Short Communication

Hepatitis B and C Viruses, Human T-Cell Lymphotropic Virus Types I and II, and Leukemias: A Case-Control Study

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Abstract

The relationship between acute myeloid leukemia (AML), acute lymphocytic leukemia, chronic myeloid leukemia (CML), and refractory anemia with excess of blasts (RAEB) and antibodies to human T-cell lymphotropic virus types I and II (HTLV-I and HTLV-II), and hepatitis B virus and hepatitis C virus (HCV) was investigated in a multicenter case-control study. There were 431 cases enrolled in the study at the time of diagnosis of hematological malignancies, and 862 controls ages 15 years or older were recruited in three hospitals. Antibodies to HTLV-I and HTLV-II, antibody to HCV, hepatitis B surface antigen, and antibody to hepatitis B core antigen were assayed.

All cases and controls were negative for HTLV-I antibodies; one case (1 of 431; 0.2%), and one control (1 of 862; 0.1%) were found positive for HTLV-II antibodies. A nonsignificant excess of risk for hepatitis B surface antigen was present among RAEB cases (odds ratio, 2.40; 95% confidence interval, 0.46–12.0), CML

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Introduction

Viruses have long been considered potential risk factors for leukemias (1). Animal experiments have established causal relationships between viruses and leukemia and lymphoma (2, 3).

Human T-cell HTLV-I has been implicated in the etiology of ATL in many parts of the world, particularly in the Caribbean (4). There is close agreement worldwide between areas of HTLV-I endemcity and ATL occurrence. Recently, Manns et al. (5), in a case-control study performed in Jamaica and Trinidad and Tobago, found that HTLV-I was associated with non-Hodgkin's lymphoma but not with other hematological malignancies (ALL and chronic lymphocytic leukemia or AML and CML).

An association between HTLV-II and malignancy has been suggested by the isolation and detection of HTLV-II DNA in malignant cells from hairy-cell leukemia patients, and by its ability to transform T lymphocytes in vitro (6–8); however, HTLV-II has not yet been shown to be the cause of any disease (9).

A causal role of hepatitis viruses, particularly non-A-non-B virus, has been hypothesized for aplastic anemia, and case reports suggest an association between non-A-non-B hepatitis and leukemias (10–12). Recent advances in the molecular biology of HBV have identified viral genome or proteins not only in hepatocytes but also in a number of extrahepatic sites, such as lymphoblastic cells, lymph nodes, and vascular elements in the liver (13). The permissiveness of these extrahepatic cells for viral replication might also promote tumor development in hematopoietic cells (13).

It has been shown that HCV is also a lymphotropic virus. Positive and negative stranded (replicative) forms of HCV have been observed in peripheral blood mononuclear cells of patients with chronic liver disease (14). Furthermore, recent reports describe the presence of HCV infection in patients presenting...
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IgM monoclonal gammapathies (15) and in patients affected by mixed cryoglobulinemia-related lymphoproliferative disorders (low-grade non-Hodgkin’s lymphomas; Ref. 16). These observations suggest that HCV infection may play a role in both liver disease and lymphoproliferative disorders.

Within a multicenter case-control study of risk factors for acute leukemias (17), we investigated the relationships between AML, ALL, CML, and RAEB; antibodies to HTLV-I and HTLV-II; and HBV and HCV.

Materials and Methods
This study was conducted between November 1, 1986, and March 31, 1990, in Rome, Bologna and Pavia, Italy.

Recruitment of Study Subjects. The method of recruitment of study subjects and data collection is described in detail elsewhere (17). Briefly, cases were 15 years or older with newly diagnosed AML, ALL, CML, or RAEB. Diagnostic criteria were based on the revised French-American-British classification of bone marrow aspirates for acute leukemias and RAEB, whereas diagnosis for CML was based on typical clinical and cytogenetic laboratory features. Controls were recruited in the region of the three hospitals during the study period among outpatients without hematological malignancies who were seen in the same hospitals at which cases had been identified. The control group was selected by taking the first five outpatients in Rome and the first three in Bologna and Pavia, seen on a random day each week. Controls having platelet disorders, leukocyteosis, leukopenias, or monoclonal gammopathies of undetermined significance were excluded from the study because of possible shared risk factors with the case diseases. Subjects evaluated in the study hospitals for chronic hepatitis and hyperbilirubinemia were also excluded.

A standard, precoded questionnaire was administered to both cases and controls to collect data on medical history and behavioral and environmental exposure. In particular, questions were included regarding i.v. drug use, homosexual or bisexual activity, and receipt of blood products.

Subjects who had received blood product transfusions (i.e., blood and pooled plasma products) were excluded from the study.

