Characterization of Overt B-Cell Lymphomas in Patients With Hepatitis C Virus Infection

By Salvatore De Vita, Cosimo Sacco, Domenico Sansonno, Annunziata Gloghini, Franco Dammacco, Marina Crovatto, Gianfranco Santini, Riccardo Dolcetti, Mauro Boiocchi, Antonino Carbone, and Vittorina Zagonel

A pathogenetic role of the hepatitis C virus (HCV) has been hypothesized for a subset of B-cell non-Hodgkin’s lymphomas (NHLs). However, the preliminary characterization of B-cell NHLs in HCV-infected individuals has been poorly addressed. In the present study, we report detailed information on 35 consecutive patients with overt B-cell NHL of recent onset and HCV infection; all patients referred to a single oncological center in Northeast Italy. Histopathologic evaluation was performed by a single reference hemopathologist, and the link with the two relevant autoimmune diseases predisposing to B-cell NHL and in which HCV has been implicated, "essential" mixed cryoglobulinemia (EMC) and Sjögren’s syndrome, was investigated. Control groups included 122 consecutive HCV-negative patients with B-cell NHL and 464 consecutive histopathologic cases of B-cell NHL referred to the same center, as well as 127 consecutive patients with HCV infection and without lymphoma referred to a different center in the same geographical area. B-cell NHLs in HCV-infected patients frequently presented at onset (1) an extranodal localization with peculiar target organs of HCV infection (ie, the liver and major salivary glands) being significantly overrepresented; (2) a diffuse large cell histotype without any prior history of low-grade B-cell malignancy or bone marrow involvement; and (3) a weak association with a full-blown predisposing autoimmune disease, although serum autoimmune features were common and cryoglobulins were always present. Therefore, the HCV-related B-cell NHLs in this oncological series presented distinctive features compared with B-cell NHLs in HCV-negative patients, and they differed from bone marrow low-grade NHLs frequently diagnosed in HCV-positive patients with EMC. Such novel information may be relevant for future research aimed at clarifying the possible link between HCV infection, autoimmunity, nonmalignant B-cell lymphoproliferation, and overt B-cell malignancy.

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THE HEPATITIS C virus (HCV) has been recently implicated as the possible etiologic factor of type II “essential” mixed cryoglobulinemia (EMC),1,4 a chronic immune-complex–mediated disease with underlying B-cell clonal proliferation that predisposes to overt B-cell malignancy.5 This has led several groups to investigate the association between HCV infection and overt B-cell malignancies.6–16 Preliminary studies have focused on the prevalence of HCV infection in patients with different B-cell tumors. HCV seroprevalence appeared to be significantly increased in the B-cell non-Hodgkin’s lymphoma (NHL) subgroup.9,11,14 Models of HCV-related B-cell lymphomagenesis have been then speculated.9,10,11,17 although substantial information to strongly support this hypothesis is still lacking. Importantly, the rigorous characterization of the B-cell NHLs in HCV-infected individuals has been poorly addressed in previous studies, and heterogeneity in case selection and pathological evaluation cannot be excluded. Furthermore, the first attempts to localize HCV within the malignant NHL lesion are quite recent.6,9 These points are crucial to investigate the putative pathobiologic role of any infectious agent in a given tumor microenvironment.16–20 Finally, based on reported data, it is not yet clear to what extent the association between HCV infection and B-cell NHL may be linked to lymphomas complicating HCV-associated EMC or whether different entities exist.

In this study, we report more detailed information on a large series of patients with overt B-cell NHL of recent onset and HCV infection, who all referred to a single oncological center in Northeast Italy.

