Lack of Preferential Localization of Tumoral Mass in B-Cell Non-Hodgkin’s Lymphoma Associated With Hepatitis C Virus Infection

To the Editor:

The possible association between hepatitis C virus (HCV) infection and B-cell–type non-Hodgkin’s lymphoma (NHL) has previously been suggested. 1-6 HCV tropism for hepatocytes and also, according to recent data, for the salivary gland ductular cells 7 may imply the existence of some preferential sites of neoplasia.

We report here the clinical and virologic features of 150 patients with B-cell–type NHL (71 men) consecutively seen at either a hematology or internal medicine outpatient clinic at our university hospital. Only subjects at first diagnosis of lymphoma were included in the study. Biopsy material was classified according to the Working Formulation for Clinical Usage. 6 The NHL grade was low, intermediate, or high in 37%, 49%, and 14%, respectively. All patients underwent clinical examination, routine blood analysis, chest x-ray or CT chest scan, abdominal ultrasonography and/or abdominal CT scan, and bone marrow biopsy. They were classified according to the Ann Arbor staging system 6 and 27% of patients were stage I, 33% were stage II, 18% were stage III, and 21% were stage IV. One hundred and fifty patients were anti-HCV–negative, age- and sex-matched subjects and 80 Hodgkin’s lymphoma (HL) patients were also studied as controls. Twenty-six NHL patients were anti-HCV positive (RIBA II; Chiron, Emeryville, CA). 34 were HCV RNA positive using one-tube nested polymerase chain reaction (PCR). 1, 4 and 37 (25%) were anti-HCV and/or HCV RNA positive. The latter prevalence was significantly higher than that observed in healthy subjects (25% v 1% and HL patients (25% v 8%; 0.01) as well as in the general Italian population (approximately 1.3%).

Samples of peripheral blood mononuclear cells (PBMC) were also tested by HCV PCR using previously described methods. 19 HCV RNA sequences were detected in PBMC in 30 of 34 (90%) serum HCV RNA-positive, in 3 of 15 (20%) serum HCV RNA-negative (2 of them also being anti-HCV–negative) NHL patients and also in neoplastic lymph node and bone marrow samples in 3 cases in whom this material was available. The results of HCV genotyping, performed with previously described methods in serum and/or PBMC samples, 10, 11 are shown in Table 1. In particular, in 3 patients a mixed infection (1b + 2) was detected in PBMC and only HCV type 1b in serum. No significant differences were observed between HCV-positive and HCV-negative patients with respect to the extradural sites of the neoplasia (Table 2) as well as to the range of age and the grade of lymphoma. In particular, among patients with low-grade, intermediate-grade, or high-grade NHL, HCV-positive subjects were 14 of 56 (25%), 18 of 73 (25%), and 5 of 21 (24%) respectively.

We previously showed the existence of a high prevalence of HCV infection in patients with both mixed cryoglobulinemia-associated and idiopathic B-cell–type NHL, suggesting that HCV may play a role in lymphomagenesis in humans. 2, 3 This study further confirms these previous results as well as the almost constant involvement of the lymphatic system by HCV infection. No specific or more probable extradural tumoral localization were identified in HCV-positive patients when compared with non-HCV–associated forms of NHL, suggesting that systemic and not local factors may trigger malignant lymphoproliferation. In this respect, the possibility that direct infection of lymphatic cells, probably in association with extralymphatic HCV epitope stimulation, plays a role in lymphomagenesis ought to be taken into account and deserves further analysis. 13 The demonstration of HCV RNA sequences in lymphoid cells from the great majority of HCV-positive NHL patients tested, even in the absence of serum HCV markers, is not sufficient to demonstrate the validity of this hypothesis. However, it does point in the same direction. The identification in lymphoid cells of genotypes not detectable in serum suggests clustering into extrahepatic sites of more lymphotropic types, which may have pathogenetic relevance. In synthesis, it is possible that HCV infection may induce a chronic B-cell prolifera-
Of the RNA nature of the HCV genome, integration of viral sequences in the host’s cellular DNA is not possible. Consequently, it may be hypothesized that, even in the case of a neoplastic transformation initially related to HCV infection, resulting cells may no longer be permissive to HCV infection and replication.

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**REFERENCES**


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Successful Treatment of Iron Overload by Phlebotomies in a Patient With Severe Congenital Dyserythropoietic Anemia Type II

To the Editor:

A 32-year-old woman of Greek origin was referred to the hospital for evaluation of fatigue and symptomatic anemia. The anemia was documented 10 years ago during her first pregnancy. During the last 10 years the patient received an unknown number of red blood cell transfusions. Otherwise, the medical history was unremarkable. There was no family history of anemia. Physical examination showed moderate hepatosplenomegaly. No jaundice was found.

