

## Prospective study of hepatitis C viral infection as a risk factor for subsequent B-cell neoplasia

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Several case-control studies have found increased prevalence of hepatitis C virus (HCV) in patients with non-Hodgkin lymphoma (NHL) and other B-cell lymphoproliferative disorders. We examined whether HCV infection preceded the development of these neoplasms in a prospective cohort study of 48 420 individuals in northern California. Stored sera from 95 subjects with NHL (n = 57), multiple myeloma

(n = 24), or Hodgkin disease (n = 14) diagnosed a mean of 21 years after phlebotomy were screened for antibodies to HCV as well as viral RNA, based on previous reports of antibody-negative viremia. Sera from 4 cases and one of 95 age-, sex-, and race-matched controls were repeatedly reactive by enzyme immunoassay, but none were confirmed by recombinant immunoblot assay; none of the case

sera had HCV RNA by reverse transcription-polymerase chain reaction. Although acquisition in later life cannot be ruled out, these prospective data do not support a substantial role of chronic HCV infection in the etiology of B-cell neoplasia. (Blood. 2002;99:4240-4242)

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### Introduction

Hepatitis C virus (HCV) infection has been variably associated with increased risk of various B-cell lymphoproliferative disorders in case-control studies, primarily in studies from Italy.<sup>1-8</sup> In several of these studies, antibody-negative viremia has reportedly accounted for a substantial fraction of HCV-associated tumors, in apparent contrast to the usually robust serologic response to this infection.<sup>1,3,6</sup> HCV infection is also the predominant cause of mixed cryoglobulinemia, a benign monoclonal lymphoproliferation that sometimes evolves to overt B-cell non-Hodgkin lymphoma (NHL).<sup>8</sup> Although the link with mixed cryoglobulinemia-related lymphoma is well established, the full scope of HCV's role as an etiologic factor for B-cell neoplasms is not yet clear.

A disadvantage of case-control studies in establishing causation is their inability to determine whether the exposure of interest occurred before or after onset of disease. Because incident HCV infection is uncommon after age 40,<sup>9</sup> we have examined HCV infection status in young adulthood as a predictor of subsequent B-cell malignancy in a prospective cohort study in northern California. We examined both serologic and molecular markers of HCV infection in banked sera with more than 30 years' follow-up to test the hypothesis that the risk of B-cell neoplasms is increased in chronic HCV infection.

### Study design

Study subjects were from the Child Health and Development Study (CHDS) cohort established in 1959 at the Kaiser Foundation Health Plan, Oakland, CA, an ethnically and socioeconomically diverse employed

population. The CHDS was originally designed to investigate the association of biologic, genetic, medical, and environmental factors during pregnancy with the development of the offspring. The cohort is comprised of the gravida, male partners, and offspring from 20 754 pregnancies recruited between June 1959 and September 1966. A total of 48 420 parents and children had data collected from interviews and medical records abstraction. In addition, 92% of the mothers, 76% of the fathers, and 16% of the children had blood samples drawn for long-term storage of serum at -20°C. The follow-up study was reviewed and approved by the Institutional Review Boards of the University of California at Berkeley, the Western Consortium for Public Health, and the Public Health Institute (Berkeley, CA).

Cases of B-cell malignancy diagnosed in cohort members were identified by computerized record linkage with the statewide California Cancer Registry and California death registrations through 1996. Tumors were classified according to the *International Classification of Diseases for Oncology*, second edition, as NHL (histologic classifications 9590 through 9642 and 9670 through 9698), multiple myeloma (9730 through 9732), or Hodgkin disease (9650 through 9667). Individual records were matched by a probabilistic algorithm using name, sex, race, and date of birth, with indeterminate matches resolved by manual review. Controls were selected from CHDS subjects free of any cancer diagnosis through 1996, individually matched to the cases by race/ethnicity, sex, decade of birth, and smoking status.

Sera from case and control subjects were screened for HCV antibodies with 2 different third-generation enzyme-linked immunosorbent assays (EIA-3; Ortho Diagnostic Systems, Raritan, NJ, and EIA-3; Abbott, Abbott Park, IL). Serum samples dually reactive by both HCV EIAs were confirmed with a third-generation strip immunoblot assay (RIBA-3; Chiron, Berkeley, CA). Case sera were also tested for HCV RNA by reverse transcription-polymerase chain reaction (RT-PCR) assays (Amplicor HCV PCR; Roche Molecular Diagnostic Systems, Nutley, NJ) at an analytical sensitivity of less than 100 copies/mL of serum.

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## Results and discussion

B-cell lymphoproliferative malignancies were diagnosed in 131 cohort members, of whom 36 were excluded because they lacked stored sera ( $n = 35$ ) or baseline data ( $n = 1$ ). Of the 95 remaining subjects, 57 had B-cell NHL, 24 had multiple myeloma, and 14 had Hodgkin disease. Sixty-seven were non-Hispanic white, 19 were African American, 5 were Asian, and 4 were Hispanic. Case subjects had a mean age at blood draw of 32.5 years (range, 19-55 years) and a mean age at diagnosis of 53.3 years (range, 19-76 years). Accordingly, there was a mean interval of 21 years (range, 0-35 years) between blood draw and cancer diagnosis.