MATERIALS AND METHODS

B-cell NHLs in HCV-infected individuals. Thirty-five consecutive, unselected patients with a full-blown B-cell NHL of recent onset (usually less than 1 to 2 months) and serological evidence of HCV infection were investigated. Other lymphoid neoplasms,21 namely B-cell neoplasms (B-cell chronic lymphocytic leukemia, acute leukemia, and myeloma), T-cell and putative natural killer (NK)-cell neoplasms, and Hodgkin’s disease, were not included in the study. All the patients were referred to the Centro di Riferimento Oncologico di Aviano, Italy. They were all Italian, human immunodeficiency virus (HIV)-negative heterosexuals, with no history of intravenous drug abuse. Most patients were observed from January 1994 to December 1996 in the course of a prospective clinical study. Bone marrow biopsy was performed in all patients to stage the dissemination of a B-cell NHL that had been histopathologically diagnosed in tissue biopsy specimens from other sites, extranodal or nodal. All the patients also underwent thorax and abdomen computed tomography (CT) scanning. In particular, liver and spleen were considered to be involved only when definite nodular lesions could be detected by CT, and not on the sole evidence of organomegaly or diffuse parenchymal inhomogeneity (with the only exception of marked splenomegaly completely regressing after treatment). Neck and abdomen ultrasonographic studies and esophagogastroduodenoscopy with tissue biopsy were also performed in the large majority of cases. Routine liver function tests, as well as hematologic parameters, were determined at the time of NHL diagnosis. Serum rheuma-
Virologic studies. HCV infection was screened by searching for serum antibodies against HCV by enzyme-linked immunosorbent assay (ELISA; HCV 3.0; Ortho Diagnostic Systems, Raritan, NJ). Confirmatory test included recombinant-based immunoblot assay (Chiron RIBA 2nd generation; Ortho Diagnostic Systems). Serum HCV RNA determination and genotype characterization were performed according to previously described procedures. Briefly, reverse transcription of HCV RNA was followed by nested polymerase chain reaction (PCR) using primers for the 5’ noncoding region. HCV genotypes were determined using primers for the core region with reverse transcription extended for 2 hours, and they were classified according to the Simmonds classification. A set of two universal primers was used in the first step of amplification, and a mixture of one universal primer and four type-specific primers was used in the second step. For type 1a HCV, the original primer was replaced by a new primer. Types 2c and 3a were determined in separate and highly specific nested-PCR protocols. The amplification products were visualized on NuSieve 2% + SeaKem 2% gel (FMC Bioproducts, Rockland, ME) in Tris-acetate-EDTA (TAE) stained with ethidium bromide. The HCV genotypes were identified on the basis of the size of the amplification products.

Histopathology. All the nodal or extranodal tissue lesions and bone marrow biopsy specimens were evaluated and then carefully reviewed by a single hematopathologist (A.C.). Pathological specimens were classified according to the Working Formulation for NHL and to the revised European-American classification of lymphoid neoplasms (REAL).

Dedifferentiated and cryostat sections were used for immunophenotyping and lineage assignment of lymphoma cases with MoAbs (CD3, CD4, CD8, CD10, CD15, CD19, CD20, CD21, CD22, CD24, CD30, CD38, CD43, CD45, CD45RA, CD45RO, CD68, CD74, CD75, L3, MB2, DDBB42, DBA44, POD4, DR1, Leu-8, anti-κ and anti-λ immunoglobulin [lg] light chains, epithelial membrane antigen, vimentin, and cytokeratin [MNF116]).

In selected cases, B-cell clonal expansion was also investigated by multiple molecular studies. In all the cases in which the bone marrow was not involved by bone marrow biopsy, α/δ restriction on bone marrow B cells was assessed by flow cytometry analysis.

Relationship with a full-blown autoimmune disease. The possible association between B-cell NHL in HCV-infected patients and a previous history of EMC and Sjögren’s syndrome, ie, the two major autoimmune diseases predisposing to B-cell malignancy and in which HCV has been implicated, was investigated in the present oncological series by a clinical expert (S.D.V.). An accurate clinical history could be recorded in 34/35 patients followed by a complete physical examination. All the available laboratory and instrumental tests performed in the years preceding NHL onset were reviewed.

For EMC, symptoms and signs consistent with purpura, asthenia, arthralgia, renal involvement, and peripheral neuropathy were investigated, as well as serum cryoglobulins, C3 and C4, and rheumatoid factor levels. By means of a validated questionnaire the patients were asked whether Sjögren’s syndrome-related symptoms of dry eye and dry mouth had occurred before NHL onset. Objective tests for ocular and oral involvement before lymphoma onset were reviewed. EMC and Sjögren’s syndrome symptoms and signs were also investigated at the time and after NHL onset.