The initial hemoglobin (Hb) concentration was 7 g/dL. Serum ferritin was 1,600 µg/L. The bone marrow cytology demonstrated hypercellularity and erythroid hyperplasia (E/G ratio was 1.075). Binuclear and polynuclear erythroblasts were the predominant morphologic features. A normal number of mature megakaryocytes and orderly myeloid maturation was present. Marrow iron was increased and pathological sideroblasts could not be observed. The incorporation of 59Fe into erythrocytes was low, at a level of 44% (normal range, 70% to 90%). Intestinal iron absorption [measured after administration of a test dose of 10 µmol 59Fe (II) by whole body counting] was increased to 77% after 14 days (normal range, 10% to 50%). The liver-iron content amounted to 3.3 mg Fe per gram of liver tissue (wet weight; normal range, 0.1 to 0.5 mg/g).

Liver iron concentration was measured using a SQUID (Super Conducting Quantum Interference Device) biomagnetometer (Perri- motometer, Bii, San Diego, CA) as described elsewhere. For measurement, the patient was lowered in a known, constant magnetic field (Bmag = 20 mT, gradiometer first order) and the magnetic flux change versus a water reference medium is detected by a second order gradiometer pickup coils. The individual liver volume is assessed by sonography and the actual total liver iron is calculated from the respective liver iron concentration and liver volume.

Increased agglutination with anti-i exceeding that on cord red blood cells was not observed. Based on these findings, a congenital dyserythropoietic anemia (CDA) type II was diagnosed.

For treatment of the iron overload, phlebotomy was started despite the severe anemia with an amount of 50 mL of removed blood in week 1, followed by a stepwise increase to 100 mL, 200 mL, and 300 mL of blood in weeks 2, 3, and 4. This procedure was followed by phlebotomies of 400 mL blood every 4 weeks for 6 months. After 3 months, a considerable improvement of erythropoiesis could be observed. Hb levels increased to between 9 and 10 g/dL. Serum ferritin levels decreased to 380 µg/L. After 6 months of phlebotomy therapy (total amount, 2,900 mL of blood), the liver iron concentration was reduced to 2.9 mg Fe/g liver tissue (Fig 1).

CDA impairments of the red blood cell characterized by ineffective erythropoiesis and a variable anemia. On the basis of morphologic features, Heimpel and Wendt classified these disorders into three types. In 1971, an additional subtype was defined by McBride using serological findings. Type II, which is the most frequent type, with more than 100 reported cases, is called the hereditary erythroblast multinuclearity associated with a positive acidified serum lysis test (HEMPAS), as described by Crookston et al. In vitro cultures of bone marrow cells from patients with CDA showed a defective growth of the earliest committed erythroid progenitors, the erythroid burst-forming cells (BFU-E). Abnormalities in glycosylation of erythrocyte membrane proteins and lipids are also described. Furthermore, a deficiency of erythrocyte membrane CD44 was detected. The diagnosis of CDA II should be considered on bone marrow examination when erythroid hyperplasia and multiple binuclear and multinuclear erythrocyte cells are found. The iron incorporation into erythrocytes is significantly decreased, indicating a high grade of ineffective erythropoiesis.

CDA usually has a relatively benign course. Iron overload due to transfusion requirements may become a problem in patients with CDA, mostly in CDA type II. Additionally increased intestinal iron absorption due to the ineffectiveness of the erythropoiesis in CDA II in transfusion independent patients may contribute to iron overload [reported case: distinct increase to 77% of 59Fe (II) test dose absorption]. Irrespective of the cause of iron overload, patients who are not anaemic can usually be treated effectively by phlebotomy, whereas patients with iron-loading anemias such as CDA usually require an iron chelation therapy. The clinical use of deferoxamine with daily subcutaneous injections requires is cumbersome. Oral active iron chelating compounds (such as 1,2-dimethyl-3-hydroxyxypyridine-4-one, also known as L1 or CP20) with, in animals, documented side effects such as bone marrow toxicity and teratogenicity are not yet available for clinical use.

To mobilize the surplus tissue iron, a careful phlebotomy treatment was started in our patient despite the severe anemia. The procedure was well tolerated, and the Hb concentration was relatively constant between 7 and 8 g/dL during the first 3 months of treatment. No increase of anaemia-related symptoms was noted by the patient. After 3 months, an improvement of effective erythropoiesis was noticed, as indicated by an Hb increase up to 10 g/dL. There was a good correlation between the quantity of blood removed (2,900 mL corresponding to 1,200 mg Fe) and the measured decrease of liver iron concentration (loss of approximately 1,100 mg Fe).

We conclude that regular phlebotomy may be well tolerated in patients with iron-loading anemias such as CDA. It seems to be possible to correct serious iron overload in both transfused and non-transfused CDA patients and to prevent these patients from late complication of secondary hemochromatosis. In addition, there was also a considerable improvement of the erythropoiesis by a yet unknown mechanism.

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