

## REGRESSION OF SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES AFTER TREATMENT OF HEPATITIS C VIRUS INFECTION

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## ABSTRACT

**Background** Some epidemiologic studies suggest a link between hepatitis C virus (HCV) infection and some B-cell non-Hodgkin's lymphomas. We undertook this study after a patient with splenic lymphoma with villous lymphocytes had a hematologic response after antiviral treatment of HCV infection.

**Methods** Nine patients who had splenic lymphoma with villous lymphocytes and HCV infection were treated with interferon alfa-2b (3 million IU three times per week) alone or in combination with ribavirin (1000 to 1200 mg per day). The outcomes were compared with those of six similarly treated patients with splenic lymphoma with villous lymphocytes who tested negative for HCV infection.

**Results** Of the nine patients with HCV infection who received interferon alfa, seven had a complete remission after the loss of detectable HCV RNA. The other two patients had a partial and a complete remission after the addition of ribavirin and the loss of detectable HCV RNA. One patient had a relapse when the HCV RNA load again became detectable in blood. In contrast, none of the six HCV-negative patients had a response to interferon therapy.

**Conclusions** In patients with splenic lymphoma with villous lymphocytes who are infected with HCV, treatment with interferon can lead to regression of the lymphoma. (N Engl J Med 2002;347:89-94.)

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**H**EPATITIS C virus (HCV) is the chief etiologic agent of non-A, non-B chronic hepatitis,<sup>1</sup> and several epidemiologic studies suggest that HCV may be involved in the pathogenesis of several B-cell lymphoproliferative disorders. Antibodies against HCV, HCV RNA, or both have been detected in most patients with type II cryoglobulinemia, which is characterized by the clonal expansion of B cells that may evolve into low-grade or high-grade non-Hodgkin's lymphoma.<sup>2</sup> Studies in Italy, southern California, and Japan have reported a high prevalence (9 to 32 percent) of chronic HCV infection among patients with B-cell non-Hodgkin's lymphoma.<sup>3,4</sup> B-cell non-Hodgkin's lymphoma is a

heterogeneous group of diseases that originate from B-cell precursors at various stages of development and that may have different causes.<sup>5</sup> HCV infection is most frequently encountered in patients with lymphoplasmacytoid lymphoma or immunocytoma-lymphoma,<sup>6-9</sup> which are characterized by an infiltration of small B cells and plasmacytes in the bone marrow and lymph nodes and are often associated with a serum IgM $\kappa$  component. Marginal-zone lymphomas of the lymph node and diffuse primary hepatosplenic large-B-cell lymphomas have also been reported to be related to HCV infection.<sup>3,9</sup> However, other epidemiologic studies have not shown any association between HCV and non-Hodgkin's lymphoma.<sup>10-14</sup>

Splenic lymphoma with villous lymphocytes is a chronic B-cell lymphoproliferative disorder characterized by splenomegaly and a clonal expansion of B cells, with villous projections, in peripheral blood. The B cells probably originate from lymphomas of the marginal zone of the spleen.<sup>15</sup> This type of non-Hodgkin's lymphoma has an indolent course characterized by the slow progression of splenomegaly and a gradual increase in tumoral B cells in peripheral blood.

Because splenic lymphoma with villous lymphocytes regressed in one patient who received interferon alfa for a symptomatic cryoglobulinemia associated with HCV infection (Patient 1) and because some studies suggest that marginal-zone lymphoma and splenic lymphomas are associated with HCV infection,<sup>3,9</sup> we tested the hypothesis that HCV infection has a role in splenic lymphoma with villous lymphocytes. Although there is no evidence that interferon alfa therapy is effective in this subtype of lymphoma,<sup>15</sup> we also investigated the effect of antiviral therapy on the

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course of splenic lymphoma with villous lymphocytes in patients with HCV infection and in those without HCV infection.

