High prevalence of hepatitis B virus infection in B-cell non-Hodgkin's lymphoma

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In this hospital-based, multicenter case-control study we investigated the prevalence of hepatitis B virus (HBV)-related markers and HBV/hepatitis C virus (HCV) co-infection among B-cell non-Hodgkin's lymphoma (B-NHL) cases and controls. Four hundred newly diagnosed B-NHL cases and 392 controls from other departments of the same hospitals were studied. The prevalence of positivity for hepatitis B surface antigen (HBsAg) was 8.5% among B-NHL cases and 2.8% among controls (adjusted odds ratio, 3.67; 95% confidence interval, 1.75-7.66). HBV/HCV co-infection was found in four cases, but in no controls. The finding of a positive association between HBV infection and B-NHL raises the possibility that HBV may play an etiologic role in the induction of B-NHL.

Key words: HBV, B-NHL, prevalence, HBV-HCV co-infection.

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses that can also replicate in lymphoid cells.[1,2] This has led to evaluation of potential associations between HCV or HBV infection with B-cell non-Hodgkin's lymphoma (B-NHL) as well as other hematologic malignancies. A large number of studies have investigated the association between HCV infection and B-NHL[3,4] demonstrating a positive association in countries where the prevalence of HCV infection is relatively high and, most recently, upon analysis of sufficiently large sample sizes, also in countries with very low prevalence of HCV infection.[5-7] On the other hand, relatively few studies have investigated the association between HBV infection and B-NHL. Three studies were designed as formal case-control studies with adequately described inclusion criteria for the control group and odds ratio (OR) adjusted for possible confounders. All of these studies reported a significantly higher risk of all types of NHL for hepatitis B surface antigen (HBsAg)-positive individuals than for controls.[8-10] We have previously reported on the positive association between HCV infection and B-NHL in a multicenter Italian case-control study using only newly diagnosed B-NHL patients (i.e., incident cases), with strict inclusion criteria for the control group, and with OR adjusted for potential confounders taken by interview.[11] In this report we present results on the association between HBV infection and B-NHL in the same patient population that had been previously screened for markers of HCV infection. All available sera were tested for HBsAg, antibodies to HBsAg (anti-HBs), and antibodies to hepatitis B core antigen (anti-HBc).

Design and Methods

Cases and controls

The study population was the same that had been investigated for HCV infection.[11] Briefly, it consisted of 400 B-NHL cases 15 years and older admitted from January 1998 through February 2001 to ten hospitals in different cities throughout Italy. The type and stage of B-NHL were defined according to the Revised European American Lymphoma (REAL)/World Health Organization (WHO) classifications.[12] The control group consisted of 392 patients admitted to other departments of the same hospitals with a newly diagnosed disease, unrelated to HBV or HCV. Written informed consent was obtained from all study participants. In each hospital, patients and controls were interviewed within one week of hospital admission by the same physician using a standardized questionnaire.[11]

Viral assays

Serum samples were tested for HBsAg, anti-HBs and anti-HBc using enzyme immunoassays (Auszyme Monoclonal, Corzyme, and Ausab EIA, respectively; all from Abbott Labs, IL, USA). Sera from all cases and controls were tested for HBsAg and anti-HBs. Anti-HBc was tested on sera from 396 cases and 382 controls because some of the sera were no longer available. All patients with HBsAg-positive B-NHL were tested for anti-

HIV using the Abbott HIV1/2 gO EIA (Abbott Labs). One case that tested positive was excluded from the analysis.

