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Epidemiology of hepatitis C virus in lymphomas

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Hepatitis C virus (HCV) is an RNA virus and is a member of the Flaviviridae family. It is the most common cause of sporadic non-A non-B and post-transfusion hepatitis (Kuo et al, 1989) and a major cause of chronic liver diseases, including liver cirrhosis and hepatocellular carcinoma. In addition to the direct liver injury, various extrahepatic manifestations includine autoimmune disorders, glomerular injury, vasculitis, sicca syndrome, and type-II cryoglobulinemia (MCII) may occur in these patients. MCII is a systemic vasculitis often characterized as "benign" B-cell proliferation that evolves into B-cell non-Hodgkin lymphoma (B-NHL) in 8-10% of affected cases. An estimated 40-100% of patients with MCII are chronically infected with HCV (Agnello *et al*, 1992; Pozzato *et al*, 1994). This finding 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, whether related or not to MCII. Results of these studies have been conflicting.

A positive association has been found in studies conducted in countries in which the prevalence of HCV infection is relatively high [Italy (Ferri *et al*, 1994; Luppi *et al*, 1996; Vallisa *et al*, 1999; Montella *et al*, 2001; Talamini *et al*, 2004)], and parts of the United States (Zuckerman *et al*, 1997), Japan (e.g. Iwata *et al*, 2004) — while results from other studies refuted any such association (e.g. McColl and Tait, 1996; Collier *et al*, 1999; Hausfater *et al*, 2001). These latter studies, however, had been conducted in populations in whom HCV infection is rare (reviewed in Negri *et al*, 2004). Thus, while approximately 3% of the world population, representing 170 million people, is chronically infected with HCV, the prevalence of HCV infection varies by geographic region and population subgroup from less than 0.5% to more than 20%. This suggests that studies in geographic regions with low HCV prevalence may disclose a positive association between HCV infection and B-NHL only with large sample sizes (De Sanjose *et al*, 2004; Engels *et al*, 2004; McOmber Morton *et al*, 2004).

Our own group has conducted, in collaboration with others, a hospital-based case-control study from January 1998 through February 2001 to evaluate the association betweenHCVinfection and B-NHL of different types (Mele *et al*, 2003). Cases were consecutive patients with a new diagnosis of B-NHL; controls were patients from other departments of the same hospitals. Both groups were interviewed using a standardized questionnaire. Infection with HCV was determined by measuring HCV antibodies in sera using an enzyme immunoassay and results were confirmed with a third-generation immunoblot assay. In addition, we determined HCV-RNA in all patients, and in anti-HCV-positive controls. HCV-RNA was also measured in a randomly chosen 10% sample of anti-HCV- controls. Odds ratio (OR) (crude or adjusted for possible confounding factors) and corresponding 95% confidence interval (CI) were estimated. Table 1 shows the prevalence of HCV infection by sex and age for patients and controls. Of the 400 patients, 70 were considered HCV-positive; 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-positive, all of whom were positive for HCV antibodies and 6 of whom were HCV-RNA-negative. Overall prevalence among the patients was higher than that among the controls (17.5% for patients compared to 5.6% for controls). HCV prevalence among controls was similar to that expected in the general population of comparable distribution by age group and geographic area. 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.

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

	Patients			Controls		
	No. patients	No. HCV+	% HCV+	No. patients	No. 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	50	11	22.0	40	6	15.0
Total	400	70	17.5	396	22	5.6

As to the prevalence of HCV infection by B-NHL histotype, for each B-NHL type it 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. The number of patients of each type of NHL, however, was too small to calculate OR. 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. Table 3. HCV prevalence and crude and adjusted OR (patients vs controls) by degree of severity of B-NHL. The distribution of HCV genotypes was similar among HCV+ patients and controls. The most commonly found genotypes were 1b and 2a/2c (data not shown). The results from this study confirmed an association between HCV and B-NHL and suggest that in Italy, 1 of 20 instances of B-NHL may be attributable to HCV infection. While all of the studies referred to so far, including our own, are based solely on serological evidence of HCV infection, a recent report from Turkey suggests that HCV-RNA may be detected in tissue (lymph node) samples in a higher number of B-NHL patients than in the corresponding sera (Paydas *et al*, 2004). Although the number of patients investigated in this study was too small to draw definitive conclusions, these results suggest that occult HCV infections may further strengthen the association between HCV infection and B-NHL. This observation would be of significant interest also as regards the possibilities of therapeutic intervention in HCV-infected B-NHL patients. Studies on the effects of antiviral treatment in these patients will be discussed below. These observations on the association between HCV infection and B-NHL have raised the

Table 2. HCV prevalence by type of B-NHL.