Control groups

Consecutive HCV-seronegative patients with B-cell NHL. This group included 122 consecutive patients with overt B-cell NHL of recent onset, all HCV seronegative (by ELISA) and HIV seronegative, and all referred to the Centro di Riferimento Oncologico of Aviano, Italy, from January 1994 to December 1996. They could be characterized regarding sex and age at NHL onset, NHL localization, histopathology and stage, and HBV infection. In particular, histopathologic diagnosis in nodal or extranodal tissue biopsy samples by the same pathologist was available. Bone marrow biopsy was performed in all patients to stage NHL dissemination, and NHL staging procedures were the same as in the HCV-positive patients.

Consecutive biopsy specimens from patients with B-cell lymphoma. A series of 464 consecutive histopathologic cases of B-cell NHL referred to the Centro di Riferimento Oncologico from July 1988 to December 1993 were reviewed with regard to age of the patients, tumor histotype, and site of NHL involvement (as documented by the sole tissue histopathology) at lymphoma onset. HCV infection was not characterized in this subgroup. Other B-cell neoplasms, T-cell and putative NK-cell neoplasms, and Hodgkin’s disease were not included as in the previous study and control groups.

Consecutive patients with HCV infection and without malignant lymphoma. The distribution of HCV genotypes was analyzed in 127 consecutive patients with HCV infection, with positive serum HCV RNA, and without malignant lymphoma. All the patients were referred to a different center, ie, the Hospital of Pordenone, located in the same geographical area in Northeast Italy (18 km away from Aviano, Italy).

Statistical analysis. The χ² test (Yates corrected) or the Fisher’s exact test (two-sided) were employed whenever appropriate when comparing the different clinical, pathological, and virological data in patient versus control groups. The Mann-Whitney U test was used when comparing the age at NHL onset.

RESULTS

Patients. Among the 35 HCV-infected patients with NHL, 20 were women and 15 were men (P = not significant [NS] v HCV-uninfected patients with NHL; Tables 1 and 2). Their mean age at NHL onset was 64.6 ± 10.2 years (P = NS v the 122 HCV-negative controls with NHL [Table 2] and versus the 464 histopathologic cases of B-cell NHL not characterized for HCV infection, aged 59.9 ± 16.5 years at NHL onset). NHL was stage I in 9 patients, stage II in 2 patients, stage III in 4 patients, and stage IV in 20 cases (P = NS v HCV-uninfected patients with NHL). Eight patients (22.8%) complained of B symptoms (P = NS). Previous blood transfusions were recorded in 6 patients (17.6%). Seventeen patients (48.6%) had been subjected to serological tests for HCV infection in the previous 1 to 4 years, and all were found to be HCV infected before NHL onset. In the remaining 18 patients, the diagnosis of HCV infection was formulated at the time of NHL onset. In 15 of these 18 patients there was evidence that HCV infection had preceded the onset of NHL, as highlighted by a previous history of abnormal liver function tests (usually lasting for several years) and liver ultrasonography indicative of chronic hepato-
Table 1. Clinical and Pathological Features of Patients With B-Cell NHL and HCV Infection