Serum samples from 4 of the 95 case subjects and 1 of the 95 matched control subjects had repeatedly reactive HCV EIAs. However, none were confirmed as HCV seropositive by RIBA-3. Furthermore, none of the case sera were HCV RNA positive by RT-PCR.

On the basis of the general population prevalence of HCV in the 1960s, we estimate that 0.5%, or approximately 250 CHDS subjects, were HCV infected at cohort entry.<sup>9</sup> As none of the 95 subjects with B-cell neoplasia were HCV positive, the Poisson upper 95% confidence limit for their HCV prevalence is 3.1% [1-antilog(log 0.05/95)]. Thus, these data are inconsistent with a relative risk of B-cell neoplasia for HCV infection of any more than 6.2 (0.031/0.005). The corresponding maximum fraction of B-cell neoplasia in the general population potentially attributable to chronic HCV infection is 2.5%  $\{(6.2 - 1)/[6.2 - 1 + (1/0.005)]\}$ . Nevertheless, these data do not address a possible risk from shorter-term infection, as we did not have follow-up sera to rule out seroconversion in later life.

There are a large number of published case-control studies of HCV and lymphoma, with a profound regional variation in findings that parallels levels of HCV endemicity. Most studies from Italy show strong associations, with HCV prevalence among cases ranging from 20% to 40%,<sup>1-7</sup> although a few report only 2- or 3-fold increases in relative risk, particularly in the absence of cryoglobulinemia.<sup>8</sup> Several studies from Japan have also found a positive association, with prevalences among NHL cases of 8% to 16%.<sup>10,11</sup> In contrast, the majority of studies from nonendemic areas elsewhere in Europe<sup>12,13</sup> or from North America<sup>14,15</sup> have generally failed to find an association, with HCV prevalences among NHL cases between 0% and 2%. An exception is one study from Los Angeles, CA, that found a prevalence of 22% in B-cell

NHL case subjects, 4.5% in other hematologic malignancies, and 5% in general medicine clinic control subjects.<sup>16</sup>

There has been one previous prospective cohort study of this association. Among 2162 patients with HCV and chronic liver disease in Japan, the incidence of NHL was not significantly increased. On the basis of 4 observed cases in 12 400 person-years of observation, the relative risk was 1.9 (95% confidence limits, 0.6-5.4) compared with the general population.<sup>17</sup>

Blood transfusion, which conferred risk of HCV infection in the past, has also been a risk factor for NHL in some studies. In the Iowa Women's Study cohort, a history of blood transfusion was associated with an NHL relative risk of 1.6, with stronger associations for low-grade tumors.<sup>18</sup> Similarly, blood recipients in southern Sweden had standard morbidity ratios for malignant lymphoma of 2.7 in a hospital-based cohort and 3.1 in a population-based cohort.<sup>19</sup> Moreover, a neonatal transfusion cohort from Britain had a 2-fold NHL excess at 15 to 49 years of age, although the increase was not statistically significant ( $P = .12$ ).<sup>20</sup> In contrast, blood transfusion was not a significant risk factor for NHL in a case-control study from Olmstead County, MN, with an odds ratio of 0.84 (95% confidence limits, 0.50-1.41).<sup>21</sup>

Posttransplant lymphoproliferative disease, which includes B-cell NHL as its most extreme manifestation, has been variably associated with hepatitis C infection. Patient series from several countries report increased risk of this complication in HCV-positive transplant recipients, although one (from the United States) failed to find an association.<sup>22,23</sup> However, HCV infection does not seem to further increase the B-cell NHL risk associated with HIV infection. Hemophilic AIDS patients, who are nearly all HCV infected, are not at higher risk of lymphoma than other HIV-transmission categories, and homosexual male AIDS patients with NHL do not have an increased prevalence of HCV.<sup>24</sup>

Regardless of these inconclusive epidemiologic data, there is some molecular evidence in support of a possible causal role for HCV in the etiology of B-cell lymphoproliferative disorders. HCV may infect lymphocytes, as HCV replicative forms have been detected in peripheral blood mononuclear cells from HCV-infected subjects.<sup>25</sup> HCV infection is also associated with an increased frequency of circulating DNA with the *bcl-2-JH* recombination characteristic of follicular lymphoma,<sup>26</sup> and successful antiviral therapy may decrease recombination frequency.<sup>27</sup>

In summary, HCV infection acquired by young adulthood was not a risk factor for subsequent B-cell neoplasia in this population. However, an increased risk from infection at older ages was not excluded by these data. The striking geographic variability among studies of this association remains unexplained.

