Objective: To assess the influence of hepatitis C virus (HCV) infection on disease appearance and outcome of ocular adnexal non-Hodgkin lymphoma (ONHL).

Design: Retrospective comparative study (from January 1, 1992, through December 31, 2006).

Methods: The medical records of 129 patients with ONHL were retrospectively reviewed. All the patients were tested serologically for the presence of HCV infection. Patients were divided into 2 groups according to the presence or absence of HCV infection.

Main Outcome Measures: Prevalence of HCV infection, staging to evaluate the extent of disease at the onset, and clinical outcome data on overall and disease-free survival.

Results: The prevalence of HCV infection among the patients with ONHL was 17.8%. Seropositivity for HCV infection was significantly associated with extraorbital lymphoma at the onset ($P = .006$). High prevalence of mucosa-associated lymphoid tissue disease (79.8%) was registered. Protocol therapy included radiotherapy and chemotherapy, depending on the stage of the disease. Complete remission was achieved in 99 patients (76.7%). A total of 23.6% of patients with HCV-seronegative status and 21.7% of those with HCV-seropositive status experienced relapse of the lymphomatous disease. No significant differences in the 5-year overall survival and disease-free survival between the 2 groups were observed.

Conclusions: Prevalence of HCV infection in patients with ONHL is a relevant issue, accounting for 17.8% of the examined patients. Infection with HCV may influence the initial appearance of ONHL because it is associated with more widespread disease at the onset. However, the overall and disease-free survival of the infected patients are not statistically different than that of patients who are not infected.


Author Affiliations: Department of Ophthalmological Sciences (Drs Strianese, Tranfa, Finelli, Schiemer, Cardone, and Bonavolontà), Pathology Section, Department of Biomorphological and Functional Sciences (Drs Staibano, Mascolo, and De Rosa), and Departments of Diagnostic Imaging and Radiation Oncology (Dr Pacelli) and Haematology (Drs De Renzo and Perna), University of Naples Federico II, Naples, Italy.

Hepatitis C virus (HCV) represents a viral pandemic and is well known as the causative agent of chronic viral hepatitis, which has been linked to the development of hepatocellular carcinoma. Hepatitis C virus is a hepatotrophic virus that can also replicate in lymphoid cells. The virus has been shown to sustain clonal expansion of B lymphocytes in patients with HCV infection. This observation raised the question of whether HCV is a lymphoepithelial or germ cell virus and has led to evaluation of the potential role of HCV infection within B-cell non-Hodgkin lymphoma, as well as other hematologic malignant neoplasms. A pathogenic link between HCV and non-Hodgkin lymphoma (NHL), mostly marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT), has been suggested. In contrast, other authors have not confirmed such an association.

Ocular adnexal non-Hodgkin lymphoma (ONHL) (eg, conjunctiva, lacrimal gland, and orbital soft tissue) is one of the most common forms of extranodal lymphomas and usually displays limited stages of disease, an indolent clinical course, and a tendency to remain localized for several years. Only 1 study shows a positive association between HCV infection and the development of ONHL. The authors of this previous article state that HCV infection is a risk factor for ONHL as well as other disseminated disease at the diagnosis and with poor prognosis. The current study aims to assess whether HCV infection plays a role in determining the clinical features of HCV-seropositive orbital lymphomas, especially regarding the prognosis.
Kaplan-Meier curves (patients (hepatitis C virus [HCV] seropositive and seronegative) assessed by the latest criteria of the World Health Organization classifying Formulation classification) were reclassified according to the previous classification for NHL (Workan incisional biopsy specimen. The patients with ONHL diagnosis based on pathologic examination of bone marrow biopsy. In patients with conditions diagnosed after 2002, positron emission tomography was also performed.

Each patient underwent tests for antibodies to HCV assayed by a second-generation enzyme-linked immunosassay (HCV RNA qualitative reverse-transcriptase polymerase chain reaction standardized method) and determination of HCV genotype. Furthermore, we analyzed the presence of chronic hepatic disease. The patients who had HCV-seropositive status underwent liver biopsy with the aim of evaluating the hepatic disease associated with the viral infection. The chronic liver disease was classified according to criteria developed by Ishak et al. First-line treatment consisted of radiotherapy, in proportions of 1.8 or 2.0 Gy, 5 times per week, with total doses ranging from 34.2 to 50.0 Gy, when the disease was localized at the only orbit. When the lymphomatous disease appeared to be disseminated, according to the staging, a conventional protocol therapy based on cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (CHOP) chemotherapy or a CHOP-like scheme was administrated. In selected patients whose conditions were diagnosed after 2002, rituximab was added.