Data analysis

OR and corresponding 95% confidence intervals (CI) were calculated for socio-demographic variables. Unconditional logistic regression was used to estimate adjusted OR and 95% CI for markers of HBV and HCV infection, adjusting by 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 their distributions varied between the two groups. Attributable risk (AR) was computed using the module for STATA *aflogit* as described by Greenland and Drescher.[13]

Results and Discussion

Table 1 reports the distribution of demographic variables among cases and controls. Table 2 shows the prevalence of HBV serological markers among cases and controls and the adjusted OR (cases vs controls) for all types of B-NHL as well as for B-NHL according to whether indolent or aggressive. The adjusted OR for HbsAg-positive status of all types of B-NHL was 3.67 (95% CI, 1.75-7.66). The estimated AR was 6.2% (2.78%-9.50%). The adjusted OR for anti-HBc-positive status showed a tendency towards a positive association (1.28; 95% CI, 0.92-1.78), while a significant negative association was found for anti-HBs-positive status (OR, 0.61; 95% CI, 0.44-0.85). Similar results were obtained for indolent and aggressive B-NHL. As to the different B-NHL histotypes, only diffuse large B-cell lymphoma, an aggressive lymphoma, and follicular lymphoma, an indolent lymphoma, involved sufficient numbers of patients to draw firm conclusions. For both histotypes the prevalence of HBsAg was higher than that among controls (*data not shown*).

The results showing a negative association and a tendency towards a positive association between B-NHL and anti-HBs- and anti-HBc-positive status, respectively, led us to analyze the different subgroups of patients (all types of B-NHL and controls) who were positive or negative for anti-HBs and/or anti-HBc (Table 3). As can be seen, anti-HBs-positive, anti-HBc-positive patients were equally distributed among cases and controls (adjusted OR, 0.89; 95% CI, 0.60-1.30). On the other hand, a significantly higher number of anti-HBc-positive, anti-HBs-negative patients, (adjusted OR, 2.05; 95% CI, 1.24-3.37), but a significantly lower number of anti-HBs-positive, anti-HBc-negative patients (adjusted OR, 0.37; 95% CI, 0.20-0.73) were found among cases than controls. When HBsAg-positive patients were subtracted from anti-HBc-positive, anti-HBs-negative ones, the tendency towards a higher prevalence among B-NHL cases was confirmed, although the CI overlapped the null value (adjusted OR, 1.51; 95% CI, 0.86-2.67). Finally, we evaluated the cumulative prevalence of HCV (defined as positivity for anti-HCV and/or HCV-RNA)[11] and HBV (defined as positivity for HbsAg) infection in this population of patients. The results are shown in Table 4: 25.1% of the cases and 8.2% of the controls were positive for HCV infection and/or HBV infection, yielding an adjusted OR of 3.76 (95% CI 2.46-5.76) and an AR of 23.6% (17.9%-28.8%). HCV/HBV co-infection was found in four of 399 B-NHL cases, but in none of the 392 controls.

Table 1. Prevalence and OR of demographic variables in B-NHL cases and controls.

	Cases No. (%)	*Controls No. (%)*	*OR (95% CI)*
Age as categorical variable			
15-35	34 (25.2)	101 (74.8)	1
36-45	52 (47.3)	58 (52.7)	2.66 (1.55-4.56)
46-55	77 (52.7)	69 (47.3)	3.31 (1.99-5.50)
56-65	100 (62.9)	59 (37.1)	5.03 (3.04-8.33)
66-75	86 (56.9)	65 (43.1)	3.93 (2.37-6.51)
≥76	50 (55.6)	40 (44.4)	3.71 (2.10-6.56)
Age as continuous variable			1.02 (1.01-1.03)
Sex			
Female	153 (44.9)	188 (55.1)	1
Male	246 (54.7)	204 (45.3)	1.48 (1.11-1.97)
Level of Education			
Any	189 (61.2)	120 (38.8)	1
Low	81 (50.6)	79 (49.4)	0.65 (0.44-95.7)
Medium	82 (44.3)	103 (55.7)	0.50 (0.35-0.73)
High	47 (36.2)	83 (36.8)	0.36 (0.24-0.55)
Place of Birth			
Northern Italy	50 (64.1)	28 (35.9)	1
Central Italy	78 (50.7)	76 (49.3)	0.57 (0.32-1.00)
Southern Italy and Islands	262 (48.8)	275 (51.2)	0.53 (0.32-0.87)
Foreign Country	9 (40.9)	13 (59.1)	0.38 (1.47-1.02)

