Prediction of sustained remission of chronic hepatitis C after a 12-month course of alfa interferon

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α-Interferon therapy normalizes aminotransferase levels in approximately 50% of the patients with chronic hepatitis C, but post-therapy relapses are common and predictive factors of sustained response remain largely unknown. We retrospectively assessed several parameters as predictors of sustained remission after a 12-month course of lymphoblastoid α-interferon: the Knodell histological activity index, serum levels of procollagen type III peptide, serum HCV-RNA, anti-α-interferon antibodies, and anti-HCV antibodies (C-100-3), all at month 12. Thirty-seven patients were studied. Fourteen patients were non-responders (38%), 15 patients experienced a sustained response (40.5%) and eight patients responded similarly but relapsed after α-interferon withdrawal (21.5%). A decrease in the histological activity index above 5, normalization of procollagen type III peptide levels (<12 ng/ml) and the absence of viremia after treatment were all significantly associated with a sustained response ($p=0.008$, $p=0.007$ and $p=0.037$, respectively). Anti-interferon antibodies were detected in only one non-responder patient. Anti-C-100-3 antibodies became undetectable at month 12 in 5 of the 15 sustained responders. The best prediction of sustained response was obtained from the three variables independent of multivariate analysis according to the following equation: $F=0.872+0.067\times K$ (decrease of histological index) $-0.052\times P$ (procollagen type III peptide levels at month 12) $-0.28\times R$ (HCV-RNA at month 12; $R=2$ when present and $R=1$ when absent). A score higher than 0 predicted sustained remission with a 100% sensitivity and specificity in this series of patients. The results of this study may be useful in establishing the optimal duration of α-interferon therapy. © Journal of Hepatology.

Key words: Aminotransferases; Anti-HCV antibodies; Anti-interferon antibodies; HCV RNA; Histological activity index, Procollagen III peptide

Although chronic hepatitis C may follow a mild and asymptomatic course, it may also lead to cirrhosis and hepatocellular carcinoma in a substantial proportion of patients (1,2). At present, α-interferon is the only therapy which has shown promising results for this disease. During α-interferon treatment, aminotransferase levels become normal and the hepatic necroinflammatory lesion improves in approximately 50% of patients (3–6). However, relapse of liver disease after discontinuation of treatment has been reported in about 50% of the responders in most trials (3), and it has been suggested that only sus-

tained responders may obtain long-term benefit from α-interferon therapy (7–9). Some preliminary results have suggested that a longer duration of α-interferon treatment could enhance sustained remission rates (6). Factors which could predict post-therapy outcome (sustained response or disease relapse) remain largely unknown (10). However, preliminary studies have shown that clearance of serum hepatitis C virus (HCV) RNA accompanies the biochemical response to α-interferon in most cases, and that relapse of the disease is preceded by the reappearance of viremia (11,12).

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Predictive parameters of relapse would allow treatment to be tailored for each patient, thus improving clinical results and the cost-benefit ratio. Accordingly, the aim of this study was to assess several parameters retrospectively as predictors of post-therapy relapse, in a series of chronic hepatitis C patients consecutively treated with a 12-month course of lymphoblastoid α-interferon.