## METHODS

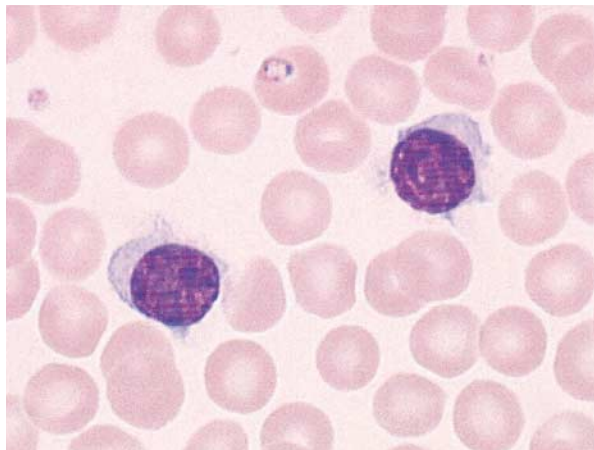
### Patients

To evaluate the effect of HCV infection and antiviral therapy on the course of splenic lymphoma with villous lymphocytes, we enrolled eight patients in addition to Patient 1 who were being treated at one of several French centers. All patients gave oral informed consent, and the study was authorized by the French Department of Health. Furthermore, using a national retrospective survey of patients who had splenic lymphoma with villous lymphocytes, we identified six patients who had received similar treatment with interferon, presumably in attempts to control the lymphoma, but who were negative for HCV infection.

The diagnosis of splenic lymphoma with villous lymphocytes was based on the presence of typical clinical, hematologic, and immunologic findings as previously described.<sup>15,16</sup> Briefly, splenic lymphoma with villous lymphocytes was defined by the presence of splenomegaly, lymphocytes with villous projections in the peripheral blood (Fig. 1), and B cells that expressed IgM, CD19, CD20, and DBA44 and were negative for CD5.<sup>15</sup> A variable degree of anemia, thrombocytopenia, and monoclonal immunoglobulin component could also be present. All blood smears and immunophenotypic findings were reviewed by two expert cytologists.

### Laboratory Analyses

Immunoblotting for cryoglobulin was performed as previously described.<sup>17</sup> An assay for rheumatoid factor and serum protein electrophoresis were performed with the use of standard methods. HCV antibodies were detected by two specific third-generation immunoassays (Monolisa anti-HCV Plus, Sanofi Diagnostic Pasteur, and AxSYM HCV, version 3.0, Abbott). Serum HCV RNA was measured by a reverse-transcription–polymerase-chain-reaction (RT-PCR) assay (Amplicor HCV test, Roche Diagnostics).



**Figure 1.** Peripheral-Blood Smear Showing Villous Lymphocytes (May–Grunwald–Giemsa Staining,  $\times 100$ ).

Two lymphocyte cells have thin, short villi concentrated at one pole. In each cell the nucleus is round and contains clumped chromatin.

### Antiviral Treatment

Both groups of patients received the same regimen of interferon alfa. Initially, they received 3 million IU of recombinant interferon alfa-2b subcutaneously three times a week for six months. Patients who had a partial response were treated for one year or more, until a complete clinical response was achieved. In HCV-positive patients with detectable HCV RNA who had no therapeutic response, ribavirin (1000 to 1200 mg per day) was added to interferon treatment. In patients with progression of lymphoma, treatment was scheduled to be stopped before six months.

### Response Criteria

The patients' responses were evaluated every three months by means of a clinical examination, ultrasonography, peripheral-blood count, examination of blood smears, and quantitation of HCV RNA in blood. Examination of bone marrow was not required for the assessment of hematologic response. Clinical manifestations associated with cryoglobulinemia were also assessed every three months before and during treatment.

A complete hematologic response was defined by the resolution of splenomegaly (the spleen was not palpable on clinical examination, was smaller than 14 cm in its larger axis on ultrasonography, or had both characteristics), a platelet count of 100,000 per cubic millimeter or more, an absolute neutrophil count of 1000 per cubic millimeter or more, a hemoglobin level of 10 g per deciliter or more (without blood transfusion), and the absence of circulating villous lymphocytes. A partial response was defined by a decrease in the size of the spleen by at least 50 percent and by an improvement in hematologic values of more than 50 percent over base-line values, resulting in an absolute neutrophil count of less than 1000 per cubic millimeter, a platelet count of less than 100,000 per cubic millimeter, or a hemoglobin level of less than 10 g per deciliter. Treatment failure and disease progression were defined as a persistent or progressive increase in the number of peripheral-blood villous lymphocytes and splenomegaly, respectively. Relapse was defined by a progressive increase in the number of peripheral-blood villous lymphocytes, increasing splenomegaly, or both after an initial complete or partial response.

