Retrospective Analysis of the Effect of Interferon Therapy on the Clinical Outcome of Patients with Viral Cirrhosis

Luisa Benvegnù, M.D.[1]
Liliana Chemello, M.D.[1]
Franco Noventa, M.D.[1]
Giovanna Fattovich, M.D.[2]
Patrizia Pontisso, M.D.[1]
Alfredo Alberti, M.D.[1]

[1] Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy.

[2] Istituto di Patologia Speciale Medica, Cattedra di Medicina Interna, University of Verona, Verona, Italy.

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Address for reprints: Prof. Alfredo Alberti, Department of Clinical and Experimental Medicine, Clinica Medica 2nd, University of Padova, via Giustiniani 2, 35128 Padova, Italy.

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BACKGROUND. Recent data suggest that interferon therapy (IFN) can reduce the risk of progression to hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV)-related cirrhosis.

METHODS. A cohort of 189 patients with Child's Stage A cirrhosis of viral etiology followed prospectively were analyzed retrospectively to assess the effects of IFN on the clinical course and development of HCC.

RESULTS. During a mean follow-up of 71.5 ± 23.6 months, 7.9% of 88 treated and 21.8% of 101 untreated patients showed worsening of the Child's disease stage ($P < 0.01$); 5.6% of treated and 26.7% of untreated patients developed HCC ($P < 0.001$); and 3.4% of treated and 19.8% of untreated patients died of liver disease or underwent orthotopic liver transplantation (OLT) ($P < 0.005$). Using Cox's regression analysis, no treatment with IFN, high bilirubin and alkaline phosphatase (ALP) levels, and low leukocyte counts and prothrombin activity (PT) were associated significantly with worsening of Child's disease stage; no treatment with IFN, long term disease, low albumin and PT, and high γ-glutamyl transpeptidase (GGT) were related significantly to HCC development; and no treatment with IFN, low albumin and PT, and high GGT and ALP were associated significantly with reduced survival. After adjustment for independent risk factors identified by multivariate analysis, the estimated cumulative probability of worsening of cirrhosis ($P < 0.05$), development of HCC ($P < 0.001$), and death or OLT ($P < 0.005$) was significantly lower in IFN-treated patients compared with untreated patients. This beneficial effect of therapy was statistically evident only in HCV positive patients.

CONCLUSIONS. These results support the hypothesis that IFN improves clinical outcomes and reduces progression to HCC in patients with HCV-related cirrhosis. These conclusions, based on retrospective data, should be confirmed prospective. ***Cancer* 1998;83:901–9.** © *1998 American Cancer Society.*

KEYWORDS: interferon, hepatitis B virus, hepatitis C virus, cirrhosis, hepatocellular carcinoma.

Cirrhosis of the liver is associated with significant morbidity and mortality and is a well-defined risk factor for the development of hepatocellular carcinoma (HCC), particularly when chronic infection with the hepatitis B virus (HBV) or the hepatitis C virus (HCV) coexists.[1–9] The risk of HCC is recognized to be higher in those cirrhotic patients who have biochemically and histologically active chronic hepatitis, suggesting that necroinflammation and the associated regenerative processes play a pivotal role in hepatic carcinogenesis.[10–13] Treatment of end-stage chronic liver disease and of HCC are currently unsatisfactory, and strategies to prevent

progression of chronic hepatitis toward cirrhosis need to be pursued for an effective control of these complications.

Interferon therapy (IFN) has been shown to induce a sustained biochemical and virologic response in a subgroup of patients with chronic HBV and HCV infection, and these cases appear to derive a definitive benefit at long term follow-up with a drastic reduction of histologic progression and improvement of clinical outcomes.[14–26] However, sustained response is achieved only in a minority of treated patients and is particularly rare in those who already have an established cirrhosis.[27,28] Indeed, the cost benefit of IFN in cirrhotic patients with HBV and HCV infection is controversial, and not all authors believe that these patients should be treated.

Recently, it has been reported that IFN may prevent or delay progression to HCC in patients with virus-related cirrhosis,[29] and such beneficial effect was found at least in one report to be independent of the biochemical and virologic response.[30] Other studies, however, have not confirmed these observations.[31] We have therefore assessed retrospectively whether treatment with interferon had influenced the outcome of virus-related cirrhosis in a large cohort of patients followed in our Institution since 1986.