Patients and Methods

Patients

Thirty-seven consecutive patients with chronic hepatitis C treated with a 12-month course of α-interferon were included in this study. Lymphoblastoid α-interferon (Wellferon®, Wellcome, Beckenham, UK) was administered in a step-down schedule: 3 million units (MU) daily for 2 months, 3 MU three times per week for 3 months, and 1.5 MU three times per week for 7 months. The mean follow up after completion of therapy was 40.7±8.3 months (range: 30–53). The patients had been entered into an open randomized controlled trial of lymphoblastoid α-interferon treatment versus no therapy, as described in detail elsewhere (6). All patients had had elevated aminotransferase levels for at least 6 months before starting therapy. Diagnosis of chronic hepatitis C was established by the presence of anti-HCV antibodies (C-100-3) (Ortho Diagnostic Systems, Raritan, NJ) after exclusion of other conditions by appropriate laboratory tests (HBsAg, autoantibodies, α1-antitrypsin, ceruloplasmin, urinary copper, serum iron and ferritin). None of the patients had received hepatotoxic drugs or had consumed alcohol for at least 6 months before starting therapy. Patients with clinically decompensated cirrhosis, systemic diseases, and anti-human immunodeficiency virus antibodies were not entered into the trial. All patients gave written informed consent prior to entry into the trial, and the study was approved by the Local Ethics Committee. Aminotransferase levels were assessed monthly during the entire follow-up period. Three groups of patients were defined according to the response to therapy; the non-response group included all patients with elevated alanine aminotransferase (ALT) levels at the end of therapy, the sustained-response group included all patients with normal ALT values at the end of therapy, and which persisted within the normal range during post-therapy follow up, and the relapse group included all patients with normal ALT levels at the end of therapy but who experienced an elevation of ALT values after α-interferon withdrawal.

Liver histology

Pretreatment liver biopsies were obtained during the 3 months before therapy began and post-treatment liver specimens were obtained during the last 2 weeks of therapy. The overall histological diagnosis was made according to standard criteria (13). All patients had chronic active hepatitis or active cirrhosis. Liver specimens were formalin-fixed and paraffin-embedded. Four-μm-thick sections stained with hematoxylin and eosin, Masson trichrome, periodic acid Schiff with previous diastase digestion and a reticulin stain were used for histological grading. A single experienced liver pathologist examined all the liver biopsies under code and applied the histological scoring system described by Knodell et al. (14) to pretreatment and post-treatment liver specimens from all patients.

Serum procollagen type III peptide

Serum procollagen type III aminoterminal peptide (PIIIP) levels were determined before and at the end of therapy using a commercial radioimmunoassay kit (RIA gnost PIIIP; Behringwerke AG, Marburg, Germany). Serum samples were stored at −40°C until assay.

Serum HCV RNA

The presence of HCV RNA in serum was studied by the reverse transcription "nested" polymerase chain reaction (PCR) in a sample obtained immediately before starting therapy and in a sample collected at the time of the second liver biopsy. In addition, serum samples from sustained responders were assessed for the presence of HCV RNA at months 6 and 12 after α-interferon withdrawal. The PCR primer sequences were derived from the highly conserved 5' noncoding region of the HCV genome (15) (Outer primers; C15 sense: 5'GTATCTCGAGGCGA-CACTCCACCATAGAT3' and C16 antisense: 5'AT-ACTCGAGGTGCACGGTCTACGAGACCT3'. Inner primers; C17 sense: 5'CCACCATAGATCTCTCCCCT-GT3' and C18 antisense: 5'CACTCTCGAGCACCCTA-TCAGGCAGT3'). The PCR was performed as described elsewhere (16), and false positive results were carefully avoided applying the recommended measures (17).

Anti-lymphoblastoid α-interferon antibodies

Neutralizing anti-lymphoblastoid α-interferon antibodies were studied by a bioassay in a serum sample collected during the last week of therapy. This bioassay is based on the protective effect of lymphoblastoid α-interferon on monkey kidney V3 cells challenged with Semliki Forest virus (18). Purified lymphoblastoid α-interferon of a standardized potency and control antisera (kindly supplied by Wellcome Research Foundation) were used to confirm the specificity and sensitivity of the assay.

Anti-HCV antibodies

Samples from each patient obtained before and at the end of therapy were tested for the presence of antibodies to HCV (C-100-3) using a commercial first-generation enzyme-linked immunoassay (ELISA) (Ortho Diagnostic Systems, Raritan, NJ).

Statistics

Student's *t*-test was used for continuous variables and Fisher's exact test for dichotomous variables. ANOVA was used for comparing the three groups. The Mann-Whitney U-test was applied to non-parametric values (decrease of the Knodell's histological activity index and sub-indices). Multivariate analysis (stepwise logistic-regression) was applied to all factors in order to obtain a discriminant function which could improve the prediction of sustained remission (19). Statistical significance was defined as a *p*-value of 0.05 or less and all *p*-values are two-tailed. The SPSS/PC+statistical package was used for the analysis. Results are presented as means±SD, unless otherwise specified.