## References

1. Ferri C, Caracciolo F, Zignego AL, et al. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Br J Haematol*. 1994;88:392-394.
2. Cavanna L, Sbolli G, Tanzi E, et al. High prevalence of antibodies to hepatitis C virus in patients with lymphoproliferative disorders. *Haematologica*. 1995;80:486-487.
3. Luppi M, Grazia Ferrari M, et al. Hepatitis C virus infection in subsets of neoplastic lymphoproliferations not associated with cryoglobulinemia. *Leukemia*. 1996;10:351-355.
4. Mazza C, Zagonel V, Monfardini S, et al. Hepatitis C virus and non-Hodgkin's lymphomas. *Br J Haematol*. 1996;94:544-550.
5. Musto P, Dell'Olio M, Carotenuto M, Mangia A, Andriulli A. Hepatitis C virus infection: a new bridge between hematologists and gastroenterologists? *Blood*. 1996;88:752-754.
6. De Rosa G, Gobbo ML, De Renzo A, et al. High prevalence of hepatitis C virus infection in patients with B-cell lymphoproliferative disorders in Italy. *Am J Hematol*. 1997;55:77-82.
7. Vallisa D, Berte R, Rocca A, et al. Association between hepatitis C virus and non-Hodgkin's lymphoma, and effects of viral infection on histologic subtype and clinical course. *Am J Med*. 1999;106:556-560.
8. Silvestri F, Barillari G, Fanin R, et al. Hepatitis C virus infection among cryoglobulinemic and non-cryoglobulinemic B-cell non-Hodgkin's lymphomas. *Haematologica*. 1997;82:314-317.
9. Armstrong GL, Alter MJ, McQuillan GM, Margolis HS. The past incidence of hepatitis C virus infection: implications for the future burden of chronic liver disease in the United States. *Hepatology*. 2000;31:777-782.
10. Kuniyoshi M, Nakamura M, Sakai H, et al. Prevalence of hepatitis B or C virus infections in patients with non-Hodgkin's lymphoma. *J Gastroenterol Hepatol*. 2001;16:215-219.
11. Izumi T, Sasaki R, Tsunoda S, Akutsu M, Okamoto H, Miura Y. B cell malignancy and hepatitis C virus infection. *Leukemia*. 1997;11:516-518.
12. Germanidis G, Haioun C, Pourquier J, et al. Hepatitis C virus infection in patients with overt B-cell non-Hodgkin's lymphoma in a French center. *Blood*. 1999;93:1778-1779.
13. Thalen DJ, Raemaekers J, Galama J, Cooreman MP. Absence of hepatitis C virus infection in non-Hodgkin's lymphoma. *Br J Haematol*. 1997;96:880-881.
14. King PD, Wilkes JD, Diaz-Arias AA. Hepatitis C virus infection in non-Hodgkin's lymphoma. *Clin Lab Haematol*. 1998;20:107-110.

15. Collier JD, Zanke B, Moore M, et al. No association between hepatitis C and B-cell lymphoma. *Hepatology*. 1999;29:1259-1261.
16. Zuckerman E, Zuckerman T, Levine AM, et al. Hepatitis C virus infection in patients with B-cell non-Hodgkin lymphoma. *Ann Intern Med*. 1997;127:423-428.
17. Ohsawa M, Shingu N, Miwa H, et al. Risk of non-Hodgkin's lymphoma in patients with hepatitis C virus infection. *Int J Cancer*. 1999;80:237-239.
18. Cerhan JR, Wallace RB, Dick F, et al. Blood transfusions and risk of non-Hodgkin's lymphoma subtypes and chronic lymphocytic leukemia. *Cancer Epidemiol Biomarkers Prev*. 2001;10:361-368.
19. Blomberg J, Moller T, Olsson H, Anderson H, Jonsson M. Cancer morbidity in blood recipients—results of a cohort study. *Eur J Cancer*. 1993;29A:2101-2105.
20. Memon A, Doll R. A search for unknown blood-borne oncogenic viruses. *Int J Cancer*. 1994;58:366-368.
21. Maguire-Boston EK, Suman V, Jacobsen SJ, et al. Blood transfusion and risk of non-Hodgkin's lymphoma. *Am J Epidemiol*. 1999;149:1113-1118.
22. Hezode C, Duvoux C, Germanidis G, et al. Role of hepatitis C virus in lymphoproliferative disorders after liver transplantation. *Hepatology*. 1999;30:775-778.
23. Zein NN, Perez RG, Wiesner RH. Hepatitis C virus infection and lymphoproliferative disorders after liver transplantation. *Hepatology*. 2000;31:808-809.
24. Levine AM, Nelson R, Zuckerman E, et al. Lack of association between hepatitis C infection and development of AIDS-related lymphoma. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1999;20:255-258.
25. Wang JT, Sheu JC, Lin JT, Wang TH, Chen DS. Detection of replicative form of hepatitis C virus RNA in peripheral blood mononuclear cells. *J Infect Dis*. 1992;166:1167-1169.
26. Zignego AL, Giannelli F, Marrocchi ME, et al. T(14;18) translocation in chronic hepatitis C virus infection. *Hepatology*. 2000;31:474-479.
27. Zuckerman E, Zuckerman T, Sahar D, et al. The effect of antiviral therapy on t(14;18) translocation and immunoglobulin gene rearrangement in patients with chronic hepatitis C virus infection. *Blood*. 2001;97:1555-1559.