After antineoplastic treatment, 6 patients with HCV-seropositive status who had ONHL were treated with interferon alfa (3 000 000 units 3 times a week for 12 months) subcutaneously. They were also given ribavirin, 1000 mg/d, orally.

All patients were followed up every 3 months with clinical evaluation and laboratory tests. They were also followed up every 6 months with ultrasonography and computed tomography (with positron emission tomography and computed tomography after 2002).

STUDY GROUP

A retrospective study of a series of 129 patients with orbital ONHL, who had been referred from January 1, 1992, through December 31, 2006, to the Orbital Unit of the University of Naples Federico II, was performed. The patients included in this study had the onset symptom of orbital localization of ONHL. Data concerning age, sex, mass localization, and follow-up were available for all the examined patients. All the patients had a diagnosis of orbital lymphoma based on pathologic examination of an incisional biopsy specimen. The patients with ONHL diagnosed according to the previous classification for NHL (Working Formulation classification) were reclassified according to the latest criteria of the World Health Organization classification by pathologic reevaluation with additional immunochemical examinations.

The staging workup consisted of physical examination, biochemical blood profile, total-body computed tomography, and

![Figure 1. Comparison of the overall survival between the 2 groups of patients (hepatitis C virus [HCV] seropositive and seronegative) assessed by Kaplan-Meier curves (P=.79).](chart)

**Table 1. Clinical Characteristics at Disease Onset**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of Patients by HCV Status (N=129)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>23 (17.8) 106 (82.2)</td>
<td>. . .</td>
</tr>
<tr>
<td>Age, median (range), y</td>
<td>64.34 (43-84) 62.88 (26-93)</td>
<td>. . .</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>4/19 45/61</td>
<td>. . .</td>
</tr>
<tr>
<td>Orbital lymphoma</td>
<td>10 (43.5) 86 (81.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Conjunctival lymphoma</td>
<td>2 (8.7) 10 (9.4)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Eyelid lymphoma</td>
<td>1 (4.3) 8 (7.5)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>9 (39.1) 22 (20.8)</td>
<td>.10</td>
</tr>
<tr>
<td>Extraocular muscles</td>
<td>1 (4.3) 1 (0.9)</td>
<td>.32</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15 (65.2) 96 (90.6)</td>
<td>.004</td>
</tr>
<tr>
<td>2</td>
<td>0 0</td>
<td>. . .</td>
</tr>
<tr>
<td>3</td>
<td>0 0</td>
<td>. . .</td>
</tr>
<tr>
<td>4</td>
<td>8 (34.8) 11 (10.4)</td>
<td>.006</td>
</tr>
<tr>
<td>Positive BMB</td>
<td>8 (34.8) 10 (9.4)</td>
<td>.004</td>
</tr>
</tbody>
</table>

Abbreviations: BMB, bone marrow biopsy; ellipses, not applicable; HCV, hepatitis C virus; M, male; F, female.

*Data are number (percentage) of patients unless otherwise indicated.*

RESULTS

The follow-up varied from a minimum of 12 months to a maximum of 185 months, with an average of 75 months. The frequency of the different histologic types of ONHL is shown in Figure 1. The clinical characteristics of the 129 patients included in this study are summarized in Table 1. The median age of the patients was 62.88 years (range, 26-93 years), and the male to female ratio was 0.61.

Twenty-three patients (17.8%) were infected with HCV. In all patients, the diagnosis of HCV infection preceded the diagnosis of lymphoma. All patients had HCV RNA detectable by polymerase chain reaction.

Mucosa-associated lymphoid tissue is the most frequent type of lymphoma, resulting in 79.8% of cases. A similar percentage was found among patients with HCV-seropositive status. No significant differences were found between the 2 groups in terms of distribution of the dif-
ferent histopathologic subtypes (Table 2). The histologic analysis of the hepatic tissue in the HCV-seropositive patients showed chronic active hepatitis in 6 patients (26.1%) and no signs of liver disease in the remaining 17 patients (73.9%). The apparent disease duration was calculated as the time passed by the first known risk of infection (eg, major or minor surgery, transfusion, tattooing, or intravenous drug abuse) to the time of the diagnosis of the orbital lymphoma. The average time was 22.6 years (SD, 13.9 years).