Results

Table 1 shows the characteristics of each group of patients and Fig. 1 shows the ALT profile in the three groups. Fourteen patients (38%) did not respond to therapy (non-response group), 15 patients (40.5%) experi-

enced a sustained response during treatment without relapsing during a mean follow up of 41±8 months (range: 30–53) after therapy (sustained-response group), and eight patients (21.5%) showed a similar complete response during therapy, but relapsed after α-interferon withdrawal (follow up: 42±8 months; range: 30–51) (relapse group). The eight post-therapy relapses occurred 1–2 months after the completion of the treatment, and later rebounds were not observed.

Histological activity index

Table 2 shows changes in the histological activity index in the sustained-response and relapse groups. Before therapy, the histological activity index in the sustained-response group was significantly higher than that of the relapse group (11.80±2.93 vs. 8.87±3.22; p=0.048). However, at the end of the treatment there were no significant differences between the two groups (5.60±2.69 vs. 6.12±2.64). The mean decrease in the histological activity index in the sustained-response group was significantly higher than in the relapse group (6.20±2.88 vs 2.75±1.58; p=0.008). The mean decrease in the relapse and non-response groups was similar (2.75±1.58 vs. 2.93±2.70). The decrease in the histological activity index in the sustained-response group was caused by a marked improvement of the periportal and lobular inflammation (Table 2). However, the periportal index was the only one which decreased significantly more in the sustained-response group

TABLE 1

Characteristics of patients at base line

Characteristic	Non-response group n=14	Sustained-response group n=15	Relapse group n=8	p*
Age (yr)	48.2±14.6	35.5±12.2	44.2±15.3	NS
Sex (M/F)	10/4	10/5	4/4	NS
Acute hepatitis	4/14	7/15	4/8	NS
Estimated duration of disease (years)	3.8±2.1	4.1±3.3	5.9±4.2	NS
Source of hepatitis:				
a) transfusions	2	7	2	
b) drug addiction	–	5	–	
c) other parenteral	1	1	2	
d) unknown	11	2	4	
Histology:				
chronic hepatitis/cirrhosis	9/5	13/2	5/3	NS
Analytical data:				
AST (U/l)[a]**	85.4±43.4	119.1±84.6	127.9±132.0	NS
ALt (U/l)[b]**	143.2±60.6	222.0±163.6	160.1±161.1	NS
Alkaline phosphatase (U/l)[c]**	156.9±67.2	134.1±46.8	152.7±38.6	NS
γGT (U/l)[d]**	42.9±39.7	49.2±35.7	47.6±34.4	NS
Bilirubin (mg/dl)	0.98±0.36	1.00±0.43	0.77±0.16	NS
Albumin (g/dl)	4.17±0.37	4.37±0.55	4.27±0.31	NS
Gammaglobulins (g/dl)	1.42±0.39	1.53±0.30	1.37±0.32	NS
Prothrombin index (%)[e]**	87.7±14.8	85.4±10.1	87.7±12.3	NS
Anti-HCV antibodies	14/14	15/15	8/8	NS