<table>
<thead>
<tr>
<th>Case, Age, Sex</th>
<th>Sites of Primary Involvement by NHL</th>
<th>Classification of B-Cell NHL REAL/W.F.</th>
<th>Bone Marrow Involvement</th>
<th>HCV Genotype</th>
<th>Cryoglobulinemia* (cryocrit level in %)</th>
<th>RF/ANA</th>
<th>Previous EMC or Sjögren Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 53, FT</td>
<td>LFN (lc)</td>
<td>LP-llc/A</td>
<td>III A 2c</td>
<td>+ type II IgMk (52%)</td>
<td>+/NS</td>
<td>+ EMC</td>
<td></td>
</tr>
<tr>
<td>2. 66, FT</td>
<td>Skin</td>
<td>LP-llc/A</td>
<td>IV A 2c</td>
<td>+ type II IgMk (1%)</td>
<td>+/-</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>3. 79, F</td>
<td>LFN (ax, hh, lc, ing) tonsil, vagina</td>
<td>Uncl/Misc A + G</td>
<td>IV B 2c</td>
<td>+ type II IgMk (3%)</td>
<td>+/NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>4. 72, FT</td>
<td>Skin</td>
<td>Uncl/Misc A + D</td>
<td>IV A 1b</td>
<td>+ type II IgMk (1%)</td>
<td>+/-</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>5. 59, FT</td>
<td>LFN (lc)</td>
<td>FC-Foll/C</td>
<td>II A 1b</td>
<td>+ type II IgMk (3%)</td>
<td>+/-</td>
<td>+ EMC</td>
<td></td>
</tr>
<tr>
<td>6. 59, F</td>
<td>LFN (lc, ax, nuc), spleen</td>
<td>Mant/E</td>
<td>IV A 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>7. 79, M</td>
<td>Parotid gland, stomach</td>
<td>Ex-MZE</td>
<td>IV A 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>8. 55, M</td>
<td>Stomach</td>
<td>Ex-MZE</td>
<td>IV A 1b</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>9. 69, F</td>
<td>Submandibular gland</td>
<td>Ex-MZE</td>
<td>IV A 1b</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>10. 70, F</td>
<td>Bronchus</td>
<td>Ex-MZE</td>
<td>IV A 2c</td>
<td>type III (2%)</td>
<td>+/-</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>11. 62, F</td>
<td>Parotid</td>
<td>Ex-MZE</td>
<td>I A NS</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>12. 68, F</td>
<td>Stomach</td>
<td>DLC/G</td>
<td>IV A 1b</td>
<td>(+1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>13. 76, F</td>
<td>Parotid gland</td>
<td>DLC/G</td>
<td>I A 1b + 2b</td>
<td>+ type II IgMk (4%)</td>
<td>+/-</td>
<td>+ EMC</td>
<td></td>
</tr>
<tr>
<td>14. 27, M</td>
<td>Liver</td>
<td>DLC/G</td>
<td>IV B 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>15. 68, F</td>
<td>Liver, LFN, spleen</td>
<td>DLC/G</td>
<td>IV B 1b</td>
<td>(&lt;1%)</td>
<td>+/−</td>
<td>+ Sjogren</td>
<td></td>
</tr>
<tr>
<td>16. 55, M</td>
<td>Spleen, lung + LR-LFN</td>
<td>DLC/G</td>
<td>IV A 2c</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>17. 55, M</td>
<td>Rhinopharynx, liquor</td>
<td>DLC/G</td>
<td>IV B 1b</td>
<td>(+1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>18. 61, M</td>
<td>Liver, spleen</td>
<td>DLC/G</td>
<td>IV A 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>19. 48, M</td>
<td>Soft tissues (neck) + LR-LFN</td>
<td>DLC/G</td>
<td>I A 1b</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>20. 66, M</td>
<td>Spleen, liver</td>
<td>DLC/G</td>
<td>IV B 2c</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>21. 74, F</td>
<td>Soft tissues (thigh) + LR-LFN</td>
<td>DLC/G</td>
<td>IV A 2c</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>22. 72, F</td>
<td>Stomach</td>
<td>DLC/G</td>
<td>I A 1b</td>
<td>(+1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>23. 66, M</td>
<td>Brain</td>
<td>DLC/G</td>
<td>I A 1b</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>24. 61, M</td>
<td>Tonsil</td>
<td>DLC/G</td>
<td>I A 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>25. 73, M</td>
<td>Small intestine</td>
<td>DLC/G</td>
<td>I A 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>26. 70, F</td>
<td>Liver, LFN (ax, ing)</td>
<td>DLC/G</td>
<td>IV A 2c</td>
<td>+ type II IgMk (1%)</td>
<td>+/−</td>
<td>+ EMC</td>
<td></td>
</tr>
<tr>
<td>27. 69, M</td>
<td>LFN (ing, il, fem)</td>
<td>DLC/G</td>
<td>I I A 2c</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>28. 59, M</td>
<td>Liver</td>
<td>DLC/G</td>
<td>I A 1b</td>
<td>+ type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>29. 72, F</td>
<td>LFN (lc, aort), tonsil</td>
<td>DLC/G</td>
<td>III A 2c</td>
<td>+ type II IgMk (4%)</td>
<td>+/−</td>
<td>+ EMC</td>
<td></td>
</tr>
<tr>
<td>30. 57, M</td>
<td>LFN (lc, ax, clav, ing)</td>
<td>DLC/G</td>
<td>III B 2c</td>
<td>type III (1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>31. 72, F</td>
<td>Spleen, LFN (hh, il, cell)</td>
<td>DLC/G</td>
<td>IV B 2c</td>
<td>&lt; type II IgMk (2%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>32. 65, F</td>
<td>Skin + LR-LFN</td>
<td>DLC/H</td>
<td>IV A 2c</td>
<td>+ type II IgMk (2%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>33. 77, M</td>
<td>LFN (lc, ax, hh)</td>
<td>DLC/H</td>
<td>III B 2c</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>34. 70, F</td>
<td>LFN (med), pleural effusion</td>
<td>DLC/H</td>
<td>IV A 1b</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>35. 57, FT</td>
<td>Breast</td>
<td>Burkitt’s/U</td>
<td>I A 1b</td>
<td>(&lt;1%)</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NS, not studied; RF, serum rheumatoid factor (positive if >40 IU/mL); ANA, serum antinuclear antibodies (positive for titers >1:40); W.F., Working Formulation; LFN, lymph nodes (hh, hepatic hylum; ax, axillary; lc, latero-cervical; aort, para-aortic; il, iliac; fem, femoral; cel, celiac; ing, inguinal; nuc, nucal; med, mediastinal; LR, locoregional); Uncl, Unclassifiable; LP-llc, Lymphoplasmacytoid Immunocytoma; FC-Foll, Follicular center, follicular; Misc, Miscellaneous Group; Ex-MZ, Extranodal marginal zone; DLC, Diffuse large cell.