PATIENTS AND METHODS

Design of the Study

Since 1986, we have been conducting a prospective follow-up study of patients with cirrhosis aimed at evaluating the course of the disease, development of HCC, and death rate. Two hundred and ninety consecutive patients were included in this study between 1986 and 1993. Among these patients, those with biopsy proven Child's stage A cirrhosis at inclusion and evidence of ongoing HBV and/or HCV infection were considered for the present analysis, which was aimed to assess the effect of IFN on the long term outcome of this type of disease. Therefore, 69 patients who had decompensated cirrhosis at inclusion and 23 patients without evidence of viral infection (12 alcoholics, 4 primary biliary cirrhosis, 7 autoimmune or cryptogenic disease) were excluded. There were 198 patients with virus-associated Child's stage A cirrhosis, and 189 of them (95.4%) had a prospective follow-up. The present analysis was performed in this group, which included 122 male (64.5%) and 67 female (35.5%) patients with a mean age of 58.2 years at inclusion (range 36–75 years; standard deviation [S.D.] 8.2). Twenty-eight (14.8%) patients were hepatitis B surface antigen (HBsAg) positive, 152 (80.4%) were anti-HCV positive, and 9 (4.8%) were both HBsAg and anti-HCV positive. A history of alcohol abuse (more than 80 g/day for males and more than 50 g/day for females) was present in 29 of these virus-infected patients (15.3%). The mean known duration of cirrhosis at inclusion was 2.1 years (range 0–11 years; S.D. 3.0).

All patients were followed up at 6-month intervals by abdominal ultrasound (US) examination. At each visit, the following tests were also performed: aspartate and alanine aminotransaminases (AST and ALT, respectively), serum albumin, prothrombin time (PT), bilirubin, γ-glutamil-transpeptidase (GGT), alkaline phosphatase (ALP), leukocyte and platelet counts, and α-fetoprotein (AFP).

Treatment with Interferon

Eighty-eight patients (46.6%) had received treatment with interferon after the diagnosis of cirrhosis was made, whereas 101 were not treated with interferon. Patients with HCV infection had received 3–6 MU of interferon thrice weekly for 6–12 months. Thirteen patients in this group had received two consecutive cycles of treatment. Patients infected by HBV, including those with HCV coinfection, had received 6–10 MU of interferon thrice weekly for 4–6 months. Long term, sustained response to interferon was defined as persistent normalization of ALT after therapy that was maintained up to the end of the follow-up period.

Events during Follow-Up

The following events during follow-up were considered for the comparison between interferon-treated and untreated patients: 1) worsening of stage of cirrhosis according to Child's classification, 2) development of HCC, and 3) death or liver transplantation. Diagnosis of HCC was made on the basis of the appearance of focal lesions at periodic US examination of the liver and was confirmed in all cases by fine needle biopsy (FNB) under sonographic guidance. Patients developing AFP levels higher than 400 ng/mL or portal thrombosis without focal lesions detectable by US were submitted to other diagnostic procedures, such as duplex US, dynamic computed tomography, and hepatic angiography with lipiodol.

Serologic Testing

Anti-HCV was determined by second-generation enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostic System, Raritan, NJ) and by second-generation recombinant immunoblotting assay (RIBA; Chiron Corporation, Emeryville, CA). HBsAg was analyzed by commercially available kits (Abbott Diagnos-

TABLE 1
Comparison of Baseline Clinical and Laboratory Findings in IFN-Treated and Untreated Cirrhotic Patients

Patients	Treated patients (n = 88)	*P*	Untreated patients (n = 101)
Sex (M/F)	59/29	n.s.[a]	63/38
Mean age (yrs)[c]	56.7 ± 8.0	<0.05[a]	59.5 ± 8.3
Mean duration of cirrhosis (yrs)[c]	1.8 ± 2.3	n.s.[b]	2.4 ± 3.5
Etiology (%)			
$HBsAg^+$	10 (11.4)	n.s.[a]	18 (17.8)
Anti-HCV^+	75 (85.2)	n.s.[a]	77 (76.2)
$HBsAg^+$/anti-HCV^+	3 (3.4)	n.s.[a]	6 (6.0)
Alcohol abuse	11 (12.5)	n.s.[a]	18 (17.8)
Laboratory findings[c]			
Albumin (g/liter)	42.4 ± 4.5	n.s.[b]	41.8 ± 4.0
Prothrombin time (% activity)	78.9 ± 12.6	n.s.[b]	82.4 ± 11.0
Bilirubin (mmol/liter)	14.3 ± 5.5	n.s.[b]	15.1 ± 5.9
ALP (IU/liter)	115.7 ± 58.5	n.s.[b]	133.9 ± 71.9
GGT (IU/liter)	99.1 ± 77.1	n.s.[b]	83.7 ± 78.2
Leukocytes (× 10.9/liter)	5.2 ± 1.4	n.s.[b]	5.1 ± 1.4
Platelets (× 10.12/liter)	134.2 ± 48.8	n.s.[b]	133.6 ± 50.1
AST (IU/liter)	141.1 ± 97.1	<0.001[b]	83.1 ± 58.5
ALT (IU/liter)	219.6 ± 160.4	<0.001[b]	117.3 ± 106.9
AFP (ng/mL)	17.1 ± 22.7)	n.s.[b]	16.9 ± 20.5