Type of B-NHL	No. patients	No. HCV+	% HCV+
Diffuse 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; ^IncludesWaldenström macroglobulinemia.

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)

question as to the role of HCV in the pathogenesis of B-NHL. This is not a trivial question because HCV contains an RNA genome which replicates in the cytoplasm, does not contain an obvious oncogene, and does not integrate into host genomes. Therefore, the mechanism of its oncogenesis remains unclear. To this regard, 2 nonmutually exclusive mechanisms have been proposed. First, neoplastic transformation may be causally linked to chronic antigen stimulation of B cells by HCV. We refer to this as the indirect mechanism of HCV contributing to lymphomagenesis. Thus, immunoglobulin variable region genes expressed by B-NHL cells from HCV-positive patients have been shown to exhibit somatic mutations, indicative of an antigen-selection process (De Re *et al*, 2000a; Ivanovski *et al*, 1998). Moreover, the histologic presentation of many B-NHL cells from HCV-positive patients is typical of germinal center (GC) and post-GC B cells (De Re *et al*, 2000b), again suggesting that lymphomagenesis occurs when B cells proliferate in response to a virus-associated antigen. The second mechanism suggests that HCV may directly impact on the cellular pathways that lead to lymphomagenesis. Accordingly, we will refer to this as the direct mechanism of HCV contributing to lymphomagenesis. Thus, studies in severe combined immunodeficiency mice (Bronowicki *et al*, 1998) have provided evidence of the persistence and low-rate multiplication of HCV infection in human mononuclear cells and expression of the virus core protein was associated with malignant lymphoma in transgenic mice (Ishikawa *et al*, 2003). As to the molecular mechanisms that may underlie direct oncogenic effects of HCV, most recently it has been demonstrated that acute and chronic HCV infection cause a 5- to 10-fold increase in mutation frequency in Ig heavy chain, BCL-6, p53, and ,-catenin genes of in vitro HCV-infected B cell lines and HCV-associated peripheral blood mononuclear cells, lymphomas, and HCCs (Machida *et al*, 2004). HCV induced error-prone DNA polymerases, and activation-induced cytidine deaminase, which together, contributed to the enhancement of mutation frequency. These results indicate that HCV induces a mutator phenotype and may transform cells by a hit-and-run mechanism.

The observation, in most studies, of a positive association between HCV infection and B-NHL implies consequences as to the possibilities of therapeutic intervention in HCV-infected B-NHL patients. In fact, if such a positive association reflects the role of HCV in inducing neoplastic transformation and in sustaining proliferation of malignant cells, then antiviral therapy may induce regression of B-NHL. This possibility was appreciated and verified first by Hermine and collaborators (2002). They observed regression in patients with splenic lymphoma after antiviral (interferon) treatment of hepatitis C virus infection. Importantly, in this study interferon was only efficacious in lymphomas associated with serologically determined HCV infection, while no hematologic response to interferon was registered when HCV infection was not present. This suggests that, in these patients, occult HCV infection, as suggested in the study of Paydas *et al*. (2004), was either absent or it did not play any role in sustaining the proliferation of malignant cells. Regression of lymphoproliferative disorder after antiviral treatment was also observed in a single patient with a partial trisomy 3, Bcl-2 overexpression, and MCII (Casato *et al*, 2002). The effect of antiviral treatment in HCV-infected B-NHL patients has been investigated also in a recently published Italian multicenter study (Vallisa *et al*, 2005). Thirteen patients with indolent, low-grade B-NHL and carrying HCV infection were submitted to antiviral treatment alone with pegilated interferon and ribavirin. Of twelve assessable patients, seven (58%) achieved complete response and two (16%) partial hematologic response at 14.1 $\pm$ 9.7 months, while two had stable disease with only one patient experiencing progression of disease. Hematologic responses were highly significantly associated to clearance or decrease in serum HCV viral load following treatmentTreatment-related toxicity did not cause discontinuation of therapy in all but two patients, one of whom, however, achieved complete response.