* Where not reported, serum cryoglobulins could not be typified due to cryocrit levels <1%.
† A histopathologic picture of either reactive lymphadenopathy or atypical lymphoproliferative disorder had been detected in the previous years.
‡ Lymphoma relapse was observed during the follow-up, with unchanged histotype in patients 1 and 35, and progression to B-cell lymphoma (F group in the W.F.) in patient 2.
§ With pathological features of Ex-MZ NHL in some residual areas of gastric mucosa, and bone marrow involvement detected only by flow cytometry.

Abnormalities at the time of NHL onset with no previous liver tests available. Other causes of hepatopathy, such as alcoholism or drugs, were excluded in all the patients. No patient had been treated with interferon before NHL onset.

Serum cryoglobulins were present in all the patients and were characterized at NHL onset in half of the cases (Table 1). Serum rheumatoid factor was detected in 18 of 35 (51.4%) patients, antinuclear antibodies in 5 of 33 (15.1%; Table 1), and low C3 or C4 levels (≤75 mg/dL and ≤15 mg/dL, respectively) in 13 of 34 (38.2%). Anti-HBs and/or anti-HBe IgG antibodies were revealed in 10 of 35 patients (28.6%), whereas HBsAg, HBeAg, and anti-HBc IgM were never detected. Anti-HBs, anti-HBe, or anti-HBc IgG antibodies were detected in 11 of 122 HCV-seronegative control patients (9%; P = .005): Three of them were HbsAg positive (P = .01).

NHL localization at onset. By histopathology and staging procedures, 23 of the 35 (65.7%) HCV-positive patients presented a primary extranodal B-cell NHL. The sites of primary involvement are reported in Table 1. By contrast, a primary extranodal NHL was detected in 23 out of the 122
Table 2. Clinical and Pathological Features of B-Cell NHLs in HCV-Positive Versus HCV-Negative Patients at Onset