### Follow-up Studies

The size of the spleen below the costal margin, and the severity of clinical manifestations associated with cryoglobulinemia, peripheral-blood count, finding on examination of blood smears, and HCV RNA load were determined every three months after the discontinuation of the interferon alfa therapy. All patients were observed for at least 12 months after the completion of therapy.

### Statistical Analysis

The two groups of patients were compared with use of Fisher's exact test and the Epi Info computer program (version 2000, Centers for Disease Control and Prevention).

## RESULTS

### Characteristics of Patients with HCV Infection

Table 1 summarizes the characteristics of the nine patients who had splenic lymphoma with villous lymphocytes and HCV infection. The median age was 55 years (range, 38 to 78). Five patients had not received prior therapy, and two had undergone splenectomy, one of whom had also received chemotherapy. Six patients presented with clinical manifestations of cryoglobulinemia: two had purpura, four had arthralgia, one had peripheral neuropathy, and one had renal

TABLE 1. CHARACTERISTICS OF THE NINE PATIENTS WITH SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES AND HEPATITIS C VIRUS (HCV) INFECTION.\*

PATIENT No.	AGE (YR)/SEX	PRIOR THERAPY	SYMPTOMS RELATED TO MC AND HCV INFECTION	SPLEEN SIZE†	ABSOLUTE NEUTROPHIL COUNT	VILLOUS LYMPHOCYTES	PLATELET COUNT	HEMOGLOBIN	ALANINE AMINOTRANSFERASE	ASSAY RESULTS
				cm below costal margin		×10 <sup>-9</sup> /mm <sup>3</sup>		g/dl	× upper limit of normal value	MC RF IgM
1	40/F	Azathioprine	Arthralgia, purpura	2	8.2	2.0	278	13.9	1.5	+ + +
2	78/M	None	None	6	4.5	4.2	120	12.6	5	+ + +
3	55/F	Splenectomy, chlorambucil, CHOP	Arthralgia, sicca syndrome	—	3.8	1.2	237	13	Normal	+ + +
4	38/M	Cyclophosphamide	Renal disease, purpura	3	3.8	2.8	136	10.5	Normal	+ + +
5	62/F	None	None	10	6.0	25	105	10.9	2	+ - -
6	40/F	None	Peripheral neuropathy	4	10.4	10	196	13.6	6	+ + -
7	57/F	None	None	3	3.1	4.1	212	12.6	1.5	- + -
8	63/F	Splenectomy	Arthralgia	—	4.5	5.9	121	8.6	5	+ + +
9	50/F	None	Arthralgia, cirrhosis	4	1.8	6.9	71	13.3	2	+ + +

\*MC denotes mixed cryoglobulinemia, RF rheumatoid factor, and CHOP cyclophosphamide, doxorubicin, vincristine, and prednisone. †Dashes indicate that the patient had undergone splenectomy.

disease. These six patients had both detectable rheumatoid factor and cryoglobulinemia. One patient had post-hepatitis cirrhosis.

**Response to Antiviral Treatment**

All nine patients had a negative HCV RT-PCR assay after antiviral therapy (Fig. 2). Seven patients (Patients 1, 2, 3, 5, 6, 7, and 8) had a sustained antiviral response, with a negative HCV RT-PCR assay after three to six months of interferon therapy. All of these patients had a progressive decrease in both spleen size and the number of villous lymphocytes in peripheral blood (Fig. 2). In these patients, the response was concomitant with the negative RT-PCR assay for HCV RNA. At six months, Patients 4 and 9 still had detectable levels of HCV RNA and no clinically significant hematologic response. However, after the addition of ribavirin to interferon alfa, Patient 4 had undetectable levels of HCV RNA and a complete clinical and biologic hematologic response. In Patient 9, the HCV RT-PCR eventually became negative, villous lymphocytes disappeared from peripheral blood, the complete blood count returned to normal, and the size of the spleen was reduced by at least 50 percent, but the spleen remained palpable (2 cm below the costal margin).