* Statistical differences between sustained-response and relapse groups.
** Normal ranges; [a] 0–25 U/l, [b] 0–29 U/l, [c] 73–207 U/l, [d] 5–38 U/l, [e] 75–120%.
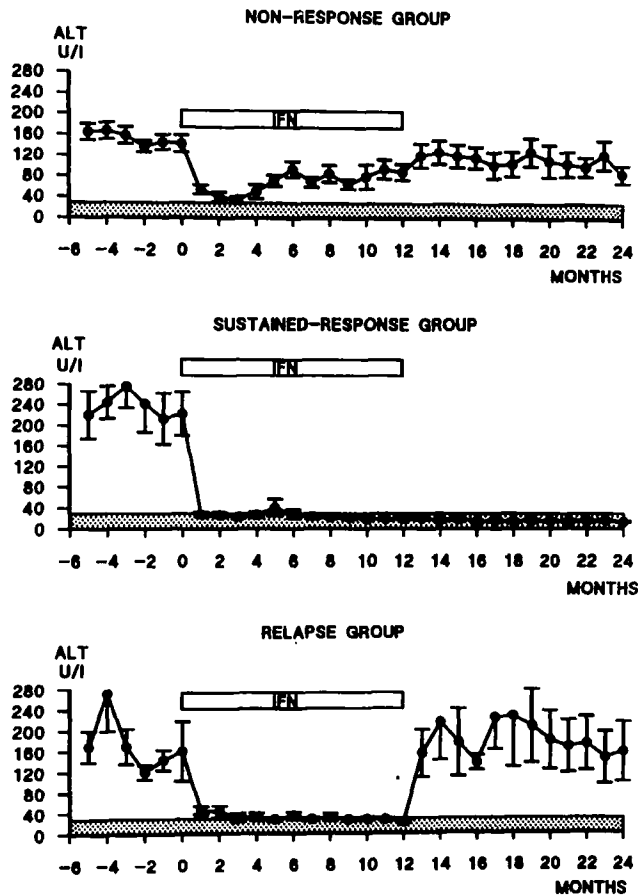
Fig. 1. Changes in alanine aminotransferase (ALT) levels in the non-response, sustained-response and relapse groups. ALT levels are presented as means±SEM.

than in the relapse group (2.60±1.45 vs. 0.88±1.13; $p=$ 0.009). It was observed that a decrease in the histological activity index higher than or equal to 5 predicted a sustained remission after therapy with a sensitivity of 73% and a specificity of 87.5% (positive predictive value: 92%, negative predictive value: 64%).

Procollagen type III aminoterminal peptide levels

Base line PIIIP levels were similar in the three groups of patients (non-response: 16.9±4.7, sustained-response: 17.7±4.5, relapse: 15.5±4.2). However, as shown in Fig. 2, at month 12 significant differences were observed between the sustained-response and the relapse groups (10.9±2.4 vs. 14.4±2.7; $p=0.007$), and between the sustained-response and the non-response groups (10.9±2.4 vs. 17.4±4.6; $p<0.001$). Differences between the relapse and the non-response groups were not statistically significant. PIIIP concentrations reached the normal range (6–12 ng/ml) at the end of therapy in 73% of the sustained-responder patients, while it occurred in only 25% and 14% of the patients from the relapse and non-response groups ($p=0.037$ and $p=0.002$, respectively). The normalization of PIIIP levels predicted a sustained post-therapy remission of the disease with a sensitivity of 73% and a specificity of 75% (positive predictive value: 85%, negative predictive value: 60%).