<table>
<thead>
<tr>
<th></th>
<th>HCV-Positive (n = 35)</th>
<th>HCV-Negative (n = 122)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient sex (F:M)</td>
<td>20:15</td>
<td>59:63</td>
</tr>
<tr>
<td>Patient mean age ± SD (range)</td>
<td>64.6 ± 10.2 (27-79)</td>
<td>56.5 ± 14.5 (21-86)</td>
</tr>
<tr>
<td>NHL stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II/III/IV</td>
<td>9/1/4/21</td>
<td>23/24/14/61</td>
</tr>
<tr>
<td>A/B</td>
<td>27/8</td>
<td>93/29</td>
</tr>
<tr>
<td>NHL localization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary extranodal</td>
<td>23/35</td>
<td>23/122 (P &lt; .001)</td>
</tr>
<tr>
<td>liver</td>
<td>4</td>
<td>0 (P = .202)</td>
</tr>
<tr>
<td>spleen</td>
<td>3</td>
<td>0 (P = .01)</td>
</tr>
<tr>
<td>salivary glands</td>
<td>4</td>
<td>1 (P = .009)</td>
</tr>
<tr>
<td>Other sites:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rhinofarinx/tonsil/tongue</td>
<td>1/1/0</td>
<td>2/0/1</td>
</tr>
<tr>
<td>stomach/small intestine</td>
<td>4/1</td>
<td>7/0</td>
</tr>
<tr>
<td>skin/lung/kidney/breast/bone</td>
<td>3/2/0/1/0</td>
<td>6/2/2/0/1</td>
</tr>
<tr>
<td>brain or cerebrospinal fluid</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Nodal + extranodal</td>
<td>6/35</td>
<td>39/122</td>
</tr>
<tr>
<td>Only nodal</td>
<td>6/35</td>
<td>60/122 (P = .001)</td>
</tr>
<tr>
<td>Bone marrow involvement</td>
<td>13/35</td>
<td>42/122</td>
</tr>
<tr>
<td>NHL histotype (REAL classification)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoplasmacytoid/immunocytoma</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Follicular center, follicular</td>
<td>1</td>
<td>26 (P = .01)</td>
</tr>
<tr>
<td>Extranodal marginal zone (MALT)</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Nodal marginal zone</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Diffuse large cell</td>
<td>23</td>
<td>60</td>
</tr>
<tr>
<td>Burkitt’s</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Provisional entities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burkitt’s like</td>
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<td>4</td>
</tr>
<tr>
<td>Splenic marginal zone</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Follicular center, diffuse, small cell</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Unclassifiable</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* When not reported, no significant differences were detected.

HCV-negative control patients (18.8%; P < .001; Table 2). Nodal localization without extranodal involvement was observed in 6 of 35 (17.1%) HCV-positive and in 60 of 122 HCV-negative (49.2%) patients (P = .001). In addition, nodal lymphoma was detected in 300 of 464 (65%) control biopsy cases of B-cell NHL. A primary nodal or extranodal origin could not be defined in patients showing a concomitant nodal and extranodal involvement at NHL onset, with lymph nodes distant from the extranodal NHL site(s) being always involved (6 of 35 HCV-positive patients and 39 of 122 HCV-negative patients; P = NS).

When comparing the sites of NHL involvement at onset in patients with primary extranodal NHL (HCV infected v HCV uninfected), liver, spleen, and salivary involvement was significantly more frequent in HCV-infected patients (P = .002, P = .01, and P = .009, respectively; Table 2). A higher prevalence of liver and salivary gland lymphoma in HCV-infected versus HCV-uninfected patients was also evident when patients with primary extranodal and with extranodal plus nodal involvement at onset were considered as a single group (liver involvement in 6 of 35 v 1 of 122; P = .001; salivary involvement in 4 of 35 v 2 of 122, P = .023), whereas a higher prevalence of spleen involvement was no longer observed (6 of 35 v 20 of 122, P = NS). The higher prevalence of liver and salivary NHL involvement in HCV-infected patients was finally supported by the results in the 464 control biopsy cases of B-cell NHL, in which liver and salivary lymphomas accounted for about 1% of the cases each (4 of 464 and 5 of 464, respectively).

The prevalence of bone marrow involvement was similar in HCV-infected and -uninfected NHL patients (37.1% v 34.4%; P = NS; Tables 1 and 2).

**Tumor histopathology.** According to the REAL classification, 23 of 35 HCV-positive patients (65.7%) presented a diffuse large B-cell lymphoma (Tables 1 and 2). Extranodal marginal zone NHL was observed in five cases (14.3%), lymphoplasmacytoid lymphoma/immunocytoma in two cases, and follicular lymphoma, mantle cell lymphoma, and Burkitt’s lymphoma in one patient each. Two cases of NHLs were unclassifiable, according to the REAL classification, being composite lymphomas, ie, belonging to the Miscellaneous group by the Working Formulation (A + D in one case, A + G in the other; Tables 1 and 2). An identical tumor histotype was noted when synchronous biopsy specimens from different involved tissues from the same patient were analyzed (Table 1). In the 22 patients in whom bone marrow was uninvolved by NHL, bone marrow atypical lymphoproliferation was observed in one case (patient 27), whereas bone marrow plasmacytosis was noted in three cases (patients 1, 14, and 24).
When comparing the NHL histotype in HCV-positive versus HCV-negative patients, follicular lymphoma showed a decreased frequency in HCV-positive patients (2.8% vs 21.3%; \( P = .01 \); Table 2). These results were confirmed when considering the 464 consecutive pathological samples of B-cell NHL, in which follicular lymphoma was observed in 92 cases (19.8%; \( P = .011 \)). No significant difference was found regarding the remaining NHL subsets.