[a] Chi-square test or Fisher's exact test.
[b] Student's *t* test.
[c] Mean ± standard deviation.
HBsAg: hepatitis B surface antigen; ALP: alkaline phosphatase; GGT: γ-glutamil transpeptidase; AST: aspartate-aminotransferase; ALT: alanine-aminotransferase; AFP: α-fetoprotein; n.s.: not significant; IFN: interferon therapy.

tics, North Chicago, IL). HCV RNA was determined in serum by nested polymerase chain reaction (PCR) after reverse transcription, using primers derived from the 5′-nontranslated region, as described elsewhere. The genotype of the infecting HCV was defined by using a dot-blot assay, in which the products of PCR amplification, immobilized on nylon filters in triplicate, are hybridized with oligonucleotide probes specific for the different HCV genotypes.[32] HBV DNA in serum was determined by spot hybridization using a ^{32}P-labelled insert of cloned HBV DNA as probe.

Statistical Analysis

Student's *t* test, Fisher's exact test, and chi-square test were performed, when appropriate, to compare baseline clinical and laboratory characteristics of treated and untreated patients. Univariate analysis with the Kaplan–Meier method and the log rank test were used to compare the cumulative probability of 1) worsening of stage of cirrhosis, 2) HCC development, and 3) death or orthotopic liver transplantation (OLT) in treated and untreated patients. Multivariate analysis with Cox's proportional hazard model (stepwise option) was performed to identify the independent role of each variable (age, sex, known duration of cirrhosis, IFN treatment, alcohol abuse, and a number of laboratory findings at inclusion: albumin, prothrombin activity, bilirubin, ALP, GGT, leukocyte and platelet counts, ALT, AST, AFP, HBsAg, and anti-HCV) in the appearance of the above-mentioned adverse events, and *P* values were calculated by using a Wald test. The estimated cumulative probability of worsening of Child's stage, of HCC development, and of death or OLT in treated and untreated patients was derived by the obtained models. *P* values of less than 0.05 for univariate analysis and of less than 0.1 for multivariate analysis were considered statistically significant. Data analysis were performed with the BMDP statistical package.[33]

TABLE 2
Worsening of Child's Stage, HCC Development, and Death or OLT during Follow-Up in IFN-Treated and Untreated Patients with Cirrhosis

Events during follow-up (71.5 ± 23.6 months)	All cases (n = 189)	Treated patients (n = 88)	Untreated patients (n = 101)
Worsening of cirrhosis's stage (%)	29 (15.3)	7 (7.9)	22 (21.8)
Development of HCC (%)	32 (16.9)	5 (5.6)	27 (26.7)
Death or OLT (%)	23 (12.2)	3 (3.4)	20 (19.8)

HCC: hepatocellular carcinoma; OLT: orthotopic liver transplantation; IFN: interferon therapy.