Table 2. Prevalence and adjusted OR (cases vs controls) of HBV-related markers in B-NHL cases (all types, indolent, aggressive) and controls.*

	Cases No. positive/ No. tested (%)	*Controls No. positive/ No. tested (%)*	*Adjusted OR# (95% CI)*
HbsAg			
All types of B-NHL	34/399 (8.5)	11/392 (2.8)	3.67 (1.75-7.66)
Indolent B-NHL	14/169 (8.3)		3.64 (1.55-8.64)
Aggressive B-NHL	20/230 (8.7)		3.75 (1.70-8.28)
Anti-HBs			
All types of B-NHL	113/399 (28.3)	145/392 (37.0)	0.61 (0.44-0.85)
Indolent B-NHL	45/169 (26.6)		0.57 (0.38-0.88)
Aggressive B-NHL	68/230 (29.6)		0.63 (0.44-0.93)
Anti-HBc			
All types of B-NHL	164/395 (41.5)	111/382 (29.1)	1.28 (0.92-1.78)
Indolent B-NHL	70/167 (41.9)		1.29 (0.85-1.94)
Aggressive B-NHL	94/228 (41.2)		1.27 (0.87-1.86)

**HBsAg, anti-HBs, and anti-HBc were tested for all sera from cases and controls that were available at the time of screening for the individual markers. #OR was adjusted by age (as a categorical variable, in 10-year groups, and as a continuous variable), sex, level of education and place of birth.*

Table 3. Prevalence and adjusted OR (cases vs controls) of anti-HBs and/or anti-HBc in B-NHL cases (all types) and controls.*

	Cases	Controls	Adjusted OR# (95% CI)
Anti-HBs⁻, anti-HBc⁻	216	217	1
Anti-HBs⁺, anti-HBc⁻	15	54	0.37 (0.20-0.73)
Anti-HBs⁺, anti-HBc⁺	94	81	0.89 (0.60-1.30)
Anti-HBs⁻, anti-HBc⁺	70	30	2.05 (1.24-3.37)
Anti-HBs⁻, anti-HBc⁺, HBsAg⁻	44	25	1.51 (0.86-2.67)

**HBsAg, anti-HBs, and anti-HBc were tested for all sera from cases and controls that were available at the time of screening for the individual markers. #OR was adjusted by age (as a categorical variable, in 10-year groups, and as a continuous variable), sex, level of education and place of birth.*

Table 4. Prevalence and adjusted OR of markers of HCV* and/or HBV° infection in B-NHL cases (all types) or controls.

	Patients No. positive/ No. tested (%)	Controls No. positive/ No. tested (%)	Adjusted OR# (95%CI)
Markers of HCV and/or HBV infection	101/399 (25.1)	32/392 (8.2)	3.76 (2.46-5.76)
Markers of HCV and HBV infection	4/399 (1.0)	0/392 (0.0)	NC^ (1.03-∞)

**Patients or controls positive for anti-HCV and/or HCV-RNA11; °patients or controls positive for HbsAg; ^NC: not computable; #OR was adjusted by age (as a categorical variable, in 10-year groups, and as a continuous variable), sex, level of education and place of birth.*

This study was designed to assess the prevalence of HBV serological markers in B-NHL. The results demonstrate a significantly higher prevalence of HBV infection in B-NHL cases than in controls. Thus, we found that the prevalence of HBsAg was 8.5% among B-NHL cases and 2.8% among controls. The prevalence in our control population should be compared with a value of ~1.5% in the general Italian population.[14] This difference may be explained by our control population having a higher median age than that of the general Italian population (51 vs. 41.8 years). The adjusted OR of all types of B-NHL for HBsAg-positive status was 3.67, while the estimated AR was 6.2%. A similar positive association was observed for both indolent and aggressive B-NHL. Since the B-NHL cases of our study were newly diagnosed, before any cytotoxic and corticosteroid treatment had been administered, these results suggest that HBV might be directly involved in inducing and/or sustaining neoplastic transformation in B-NHL.