**Predisposing autoimmune disease preceding NHL in HCV-positive patients.** A definite clinical picture of EMC or Sjögren’s syndrome preceded NHL onset in 5 of 34 (14.7%) and in 1 of 34 (2.9%) patients, respectively (Table 1), and in no case was it evident only at the time of NHL onset or later.

In patient 15, marked subjective and objective dry eye and dry mouth manifestations diagnostic for primary Sjögren’s syndrome preceded NHL onset for 6 years in the absence of other causes of oral/ocular dryness. Definite dry eye and dry mouth symptoms were lacking in all the remaining patients both before and at the time of NHL development, except for patient 11, in whom such symptoms could be caused by sarcoidosis (histologically diagnosed 13 years before NHL onset).

**HCV infection and genotype.** HCV infection was documented in all the patients with NHL by ELISA. It was then confirmed in 33 of 35 cases by positive RIBA results (undetermined in patients 6 and 13) and in all the cases by HCV RNA detection in the serum. When considering the HCV genotype, type 1b was detected in 13 cases (38.2%) and in one additional case (patient 13) as a coinfection with type 2b, whereas type 2c was detected in 20 cases (58.8%; Table 1). Among the 127 control patients with HCV infection but without lymphoma, HCV type 1a was detected in 7 cases (5.5%; \( P = NS \)), type 1b in 66 cases (51.9%; \( P = NS \)), type 2a in 3 cases (2.4%; \( P = NS \)), type 2b in 1 case (0.8%; \( P = NS \)), type 2c in 41 cases (32.3%; \( P = .008 \)), and type 3a in 3 cases (2.4%; \( P = NS \)). A coinfection by two or more HCV types was detected in 6 cases (4.7%; \( P = NS \)).

**DISCUSSION**

The issue of a possible pathogenetic role of HCV in a subset of B-cell NHLs is quite recent. HCV infection is strongly associated with EMC, a chronic autoimmune disease with underlying B-cell clonal proliferation, which in turn predisposes to overt B-cell NHL. Furthermore, HCV seroprevalence appears to be significantly increased in patients with B-cell NHL by preliminary studies. Finally, HCV has been localized within neoplastic and non-neoplastic B-cell lymphoproliferative lesions, and, by preliminary evidence, HCV eradication in EMC may be followed by decreased lymphoproliferation. Additional and more comprehensive studies on this topic are thus of interest. Several new data emerged by the present characterization of patients with HCV-infection and overt B-cell NHL of recent onset, all referred to a single oncolgical center in Northeast Italy. Patients with autoimmune diseases, such as EMC, are not followed in this center. Thus, the present oncological series shows quite distinctive features and integrates previous knowledge on B-cell lymphoproliferation underlying EMC.

Extranodal sites were primarily involved at NHL onset in the large majority of the cases similarly to NHLs in other conditions characterized by immune dysregulation. In particular, the liver and the major salivary glands were significantly over-represented in our series, whereas these primary organ localizations are extremely uncommon in unselected series of B-cell NHLs. Thus, a possible link between peculiar sites and HCV infection should be searched. Strikingly, both the liver and the major salivary glands are targets of HCV infection. HCV may infect and actively replicate within the hepatocytes as well as in the salivary gland ductal and acinar epithelium. Because nonlymphoid cells are typically infected by HCV in these organs, and because lymphoid stimulation by exogenous antigens has been related to NHL development in other extranodal sites, a local role of HCV as an exogenous trigger of B-cell proliferation should be considered at first. Furthermore, we always failed to detect HCV within the malignant B-cell component in diffuse large cell lymphomas arising in HCV-infected individuals. A putative oncogenetic role of HCV by direct infection and deregulation of B cells is not supported by present data, although additional studies are required. Of note, HCV may also localize in nonmalignant nodal tissue, and autoimmune patients with EMC evolving to lymphoma apparently presented a more frequent nodal involvement at lymphoma onset (four out of five patients in the present series; and Dammacco, personal observations in five out of six additional patients, December 1996). Therefore, a possible discrepancy in nodal lymphoproliferation in different subsets of patients deserves further investigation.