Conclusions

A large number of studies that have been performed over the last decade strongly suggest a positive association between HCV infection and B-NHL. This was first assessed in geographic areas where prevalence of HCV infection is relatively high, but most recent studies suggest that this holds true also for countries with low prevalence, if the sample size of the investigated population is large enough. The association between HCV infection and B-NHL has led to investigate possible therapeutic effects of anti-HCV therapy in patients with indolent B-NHL. Results that have been published so far have been positive and suggest that antiviral therapy may become a routinary therapeutic intervention in HCV-positive, indolent B-NHL patients.

References

Agnello V et al. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med. 1992;19:1490-1495.

Bronowicki et al. Hepatitis C virus persistence in human hematopoietic cells injected into SCID mice. Hepatology. 1998;28:211-218.

Casato et al. Regression of lymphoproliferative disorder after treatment for hepatitis C virus infection in a patient with a partial trisomy 3, Bcl-2 overexpression, and type II cryoglobulinemia. Blood. 2002;99:2259-2261.

Collier et al. No association between hepatitis C and B-cell lymphoma. Hepatology. 1999;29:1259-1261.

De Re et al. Sequence analysis of the immunoglobulin antigen receptor of hepatitis C virus-associated non-Hodgkin lymphomas suggests that the malignant cells are derived from the rheumatoid factor-producing cells that occur mainly in type II cryoglobulinemia. Blood. 2000a;96:3578-3584.

De Re et al. Pre-malignant and malignant lymphoproliferations in an HCV-infected type II mixed cryoglobulinemic patient are sequential phases of an antigen-driven pathological process. Int J Cancer. 2000b;87:211-216.

De Sanjose et al. Role of heptatitis C virus infection in malignant lymphoma in Spain. Int J Cancer. 2004;111:81-85.

Engels 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.

Ferri et al. Hepatitis C infection in patients with non-Hodgkin's lymphoma. Br J Haematol. 1994;88:392-394.

Hausfater et al. Hepatitis C virus infection and lymphoproliferative diseases: prospective study on 1,576 patients in France. Am J Hematol. 2001;67:168-171.

Hermine et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002;347:89-94.

Ivanovski et al. Somatic hypermutation, clonal diversity, and preferential expression of the VH 51p1/VL kv325 immunoglobulin gene combination in hepatitis C virusassociated immunocytomas. Blood. 1998;91:2433-2442.

Ishikawa et al S. Expression of hepatitis C virus core protein associated with malignant lymphoma in transgenic mice. Comp Immunol Microbiol Infect Dis. 2003;26:115-124.

Iwata et al. High incidences of malignant lymphoma in patients infected with hepatitis B or hepatitis C virus. Haematologica. 2004;89:368-370.

Kuo et al. An assay for circulating virus of human NANB hepatitis. Science. 1989;244:3623–3664.

Luppi et al. Hepatitis C virusinfection in subsets of neoplastic lymphoproliferations not associated with cryoglobulinemia. Leukemia. 1996;10:351-355.

Machida et al. Hepatitis C virus induces a mutator phenotype: Enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci USA 2004; 101:4262-4267.

McColl and Tait. Hepatitis C virus infection in patients with lymphoproliferative disorders. Br J Haematol. 1996;92:771-772.

McOmber Morton 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;13:425-430.

Mele et al. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study. Blood. 102:996-999.

Montella et al. HCV and tumors correlated with immune system: a case control study in an area of hyperendemicity Leuk Res. 2001;9:775-781.

Negri et al.B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. Int J Cancer. 2004;111:1-8.

Paydas et al. Detection of hepatitis C virus RNA in paraffin-embedded tissues from patients with non-Hodgkin's lymphoma. Am J Hematol. 2004;76:252-257.

Pozzato et al. Low grade malignant lymphoma, hepatitis C virus nfection, and mixed cryoglobulinemia. Blood. 1994;84:3047-3053.

Talamini et al. Non-Hodgkin's lymphoma and hepatitis C virus: a case-control study from northern and southern Italy. Int J Cancer. 2004;110:380-385.

Vallisa et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: A multicenter Italian experience. J Clin Oncol 2005;23:468-473.

Yoshikawa et al. Prevalence of hepatitis C virus infection in patients with non-Hodgkin's lymphoma and multiple myeloma. J Clin Gastroenterol. 1997;25:713-714.

Zuckerman et al. Hepatitis C virus in patients with B-cell non-Hodgkin lymphoma. Ann Intern Med. 1997;127:423-428.