**Follow-up Study**

After a median follow-up of 27 months (range, 15 to 40), seven patients still had a complete hematologic response while remaining negative for HCV RNA, one still had a partial response (Patient 9), and one (Patient 6) had had a relapse of splenic lymphoma with villous lymphocytes and detectable levels of HCV RNA (Fig. 2). Treatment with interferon and ribavirin resulted in a second virologic response, and Patient 6 had a complete hematologic response (Fig. 2). This patient still has a complete hematologic response after a follow-up of 40 months. After antiviral therapy and the loss of detectable HCV RNA, despite persistent cryoglobulinemia and detection of rheumatoid factor, the clinical manifestations of cryoglobulinemia disappeared in all symptomatic patients.

**Characteristics of Patients without HCV Infection**

The characteristics of the six patients without HCV infection were similar to those of the patients with HCV infection, except for the absence of clinical or biologic manifestations of cryoglobulinemia (Table 2). None of the six patients had a response to interferon therapy (data not shown). The size of the spleen and the villous lymphocyte count remained stable in five patients despite at least six months of interferon therapy. One patient (Patient 14) had disease progression after four months of therapy, leading to the discontinuation of interferon. The difference in response be-

tween HCV-positive patients and HCV-negative patients was statistically significant ( $P < 0.01$ ).

### DISCUSSION

Splenic lymphoma with villous lymphocytes is characterized by splenomegaly and various degrees of anemia, thrombocytopenia, or both. This disease has a relatively indolent course and is usually treated by splenectomy, chemotherapy (including fludarabine), or both; the overall five-year survival rate is 80 percent.<sup>15,18</sup> The immunologic phenotype of splenic lymphoma with villous lymphocytes is similar to that of immunocytoma-lymphoma (e.g., negative for CD5 and positive for CD19 and IgM) and, in a significant number of cases, is also associated with the presence of a serum monoclonal immunoglobulin component.

We obtained therapeutic evidence that HCV may have a critical role in splenic lymphoma with villous lymphocytes. We first observed the unexpected regression of splenic lymphoma with villous lymphocytes in a patient who was treated with interferon alfa for a symptomatic type II cryoglobulinemia (Patient 1). This prompted us to evaluate the effect of interferon alfa in eight additional patients, and we found that seven patients had both a complete virologic and a hematologic response. In contrast, the six patients with splenic lymphoma with villous lymphocytes but without HCV infection had no hematologic response. It is unlikely that this difference is due to the selection of a group of patients who are more sensitive to therapy, since more of the HCV-positive patients had previously received chemotherapy. Thus, a direct antiproliferative effect of interferon on splenic lymphoma with villous lymphocytes probably did not account for the response observed in these patients.

The relation between the course of splenic lymphoma with villous lymphocytes and the HCV RNA load was also illustrated by the observation that a complete hematologic response occurred in patients who had a partial response or a relapse after the addition of ribavirin treatment and the loss of detectable HCV RNA. In all five patients in whom molecular studies were performed, the rearrangement of the monoclonal immunoglobulin gene observed at diagnosis was still detectable in the blood even after a complete hematologic response had been achieved (data not shown). These findings differ from those of Zuckerman et al.,<sup>19</sup> who recently reported the disappearance of B-cell clones from the blood of HCV-infected patients after antiviral therapy. This discrepancy might be explained by the fact that the population of patients studied by Zuckerman et al. did not have clinically evident lymphoma and were thus probably still dependent on HCV stimulation for B-cell survival.