As reported in Table 1, the extent of the disease was significantly different between the 2 groups: 8 (34.8%) patients with HCV-seropositive status had stage IV disease at the diagnosis vs 11 (10.4%) patients with HCV-seronegative status. Infection with HCV was significantly associated with extraorbital disease at the onset (P = .006).

Radiotherapy treatment was administered in 108 patients (83.7%) as single-line therapy, whereas 21 patients (16.3%) were treated with a combination of radiotherapy and chemotherapy. The therapeutic outcome is summarized in Table 3. Ninety-nine patients (76.7%) achieved complete remission of the NHL. Thirty patients experienced relapse of the disease with extranodal localization: 25 (23.6%) of these patients had HCV-seronegative status and 5 patients (21.7%) had HCV-seropositive status (P = .99). The median time to disease progression was 57.6 and 55.0 months for the HCV-seronegative and HCV-seropositive groups, respectively. The 5-year overall survival and disease-free survival were not significantly different in the 2 groups (P = NS) (Figure 1 and Figure 2).

## Table 2. Distribution of Different Disease Subtypes Between the 2 Groups of Patients

<table>
<thead>
<tr>
<th>Subtype</th>
<th>HCV Seropositive, No.</th>
<th>HCV Seronegative, No.</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALT</td>
<td>18</td>
<td>85</td>
<td>.78</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2</td>
<td>9</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Follicular</td>
<td>0</td>
<td>9</td>
<td>.36</td>
</tr>
<tr>
<td>Mantle</td>
<td>3</td>
<td>3</td>
<td>.69</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; MALT, mucosa-associated lymphoid tissue.

## Table 3. Study Outcome Data

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Seropositive (n=23)</th>
<th>Seronegative (n=106)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse rate</td>
<td>5 (21.7)</td>
<td>25 (23.6)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Local relapse</td>
<td>0</td>
<td>12 (11.3)</td>
<td>.12</td>
</tr>
<tr>
<td>Systemic relapse</td>
<td>4 (17.4)</td>
<td>12 (11.3)</td>
<td>.48</td>
</tr>
<tr>
<td>Systemic and local relapse</td>
<td>1 (4.3)</td>
<td>1 (0.9)</td>
<td>.32</td>
</tr>
<tr>
<td>Follow-up, median, mo</td>
<td>71.7</td>
<td>77.4</td>
<td>...</td>
</tr>
<tr>
<td>TTP, median, mo</td>
<td>55.6</td>
<td>57.6</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviations: ellipses, not applicable; HCV, hepatitis C virus; TTP, time to progression.

Data are number (percentage) of patients unless otherwise indicated.

## Figure 2

Comparison of the progression-free survival between the 2 groups of patients (hepatitis C virus [HCV] seropositive and seronegative) assessed by Kaplan-Meier curves (P = .53).

©2010 American Medical Association. All rights reserved.

The association between HCV infection and NHL has been postulated in several studies.\(^1\)\(^-\)\(^10\) This correlation has been mostly observed in countries such as Italy, the United States, and Japan.\(^6\)\(^-\)\(^10\) However, this issue remains controversial based on additional studies.\(^12\)\(^-\)\(^17\) In Italy a prevalence of HCV infection in NHL ranging from 15% to 30% has been reported.\(^4\)\(^-\)\(^6\)

In the literature there are few studies\(^10\)\(^-\)\(^13\)\(^,\)\(^18\) that explore the association of HCV infection and ocular adenoidal lymphomas (eg, conjunctiva, lachrymal gland, and orbital soft tissue), even though the ocular region is one of the most common sites of extranodal lymphomas. Ferrari and coworkers\(^10\) have shown clinical implications of HCV infection in MALT-type lymphoma of the ocular adnexa. Of 55 patients, 13% tested seropositive for HCV. The concomitant HCV infection was associated with more disseminated disease and aggressive behavior in ocular adnexal lymphoma. By contrast, in an Austrian study,\(^12\) only 2 of 45 patients (4%) had a concomitant HCV infection, and neither influence of HCV on clinical course nor risk of relapse was found. In a short report,\(^11\) performed in France, of 40 analyzed patients with ONHL, only 1 patient had HCV-seropositive status.