Serum HCV RNA

Figure 3 shows changes in the detection of viremia in the three groups of patients. Before treatment, detection rates of HCV RNA were similar in the three groups (non-response: 78%, sustained-response: 85%, relapse: 87%). In contrast, at the end of the α-interferon course significant differences were observed between the sustained-response and the relapse groups, and between the sustained-response and non-response groups. No differences were found between the relapse and the non-response groups. Seventy-three per cent of the patients who had a sustained-response were not viremic at the end of the therapy, as compared to only 25% and 7% of the cases from the relapse and non-response groups respectively ($p=$ 0.037 and $p<0.001$). Accordingly, the estimated sensitivity and specificity of serum HCV RNA to predict a sustained response was 73% and 75%, respectively, for a
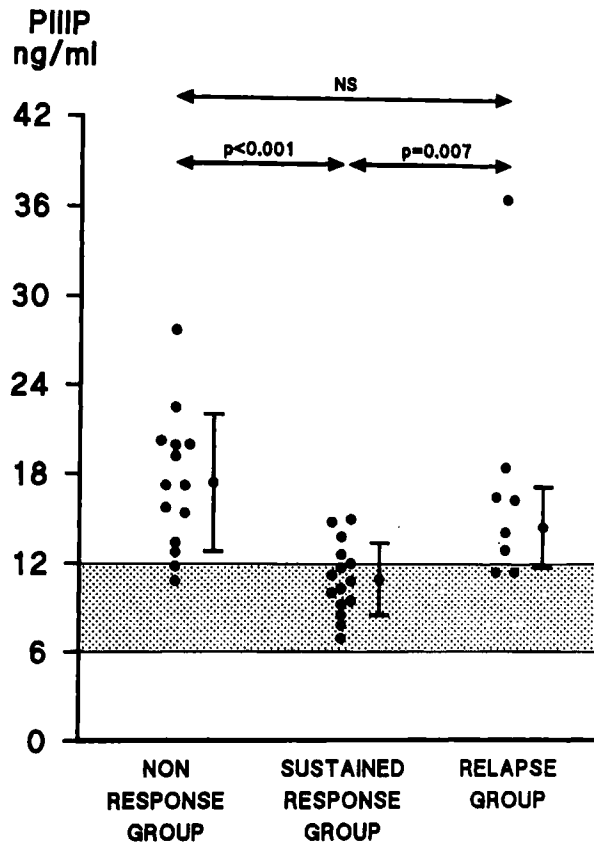
TABLE 2

Changes of the Knodell histological activity indexes in the sustained-response and relapse groups

	Sustained-response group $n=15$			Relapse group $n=8$		
	Base line	End of therapy	Mean decrease	Base line	End of therapy	Mean decrease
Periportal	3.46±1.40	0.86±0.95	2.60±1.45[a]**	1.87±1.16	1.00±0.86	0.88±1.13[a]
Lobular	2.73±1.12	0.73±0.77	2.00±1.31[b]	1.75±0.96	0.62±0.48	1.12±1.05[b]
Portal	3.53±0.49	2.46±0.95	1.06±0.99[c]	3.37±0.48	2.62±0.99	0.75±0.82[c]
Fibrosis	2.06±1.06	1.53±0.95	0.53±0.95[d]	1.87±1.16	1.87±1.16	0[d]
Total	11.80±2.93*	5.60±2.69	6.20±2.88[e]	8.87±3.22	6.12±2.64	2.75±1.58[e]

** Statistical differences between the two groups with respect to the mean decreases of each one of the histological scores: [a] $p<0.01$, [b] NS, [c] NS, [d] NS, [e] $p<0.01$.
* $p<0.05$ versus base line total histological activity index of the relapse group.

Fig. 2. Procollagen type III peptide (PIIIP) concentrations at the end of the therapy (month 12) in the three groups of patients. Significant differences may be observed between the sustained-response and relapse groups, and between the sustained-response and non-response groups. The shaded area represents the normal range of PIIIP levels.
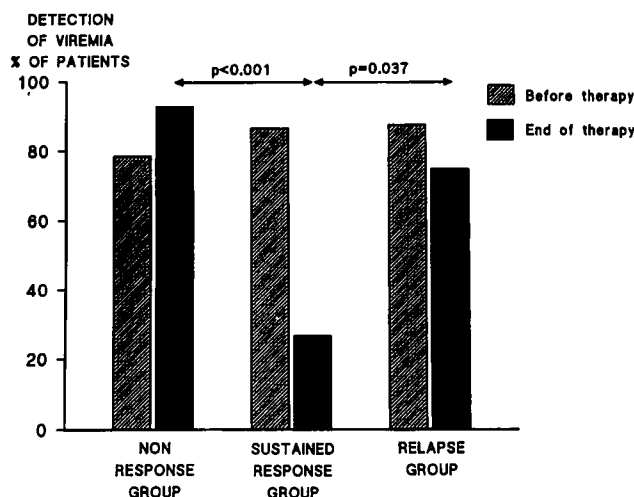