In addition to the higher prevalence of HBsAg, we also found a tendency towards a higher prevalence of anti-HBc, and a significantly lower prevalence of anti-HBs among cases than among controls. When we analyzed subgroups of anti-HBs- and/or anti-HBc-positive patients, we found that anti-HBs-positive, anti-HBc-positive patients were equally distributed among cases and controls. On the other hand, a significantly higher number of anti-HBc-positive, anti-HBs-negative patients, (adjusted OR 2.05), but a significantly lower number of anti-HBs-positive, anti-HBc-negative patients were found among cases than among controls (adjusted OR 0.37). The latter result might be due, at least in part, to a higher prevalence of vaccinated individuals among our controls. We did not collect information on the vaccination status of the patients in this study and cannot, therefore, comment on this possibility. An alternative explanation, not mutually exclusive with the previous one, is that an anti-HBs antibody response, in the absence of anti-HBc (*anti-HBs alone*), may be especially effective in the control of HBV replication in lymphoid cells, thereby preventing any contribution of HBV to neoplastic transformation. As regards the finding of a positive association of anti-HBc-positivity, anti-HBs-negativity and B-NHL, when we subtracted the number of HBsAg-positive patients from both cases and controls, the tendency towards a higher prevalence of this serological profile in B-NHL cases was confirmed, although the 95% CI of the adjusted OR overlapped the null value. It is noteworthy that the serological pattern *anti-HBc alone* has raised considerable interest because a significant proportion of patients with this profile has detectable HBV-DNA in the serum, and an even higher proportion was found to harbor HBV-DNA in the liver, thereby conforming with the definition of *occult* HBV infection[15-17] In the light of this previous knowledge, our findings suggest that positivity for HBsAg may underestimate the prevalence of HBV infection in our patient population and that determination of HBV-DNA may yield a more accurate picture of the actual prevalence of HBV infection in B-NHL patients as compared to controls.

Previous results obtained with the same population of patients investigated in this study had demonstrated a positive association between HCV and B-NHL.[11] On the basis of the results of the present study we have calculated the cumulative prevalence of HCV and HBV (patients positive for HBsAg) infection in B-NHL cases and controls. The prevalence of HCV and/or HBV infection was 25.1% among B-NHL cases and 8.2% among controls (adjusted OR 3.76, AR of 23.6%). Interestingly, four of 399 B-NHL cases were co-infected by HCV/HBV, but none of the controls was, suggesting the possibility of a higher prevalence of HCV/HBV co-infection in B-NHL patients. HCV/HBV co-infection has been shown to carry an additive risk of hepatocellular carcinoma.[18] It will be interesting to investigate, in larger studies, whether this also holds true for B-NHL.

In summary, our results demonstrate a significantly higher prevalence of HBV infection in B-NHL cases than in controls. HBV/HCV co-infection was detected in some B-NHL cases but in no controls. These findings raise the possibility that HBV may play an etiologic role, in addition to its established role in the induction of hepatocellular carcinoma, also in the induction of B-NHL. As regards the possible role of HBV in inducing and/or sustaining lymphomagenesis, given that HBV is both hepatotropic and lymphotropic,[2] it is reasonable to put foward the same, non-mutually exclusive mechanisms that have been considered to explain its role in the pathogenesis of hepatocellular carcinoma. Thus,

both viral and host factors have been implicated in this process. First, the virus, may have a direct role, for example, through the action of the HBV X protein that has been shown to transactivate cellular genes associated with cellular growth control[19] and inhibit p53 gene function *in vitro*.[20] Second, it may have an indirect role through HBV-specific, chronic immune-mediated cell injury, which has been shown to be sufficient to initiate and sustain the process of hepatocarcinogenesis.[21]

All authors declare that they participated in the design, execution, and analysis of this study, and that they have seen and approved the final version of the article. The authors also declare that they have no conflict of interest in connection with this paper.