Laboratory Methods. All sera were stored at −20°C and subsequently tested. HBV markers, HBsAg, anti-HBc were tested, in serum, by immunoenzymatic assays (Abbott Laboratories, North Chicago, IL). Cutoff of positivity was calculated according to manufacturer’s instructions.

Anti-HCV prevalence was calculated using a second-generation ELISA (Ortho Diagnostic, Raritan, NJ). Repetitive, reactive sera were further tested by third-generation supplemental RIBA (RIBA III, Ortho Diagnostic), which includes structural (C22) and nonstructural (C100, C33, NS5) viral proteins. Sera were considered anti-HCV positive when two or more bands were shown by RIBA.

Only 1 band (indeterminate result) was observed in 4.2 and 4.4% of anti-HCV ELISA reactive sera from cases and controls, respectively. No bands were detected in 18.3 and 6.1% of anti-HCV ELISA reactive sera from cases and controls, respectively. All RIBA indeterminate (3 cases and 5 controls) and nonreactive samples were excluded from analysis.

Coded samples were screened using an HTLV-I viral lysate/HTLV-I/HTLV-II recombinant protein-based enzyme-immunoassay (HTLV-I/HTLV-II ELISA, Diagnostic Biotechnology, Singapore). Samples were confirmed as seropositive if antibodies against gag (p19 or p24) and env (gp46) gene products were present by Western blot (HTLV blot 2.3, Diagnostic Biotechnology, Singapore). Anti-HTLV-I or HTLV-II reactive profiles were differentiated, based on reactivity to either HTLV-I- or HTLV-II-recombinant gp46 protein.

Statistical Analysis. Adjusted ORs for age, sex, education, history of hospitalization, and area of residence and 95% CIs were calculated by polychotomous logistic regression (18). Age was included as a continuous variable in the logistic regression model.

Results
Serum samples from 431 out of 636 recruited cases and from 862 out of 1193 recruited controls were tested. No differences regarding age, sex, and leukemia type existed between tested and untested cases, whereas a higher proportion of unbled subjects were recruited in the Bologna and Pavia centers.

The main categories of control diagnoses included iron deficiency and thalassemia minor, which accounted for 43% of the control diagnoses; 36% of the control group had no underlying hematological disorders. Controls with chronic hepatitis (n = 26), hyperbilirubinemias (n = 23), monoclonal gammopathies (n = 24), platelet disorders (n = 58), and leukocytosis (n = 69) and those who received blood products (n = 131) referred by general practitioners to the study center were excluded.

All cases and controls denied i.v. drug use, sexual contact with i.v. drug users, and homosexuality.

ALL cases were younger than other cases (41% ≤ 35 years), whereas the RAEB group was the oldest cases (75% ≥ 54 years). No differences were found between cases and controls regarding gender.

Two subjects older than 70 years, 1 AML case and 1 control, were found to be anti-HTLV-II positive; all cases and controls were anti-HTLV-I negative. The two HTLV-II subjects, born in Italy, denied i.v. drug use and sexual contact with i.v. drug users and had not received blood products.

Table I shows the crude prevalences of HBsAg, anti-HBc, and anti-HCV with adjusted ORs. The HBsAg prevalence ranged between 1.7% in AML to 4.0% in CML versus 1.4% in controls, and the anti-HBc crude prevalence ranged between 16.4% in all cases to 31.8% in RAEB cases versus 17.7% in controls. The anti-HCV prevalence was 1.6% in CML cases and 8.7% in AML cases, whereas 5.1% of controls were positive. The adjusted ORs show an association between CML and RAEB and HBsAg, and between AML and anti-HBc, but all 95% CIs overlapped the null value.

A slight, nonsignificant excess of risk was present among AML and ALL cases for anti-HCV positivity.

Discussion
To our knowledge, this is the first study on the association between acute leukemias, RAEB, and CML and HTLV-I, HTLV-II, HBV, and HCV.

HTLV-I has been firmly linked to ATL, an aggressive leukemia with a poor prognosis (19). Manns et al. (5), in a case-control study, found that patients with non-Hodgkin’s lymphoma were 10 times more likely than controls to be seropositive for HTLV-I, whereas patients with other hematological disorders (AML, CML, ALL, and chronic lymphocytic leukemia) were not associated with HTLV-I antibodies. The present study does not provide evidence for an association between HTLV-I and HTLV-II and leukemias. Surprisingly, none of the cases or controls were positive for HTLV-I antibodies. A high incidence of HTLV-I infection has been re-
ported among Italian i.v. drug users (20), and this virus has been found to be endemic in certain areas of in southern Italy (21).