Fig. 3. Changes in the detection of serum HCV RNA (before therapy/end of therapy) in the three groups of patients. A significant decrease may be observed only in the sustained-response group.

positive predictive value of 85% and a negative predictive value of 60%. However, sustained remission was attained in four patients who had viremia at the time of α-interferon withdrawal. Further follow up of these patients showed disappearance of HCV RNA in three patients at months 6 and 12, while one patient who had moderately active cirrhosis before therapy remained viremic at months 6, 12 and 24 after the completion of treatment, despite persistently normal ALT levels assessed at monthly intervals. A liver biopsy of this patient obtained 1 year after finishing therapy showed mildly active cirrhosis. The remaining 11 patients who had a sustained response and had cleared HCV RNA from sera remained without detectable viremia at months 6 and 12 after the end of the α-interferon course. Thus, post-therapy follow up showed that 93% (14/15) of the sustained responders cleared serum HCV RNA.

Neutralizing anti-lymphoblastoid α-interferon antibodies

Anti-lymphoblastoid α-interferon antibodies were detected at the end of therapy in only one non-responder patient, thus precluding any association between its presence and the post-therapy evolution of the disease.

Anti-HCV antibodies

Antibodies to the C-100-3 recombinant antigen of HCV became negative in five patients (33%) from the sustained-response group at the end of therapy. In contrast, none of the relapsers and non-responders lost anti-C-100-3 antibodies (positive predictive value: 100%, negative predictive value: 44%).

Multivariate analysis

In order to obtain a discriminant function, forward stepwise logistic-regression analysis was applied to all variables. Decrease of the histological activity index, and PIIIP levels and the presence or absence of HCV RNA, both at the end of therapy, were independently predictive and allowed to obtain the following equation: $F = 0.872 + 0.067 \ K - 0.052 \ P - 0.28 \ R$, where K corresponds to the histological activity index at base line minus the histological activity index at month 12, P to PIIIP levels in ng/ml at month 12, and R to HCV RNA at month 12 (a PCR positive result=2 and a PCR negative result=1). All sustained-responders had an F higher than 0 while all relapsers had an F of less than 0 for a sensitivity and specificity of 100%.

Discussion

Until now, most reports on α-interferon treatment of chronic hepatitis C have focussed on the short-term utility

of this therapy, i.e. in the normalization of aminotransferase levels during administration. However, the goal of α-interferon therapy should be the induction of a sustained remission of the disease or even a complete recovery of the process. Some studies have shown that α-interferon suppresses HCV replication in most patients who experience a biochemical response to therapy, as measured by the detection of HCV RNA in serum with the PCR technique (6,11,12). However, in most trials post-treatment relapses affected approximately 50% of the patients who had experienced a biochemical response during therapy (3). Therefore, disappearance of HCV RNA from sera during therapy does not preclude further reactivation of the disease when α-interferon is discontinued, which is probably due to low-level replication of HCV within the liver or in other sites such as peripheral blood mononuclear cells (20). Future studies should compare the presence of HCV RNA in serum and liver tissue before, during and after α-interferon therapy. In accordance with this series in which relapses occurred during the first 2 months after α-interferon withdrawal, other studies have shown that the vast majority of relapses take place during the early post-therapy period (6 months) (7,8). However, late relapses (>12 months after stopping therapy) have also been observed (21).

In agreement with a previous uncontrolled study (7,22) we found a high rate of sustained remissions (40% of the treated patients) in the present study.

Study of the liver biopsies showed that the post-therapy outcome of the disease was more related to the degree of liver inflammation in the pretreatment biopsy than to that observed in the biopsy obtained at the end of the therapy. However, the variable more strongly associated with post-therapy outcome was the mean decrease in the histological activity index. In the sustained-response group the decrease in the histological activity index was significantly higher than that in the relapse group. Differences were mainly due to a higher degree of pretreatment periportal and lobular damage in sustained responders, although the histological activity index was also lower at the end of therapy in these patients.