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References

1. Yoffe B, Noonan CA, Melnick JL, Hollinger F. Hepatitis B virus DNA in mononuclear cells and analysis of cell subsets for the presence of replicative intermediates of viral DNA. J Infect Dis 1986;153:471-7.
2. Bronowicki JP, Loriot MA, Thiers V, Grignon Y, Zignego AL, Bréchot C. Hepatitis C virus persistence in human hematopoietic cells injected into SCID mice. Hepatology 1998;28:211-8.
3. Musto P. Hepatitis C virus infection and B-cell non-Hodgkin's lymphomas: more than a simple association. Clin Lymphoma 2002;3:150-60.
4. Negri E, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. Int J Cancer 2004;111:1-8.
5. Duberg A-S, Nordström M, Törner A, Reichard O, Strauss R, Janzon R et al, Non-Hodgkin's lymphoma and other nonhepatic malignancies in Swedish patients with hepatitis C virus infection. Hepatology 2005;41:652-9.
6. Engels EA, Chatterjee N, Cerhan JR, Davis S, Cozen W, Severson RK et al, Hepatitis C virus infection and non-Hodgkin lymphoma: results of the NCI-SEER multi-center case-control study. Int J Cancer 2004;111:76-80.
7. McOmber Morton L, Engels EA, Holford TR, Leaderer B, Zhang Y, Zahn SH et al, Hepatitis C virus and risk of non-Hodgkin lymphoma: a population-based case-control study among Connecticut women. Cancer Epidemiol Biomarkers Prev 2004; 13:425-30.
8. Pioltelli P, Gargantini L, Cassi E, Santoleri L, Bellati G, Magliano EM, et al. Hepatitis C virus in non-Hodgkin's lymphoma. A reappraisal after a prospective case-control study of 300 patients. Am J Hematol 2000;64:95-100.
9. Kim JH, Bang YJ, Park BJ, Yoo T, Kim CW, Kim TY, et al. Hepatitis B virus infection and B-cell non-Hodgkin's lymphoma in a hepatitis B endemic area: a case-control study. Jpn J Cancer Res 2002;93:471-7.
10. Talamini R, Montella M, Crovatto M, Dal Maso L, Crispo A, Negri E, et al. Non-Hodgkin lymphoma and hepatitis C virus: a case-control study from northern and southern Italy. Int J Cancer 2004;110:380-5.
11. Mele A, Pulsoni A, Bianco E, Musto P, Szklo A, Sanpaolo MG, et al. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study. Blood. 2003;102:996-9.
12. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization classification of tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press. 2001.
13. Greenland S, Drescher K. Maximum likelihood estimation of attributable factions from logistic models. Biometrics 1993;49:865-72.
14. Stroffolini T, Bianco E, Szklo A, Bernacchia R, Bove C, Colucci M et al, Factors affecting the compliance of the antenatal hepatitis B screening programme in Italy. Vaccine 2003;21:1246-9.
15. Larsen J, Hetland G, Skaug K. Post-transfusion hepatitis B transmitted by blood from a hepatitis B surface antigen–negative virus carrier. Transfusion 1990;30:431-2.
16. Bréchot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely occult? Hepatology 2001;34:194-203.
17. Jilg W, Hottenträger B, Weinberger K, Schlottmann K, Frick E, Holstege A et al, Prevalence of markers of hepatitis B in the adult German population. J Med Virol 2001;63:96-102.
18. Tanaka H, Tsukuma H, Yamano H, Oshima A, Shibata H. Prospective study on the risk of hepatocellular carcinoma among hepatitis C virus-positive blood donors focusing on demographic factors, alanine aminotransferase level at donation and interaction with hepatitis B virus. Int J Cancer 2004;112:1075-80.
19. Maguire HF, Hoeffler JP, Siddiqui A. HBV X protein alters the DNA binding specificity of CREB and ATF-2 by protein-protein interactions. Science 1991; 252:842-4.
20. Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc Natl Acad Sci USA 1994;91:2230-4.
21. Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. J Exp Med 1998;188:341-50.