to calculate the OR. With regard to a possible association between B-NHL and specific HCV genotypes, in our study the genotype distribution did not differ when comparing patients and controls, suggesting that the association with HCV infection is not genotype specific. Indeed, the most commonly identified genotypes (1b, 2a/2c) are the same as those identified most frequently in the general population in Italy, according to several seroepidemiologic population-based studies.²⁶⁻²⁹ These results are consistent with those of a previous study on HCV prevalence among patients with lymphoproliferative disorders in whom no association with a specific HCV genotype was found.⁵ In 2 studies, however, genotype 2a was more common among patients with monoclonal gammopathy than in patients without this disease.^{30,31} Approximately two thirds of the lymphoma patients were

male, consistent with a known higher incidence of the disease among males than females.³²

In interpreting the results of this study, several limitations must be taken into account. Selection bias is always difficult to rule out completely in case-control studies. To minimize this possibility, the controls were recruited in the same hospitals as the patients, although from different departments, and they included a broad range of persons with different newly diagnosed diseases unrelated to HCV infection. HCV prevalence among controls (5.6%) was similar to that expected in the general population of comparable distribution by age group and geographic area. In fact, this prevalence is similar to the 5.1% prevalence found in a multicenter, hospital-based, case-control study on HCV and leukemia carried out between 1986 and 1990.³³ Controls were younger than patients, but anti-HCV prevalence was higher in patients of all age groups. Furthermore, when the adjustment was made, we considered age a categorical variable and, within each age group, a continuous variable. In addition to adjusting the OR for age, sex, level of education, and place of birth, other possible confounders were evaluated (history of intravenous drug use, blood transfusion, chronic illness, surgery).

Our findings support the hypothesis that HCV plays a pathogenic role in inducing B-NHL.⁵⁻⁶ Although the mechanisms underlying its contribution to neoplastic transformation are unknown, 2 nonmutually exclusive pathogenic mechanisms have been proposed. First, neoplastic transformation may be causally linked to chronic antigen stimulation of B cells by HCV. Immunoglobulin variable region genes expressed by B-NHL cells from HCV⁺ patients have been shown to exhibit somatic mutations, indicative of an antigen-selection process.^{34,35} Moreover, the histologic presentation of many B-NHL cells from HCV⁺ patients is typical of germinal center (GC) and post-GC B cells,³⁶ again suggesting that lymphomagenesis occurs when B cells proliferate in response to a virus-associated antigen. Second, neoplastic transformation may also be the result of direct antiapoptotic pathways activated by HCV within B cells. In fact, HCV sequences have been detected in lymph node biopsy specimens from patients with B-NHL,⁸ and the presence of HCV-associated proteins within lymphoma cells has been demonstrated.⁶ Moreover, studies in severe combined immunodeficiency (SCID) mice³⁷ have provided evidence of the persistence and low-rate multiplication of HCV infection in human mononuclear cells. Finally, some HCV proteins have been shown to exert antiapoptotic effects in infected cells in transgenic mice.^{38,39}

Whereas the approximately 3-fold increased risk for B-NHL in HCV-infected patients in our study is consistent with risk estimates from other parts of Italy¹⁰⁻¹³ and elsewhere,^{14,15} the fraction of B-NHL attributable to HCV infection varies according to HCV prevalence in the different populations. Our findings suggest that in Italy approximately 1 of 20 cases of B-NHL may be attributable to HCV, and this points to some important new treatment options for patients. Interferon (IFN), in combination with ribavirin, has been shown to be effective in 50% of patients with chronic HCV-related liver disease⁴⁰ and to induce regression of splenic lymphoma in

Table 4. Distribution of HCV genotypes among patients with B-NHL and controls

| HCV genotype | Patients | | Controls | |
|--------------|----------|-------|----------|-------|
| | No. | % | No. | % |
| 1a | 3 | 4.9 | 1 | 6.2 |
| 1a/1b | 1 | 1.6 | 0 | 0.0 |
| 1b | 25 | 41.0 | 8 | 50.0 |
| 2a/2c | 28 | 45.9 | 7 | 43.8 |
| 4c/4d | 2 | 3.3 | 0 | 0.0 |
| Unknown | 1 | 1.7 | 0 | 0.0 |
| Total | 60 | 100.0 | 16 | 100.0 |

Excludes 10 HCV⁺ patients and 6 HCV⁺ controls who were HCV-RNA⁻.

HCV-RNA⁺ patients.⁴¹ Adequately large randomized clinical trials that evaluate the efficacy of IFN and ribavirin in HCV-infected patients with all types of B-NHL are strongly recommended.

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