The mechanisms by which HCV infection leads to the development of B-cell lymphoma remain to be de-

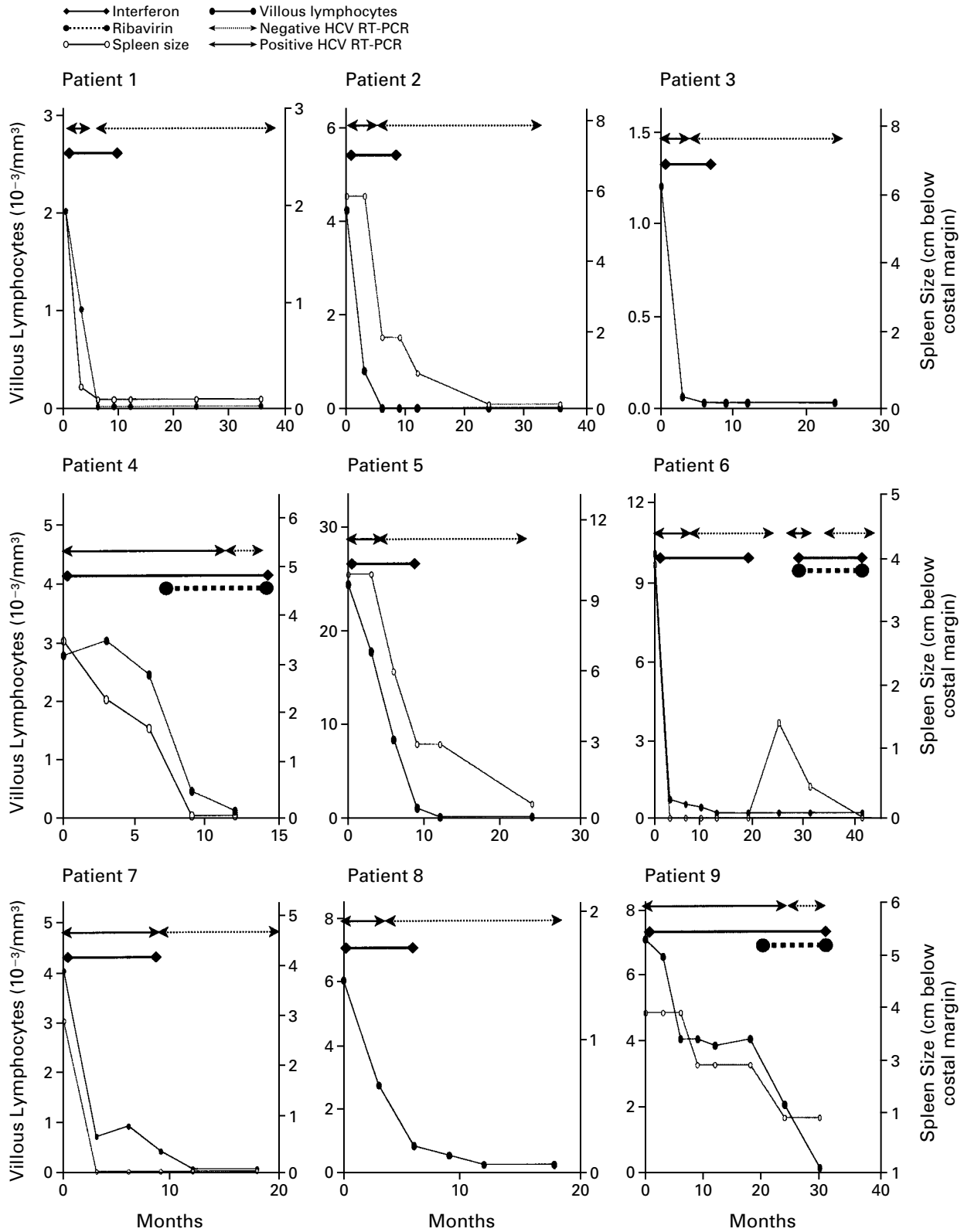
termined. Our results bring to mind the finding that *Helicobacter pylori* infection has a pathogenic role in the development of gastric lymphoma involving mucosa-associated lymphoid tissue (MALT), another subtype of marginal-zone lymphoma that originates in marginal B cells of the mucosa. Most cases of MALT gastric non-Hodgkin's lymphoma regress after the eradication of *H. pylori* infection,<sup>20</sup> as was also the case in our patients with splenic lymphoma with villous lymphocytes who had a complete hematologic response after antiviral therapy and the loss of detectable HCV RNA.<sup>20</sup> In the case of *H. pylori* infection, non-Hodgkin's lymphoma is supposed to be due to the chronic stimulation of immunologic tissues by bacterial antigens, resulting in clonal expansion of B cells and secondary progression to non-Hodgkin's lymphoma. Stimulation of marginal-zone B cells in the spleen by persistent HCV antigens, particularly the E2 viral antigen, might also be involved in the pathogenesis of splenic lymphoma with villous lymphocytes,<sup>21,22</sup> since, on antigenic stimulation, the normal marginal-zone B cells exhibit somatic hypermutation and expand clonally.<sup>23,24</sup> These CD5-IgM+ clones may persist and enter the circulation. Alternatively, HCV may have a direct oncogenic effect on B cells. Indeed, HCV is capable of infecting B cells, and low-density lipoprotein receptors<sup>25</sup> and CD81<sup>26</sup> are candidate receptors for the virus. Moreover, some HCV proteins, such as HCV protein core and nonstructural protein 5A, apparently have a direct effect on cellular proliferation and viability.<sup>27-29</sup> However, we were unable to detect HCV proteins in villous lymphocytes or any particular viral genotype (data not shown).