These observations might suggest that periportal and lobular inflammation could reflect the involvement of the immune system in the clearance of hepatocytes infected with HCV, as has been documented in chronic hepatitis B (23). Other studies also have suggested the implication of the immune system in liver damage in chronic hepatitis C (24–26). Hence, although α-interferon seems to have a direct antiviral effect in HCV infection, a competent immune system is probably a prerequisite for achieving sustained remission or eradication of the infection. In support of this view, among patients undergoing liver transplantation for end-stage HCV-related liver disease, α-interferon seems less efficacious in suppressing HCV replication (27). In any case, our findings show that long-term response is associated with a marked histological improvement due to a better response to IFN.

Procollagen III peptide levels have been related to the extent of liver fibrosis and inflammatory activity, probably reflecting liver fibrogenesis and fibrolysis (28,29). Thus, the higher degree of periportal and lobular inflammation at baseline in the sustained-response group compared to the relapse group could explain the trend towards higher PIIIP levels in sustained responders. Most sustained responders (73%) attained normalization of PIIIP levels at the end of therapy, compared to only 25% of those who relapsed. This may indicate a more profound effect of α-interferon in suppressing viral replication and inflammation in patients who experience a sustained remission of the disease. Thus, measurement of PIIIP values at the end of α-interferon treatment might help predict post-therapy outcome. Similar results have been observed in a recent study (8).

The PCR technique used in this study detected viremia in most patients before α-interferon therapy. However, although the sustained-response and relapse groups showed a similar biochemical response to α-interferon, viremia was detected at the end of therapy in most patients who relapsed, despite normal aminotransferase levels during the treatment period. In contrast, most sustained-responders cleared HCV RNA. One study (11) showed that HCV RNA was cleared from serum in all the biochemical responder patients at weeks 1 or 2 after starting a 4- to 8-week course of α- or β-interferon therapy. In this short-term trial, the absence of viremia at the end of therapy was unrelated to post-treatment outcome. Recently, serum HCV RNA at the end of therapy was found to be predictive of post-therapy outcome (30,31). In the present study the presence of viremia was significantly associated with post-therapy relapse. However, two patients who did not have detectable viremia at the end of therapy still relapsed, and even more striking, four patients in whom HCV RNA was detected at the time of the completion of the α-interferon course experienced a long-term sustained response. In three of these cases, HCV RNA was not detected at months 6 and 12 after finishing therapy. The fourth case had detectable viremia at months 6, 12 and 24 after therapy, in spite of having normal ALT values in monthly assessments. Thus, "residual" or low-level replication of HCV, might not preclude long-term remission of chronic hepatitis C. Further studies are needed to clarify whether healthy carriers exist or whether a smoldering hepatic disease persists in viremic patients with normal ALT values, as suggested by the presence of periportal

inflammation 1 year after finishing therapy in one case in our study with persistent serum HCV RNA and also by one study of blood donors with confirmed anti-HCV antibodies and persistently normal ALT levels (32). However, another study indicates that persistent viremia may be found in individuals with normal histology and normal aminotransferase values (33).

Anti-lymphoblastoid α-interferon antibodies were detected in only one patient, which supports another study showing a very low rate of development of neutralizing antibodies (1.2%) when using lymphoblastoid α-interferon preparations (34). Our observation that one third of long-term responders lost anti-C-100-3 antibodies has been also reported by others (7,35). Other antibodies, such as those directed to the core protein, however, tend to persist in most treated patients (36).

Since the decrease of the histological activity index, PII-IP levels and viremia by the end of therapy were independent of the prediction of sustained remission on multivariate analysis, we obtained a linear discriminant function which had a 100% sensitivity and specificity in this series of patients. This function, however, needs to be prospectively assessed in a clinical trial and might not be useful with other interferon schedules. If a second liver biopsy cannot be performed, sustained remission could be predicted on the basis of PIIIP levels and serum HCV RNA. Normalization of PIIIP levels and/or clearance of HCV RNA at the end of therapy predicted sustained remission with a positive predictive value of 82.5% and a negative predictive value of 83%.

Acknowledgements

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