In the present study, the prevalence of HCV infection in the examined population with ONHL was 17.8%, similar to what has been reported in other Italian studies on patients from other districts with NHL localized in different sites.\(^3\)\(^-\)\(^12\) The variable worldwide prevalence of HCV together with different environmental and genetic factors could explain these contrasting results. In fact, the prevalence of HCV infection in Italy is high (3%-5%).\(^3\) For comparison, the role of Chlamydia psittaci in ocular adnexal lymphoma appears to be especially pronounced in Italy,\(^18\) whereas widely varying rates of infection could be found in different geographic regions.\(^20\)

Recently, an increasing amount of biologic data have been found that support the role of HCV in lymphomagenesis.\(^21\)\(^-\)\(^23\) It seems that the long-term antigenic stimulation provided by these agents may elicit host immune
responses able to promote and sustain clonal B-cell expansion. A significant increase of NHL in patients with more than 15 years of HCV infection has been reported. In our study the median duration of HCV infection was 22 years, with 34.8% of patients with HCV-seropositive status having disseminated disease at onset and only 9.4% of patients with seronegative status having widespread disease at onset ($P = 0.004$) (Table 1). This finding supports the hypothesis that HCV infection allows spreading of the lymphoma, sustaining its clonal expansion. Although the HCV genome and its replicative intermediates have been detected in peripheral blood mononuclear cells and in lymphoid tissues of chronically infected patients, its role as a trigger agent of a frank B-cell neoplastic disorder is not clear yet. In fact, because the virus lacks reverse transcriptase, it is unable to integrate into the host genome and to encode for any known oncogene. Considering this possible pathogenic mechanism, the need for analysis of the biopsied tissue for the presence of HCV is uneventful, as advocated by some authors.

A history of liver disease may be considered a possible pathogenic factor on the progression of lymphoma in patients with HCV-seropositive status. However, only 6 of 23 patients in our cohort had an active viral liver disease. Considering these data, we were not able to state that liver disease gravity may have conditioned lymphoma staging in our group of patients.

Patients included in this study received radiotherapy alone or with adjuvant chemotherapy, depending on the stage of the disease. As we previously reported in primary NHL with different site distribution, the use of an appropriate therapeutic option also resulted in a favorable outcome for this subset of patients with extranodal lymphomas. The overall outcome resulted in a complete remission of NHL in 99 patients (76.7%). A total of 25 (23.6%) of the patients with HCV-seronegative status and 5 (21.7%) of those with HCV-seropositive status experienced relapse of the disease; however, we did not observe a statistically significant difference in the 5-year overall survival and disease free survival between the 2 groups (Tables 1 and 3 and Figures 1 and 2). It can be argued that the appropriate use of the protocol therapy based on a careful evaluation of the stage or extent of the disease can minimize the negative effect of HCV infection.

The results of this study were compared with those of the only previously published large Italian study in the PubMed database, to our knowledge, which concerned the positive link between HCV infection and ocular adnexal lymphoma. The authors of the other study similarly found that HCV infection is associated with a more aggressive clinical stage at onset, but they conversely reported that patients with HCV-seropositive status had a poor prognosis over time compared with those with HCV-seronegative status. Possible explanations of the difference regarding the outcome between these reported data and our own data may be ascribed to differences in the first-line therapy choices.

In a study performed with a French population that demonstrated the link between HCV infection and the diffuse large cell lymphoma, the authors suggested that specific protocols, including antiviral therapy, should be designed for patients affected by lymphoma. Recent reports, attesting to the efficacy of antiviral treatment on the course of HCV-related B-cell NHL, strengthen the hypothesis of a link between HCV infection and B-cell lymphoma. The value of the antiviral therapy was ruled out by the statistical analysis of this study because of the paucity of patients with HCV-seropositive status treated. Nevertheless, the use of antiviral therapy may improve the outcome of patients infected with NHL, as we reported in a previous study.

To summarize, HCV infection has a potential negative effect on patients infected with ocular lymphoma because it is associated with a more diffuse dissemination at the onset. Discrepancy with previous studies in terms of the incidence of HCV-seropositive cases among patients infected with ONHL may be explained by the geographic variations of this infection. Therefore, although an influence of HCV infection on lymphoma exists, the different prevalence can modify its role as a prognostic factor. Outcome seems to be slightly modified by the presence of this infection, probably because the effectiveness of the recent first-line treatment could minimize the influence of this risk factor. However, future studies will be necessary to better assess the influence of infectious agents and malignant neoplasms and the possible role of antiviral therapy. It must be considered that the data reported herein were collected in a single referral center.

Submitted for Publication: April 25, 2009; final revision received November 20, 2009; accepted November 28, 2009.

Correspondence: Diego Strianese, MD, PhD, Department of Ophthalmological Sciences, University of Naples Federico II, Via Pansini 5, Naples 80131, Italy (strianese@unina.it).

Financial Disclosure: None reported.

REFERENCES