The issue of bone marrow NHL localization in HCV-infected patients is important in terms of tumor pathobiology and should be considered in conjunction with the NHL histotype. HCV may localize in the bone marrow, and in EMC, ie, in a prelymphomatous phase of B-cell lymphoproliferation associated with HCV infection, the bone marrow is frequently a site of florid B-cell lymphoproliferation. A clear-cut pathobiological and clinical distinction between nonmalignant and low-grade malignant B-cell proliferation limited to the bone marrow may be problematic and, in part, subjective in selected cases. On the other hand, only a minority of EMC patients develop a fully malignant lymphoma. Because diffuse large cell lymphomas represented the major subgroup in the present oncological series, and neither a previous EMC/low-grade malignancy nor bone marrow malignancy involvement were present in the large majority of patients (16 of 23: 70%), the possible links between overt lymphoma and nonmalignant (rather than low-grade malignant) lymphoproliferation involving the bone marrow should be better focused. Secondly, in most diffuse large cell lymphomas, mechanisms of full-blown tumor progression conceivably occur in sites other than the bone marrow. Other studies detected a higher number of low-grade NHLs (immunocytomas) in HCV-positive patients. The discrepancy with our present results may be explained by considering that such low-grade NHLs, including cases limited to the bone marrow, have been reported by centers following patients with EMC who were enrolled in the study groups. Thus, the evidence that immunocytoma is a B-
cell NHL histotype closely associated with HCV infection is not at all surprising, and likely reflects the well-known association between HCV infection and indolent bone marrow B-cell lymphoproliferation as observed in EMC. On the other hand, the present study on overt NHLs from a center in which hepatitic or EMC patients are not followed, and in which overt lymphomas are referred, is the first study that definitely points out an additional subset of NHLs in HCV-infected individuals, i.e., diffuse large B-cell lymphomas in patients without a previous history of full-blown predisposing autoimmune disease or low-grade B-cell malignancy. Other groups as well have reported HCV infection in patients with intermediate or high-grade B-cell NHL, but these cases were poorly characterized. These NHL subgroups accounted for about two thirds of the whole series of HCV-associated NHLs by Ferri et al. and in the study by Silvestri et al. Centroblastic lymphomas were the second NHL histotype (after immunocytoma) showing an increased relative risk for HCV infection. Furthermore, HCV infection is significantly increased in patients with diffuse large cell lymphoma referred to our center. Thus, based on the present and previous evidence, the possible pathogenetic role of HCV should be better addressed both in indolent stages of B-cell lymphoproliferation (putatively antigen-dependent) and in aggressive NHLs (putatively antigen-independent). Of note, a common origin from B cells selected by the triggering antigen may be supposed in both low-grade and high-grade NHLs. Furthermore, the relationship between autoimmunity, benign B-cell proliferation, and B-cell malignancy in the course of HCV infection remains of major interest. Autoimmune serological features are common in patients with HCV infection in the absence of full-blown autoimmune disease or organ involvement, and this was also noted in the present series of patients with NHL.

Finally, the identification of the infectious HCV subtype might have biological and clinical implications in patients with B-cell NHL as reported for other clinical settings. In accordance with a previous study, type 1b or 2 were the only HCV genotypes detected in patients with NHL. However, this might simply reflect the HCV genotype distribution in our geographic area. Larger studies are, therefore, required. Furthermore, the full pathogenic potential of the HCV 2c subtype should be better addressed. The prevalence of this strain is relatively high in Italian patients, is further increased in our region (Crovatto et al., manuscript in preparation), and, by the present preliminary results, proved to be significantly higher in patients with NHL.

In conclusion, recent-onset B-cell NHLs in patients infected by HCV and referred to a single oncological center have been extensively characterized. The novel information regarding peculiar NHL localizations at onset, tumor histotype, and association with predisposing autoimmune disease may be relevant for future research aimed at clarifying the possible link between HCV infection and B-cell lymphoproliferation.

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Characterization of Overt B-Cell Lymphomas in Patients With Hepatitis C Virus Infection

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