Two subjects were found to be positive for anti-HTLV-II antibodies: 1 out of 431 (0.2%) cases and 1 out of 862 (0.1%) controls. The seroprevalence observed in our leukemia patients (0.2%) is similar to that of a recent study (22), including 317 patients receiving allogeneic or syngeneic marrow transplants for hematological malignancies; only 1 patient (0.3%) was positive for HTLV-I/HTLV-II antibodies. The prevalence of anti-HTLV-II was 1 and 7% among injecting drug users in Europe and in the United States, respectively (20, 23, 24).

The present study suggests a possible association (elevated ORs) between AML, CML, RAEB, and HBV. However, because all CI s overlapped the null value, these findings should be regarded as preliminary and need to be confirmed in larger case-control studies. The inconsistency between HBsAg and anti-HBC prevalences may be due to the inability of these patients to mount an antibody response.

A higher prevalence of HBV markers has been found in patients with lymphoma and leukemia as compared to the general population (25–28). The high frequency of HBV infection in these patients may result from exposure to blood products, especially when bone marrow is replaced by neoplastic cells and during chemotherapy. In our study, subjects with hematological malignancies were recruited at admission to the hospital and had not received chemotherapy treatment or blood products.

The carcinogenic role of HBV in the liver is well known (29), but HBV DNA has also been found in extrapathic tissues, including cells of hematopoietic origin (30, 31), and sequences of HBV-DNA have been detected in the bone marrow of patients with leukemia (32). Furthermore, HBV has in vitro inhibitory effects on the differentiation and proliferation of human bone marrow progenitor cells (33, 34).

Hematopoietic cells that are permissive for HBV replication are most likely the major extrapathic site for HBV production (13). The bone marrow may therefore be considered a reservoir for HBV outside the liver. Galun et al. (13) detected HBsAg in bone marrow cells of patients with leukemia and lymphoma. HBsAg was also detected in the endothelial cells of blood vessels of tumor tissue. These authors conclude that the presence of HBV gene product in endothelial cells suggests a role for HBV infection in the development of certain hematopoietic tumors, possibly through activation of cytokines or growth factors, which may stimulate cell proliferation.

We found a weak, nonsignificant association between anti-HCV positivity and AML, ALL, and RAEB. HCV is also a carcinogenetic agent (35); an association of non-A-non-B hepatitis with aplastic anemia and leukemias has been reported (36), but a study on HCV and aplastic anemia showed no role of HCV in that disease (37). However, our data should be reconsidered when an HCV antigen assay becomes available. Among our control group, we found an anti-HCV prevalence of 5.1%, much higher than that found in the general population (about 1%; Ref. 38), but similar to that observed in Italian individuals with nonhepatic diseases seen in a general hospital (35).

The present work is a hospital-based case-control study. Hospital controls were recruited from the same clinical sources as the case patients in order to prevent selection bias. Patients with chronic hepatitis and cirrhosis were excluded from the controls because of a possible excess of HBV and HCV markers in these subjects. In addition, subjects reporting administration of blood products were excluded from both cases and controls because of their possible association with hepatitis parenteral viruses.

Analysis of case charts showed that no patient with AML had been previously diagnosed with RAEB.

Furthermore, the data were adjusted for history of hospitalization to take into account patients with smoldering hematological malignancies (i.e., RAEB and CML) who may have been exposed to parenteral risk factors, including diagnostic and therapeutic procedures.

In conclusion, the present study encourages further, larger case-control studies on possible association between HBV and leukemia.

### Table 1: Prevalences of hepatitis B and C with adjusted ORs by leukemia type

<table>
<thead>
<tr>
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<th>HBsAg</th>
<th>anti-HBc</th>
<th>anti-HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive (%)</td>
<td>Total</td>
<td>OR (95% CI)</td>
<td>Total</td>
</tr>
<tr>
<td>AML</td>
<td>3 (1.7)</td>
<td>173</td>
<td>1.3 (0.35–4.7)</td>
</tr>
<tr>
<td>ALL</td>
<td>0 (0)</td>
<td>67</td>
<td>11 (16.4)</td>
</tr>
<tr>
<td>CML</td>
<td>5 (4.0)</td>
<td>125</td>
<td>2.7 (0.86–8.5)</td>
</tr>
<tr>
<td>RAEB</td>
<td>2 (3.0)</td>
<td>66</td>
<td>2.4 (0.46–12)</td>
</tr>
<tr>
<td>Controls</td>
<td>12 (1.4)</td>
<td>862</td>
<td>153 (17.7)</td>
</tr>
</tbody>
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* Adjusted by age, sex, education, history of hospitalization, and study area.

### References

Hepatitis B and C viruses, human T-cell lymphotropic virus types I and II, and leukemias: a case-control study. The Italian Leukemia Study Group.

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