In conclusion, our observations in patients with splenic lymphoma with villous lymphocytes extend those of previous epidemiologic studies suggesting that HCV infection has a role in lymphomagenesis. Therefore, systematic screening for HCV infection should be performed in patients who have been given a diagnosis of splenic lymphoma with villous lymphocytes. In HCV-positive patients, antiviral therapy may be an alternative to splenectomy, chemotherapy, or both. Epidemiologic studies are warranted in larger groups of patients with splenic lymphoma with villous lymphocytes and other low-grade B-cell non-Hodgkin's lymphomas, particularly marginal-zone lymphomas. Larger therapeutic trials of antiviral therapy are

**Figure 2 (facing page).** Response to Antiviral Treatment of Patients with Splenic Lymphoma with Villous Lymphocytes and Hepatitis C Virus (HCV) Infection.

Serum HCV RNA was measured by a reverse-transcription-polymerase-chain-reaction (RT-PCR) assay. Patients 3 and 8 had undergone splenectomy.

SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES AND HEPATITIS C VIRUS



**TABLE 2.** CHARACTERISTICS OF THE SIX PATIENTS WITH SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES WITHOUT HEPATITIS C VIRUS INFECTION.

PATIENT NO.	AGE (YR)/SEX	PRIOR THERAPY	SPLEEN SIZE	ABSOLUTE NEUTROPHIL COUNT	VILLOUS LYMPHOCYTES	PLATELET COUNT	HEMOGLOBIN	DURATION OF INTERFERON THERAPY	OUTCOME AFTER INTERFERON THERAPY
			cm below costal margin		$\times 10^{-3}/\text{mm}^3$		g/dl	mo	
10	54/F	None	8	1.4	4.5	88	11	7	Treatment failure
11	72/F	None	0	2.1	44	139	12	9	Treatment failure
12	61/M	None	10	2.3	2	106	10.6	6	Treatment failure
13	61/F	Splenectomy, chlorambucil	—*	1.9	94	265	13	6	Treatment failure
14	69/M	None	10	1.1	100	88	10	4	Progression
15	58/M	None	6	1.2	19	89	9.8	6	Treatment failure

\*Patient 13 had undergone splenectomy.

needed to determine the role of antiviral therapy in HCV-infected patients with low-grade lymphoma.

We are indebted to J.P. Hayman (Hôpital Tenon, Paris), F. Lejeune (Hôpital Jean Verdier, Bondy, France), K. Edric, G. Damaj, and C. Belanger (all at Hôpital Necker, Paris), and F. Dreyfus (Hôpital Cochin, Paris) for their help in caring for and following the patients; to C. Besson (Hôpital Necker, Paris) for help with the statistical analysis; and to A. Tu for assistance in the preparation of the manuscript.

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