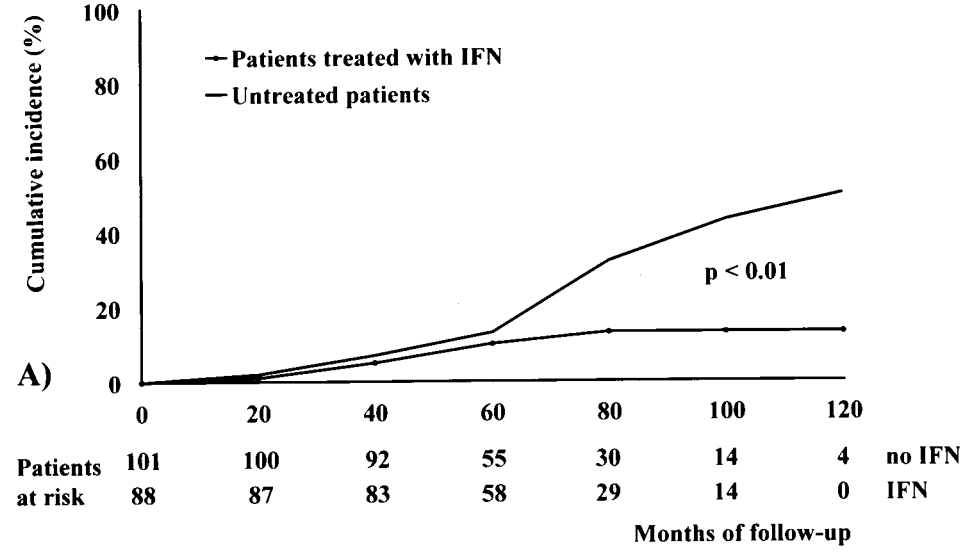
RESULTS

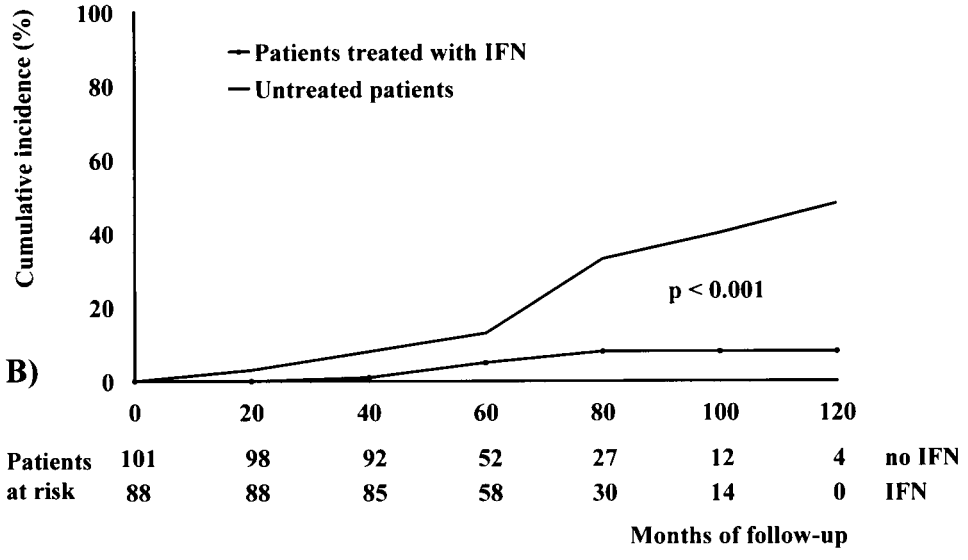
Baseline Comparison of IFN-Treated and Untreated Patients

Pretreatment baseline features in the 88 patients who received IFN were compared with those of the untreated cases at inclusion in the follow-up. Table 1 shows that there were no significant differences between the two subgroups with regard to sex distribution, known duration of cirrhosis, etiologic factors, and laboratory findings except for mean ALT and AST levels, which were significantly higher in treated patients, and mean age, which was significantly higher in the untreated cases. Mean follow-up after inclusion in the prospective study was similar in untreated (71.4 ± 26.0 months) and IFN-treated (71.9 ± 20.7 months) patients.

Outcomes of Liver Disease during Follow-Up in IFN-Treated and Untreated Patients

During a mean follow-up of 71.5 ± 23.6 months (mean ± S.D.; range 23–125 months), 29 patients (15.3%) showed worsening of stage of cirrhosis according to Child's classification, 32 patients (16.9%)





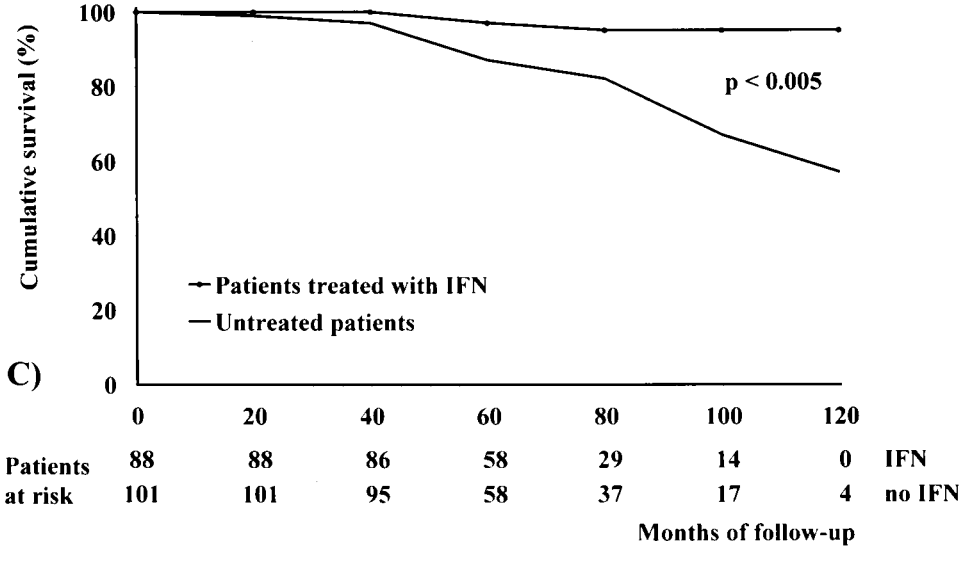


FIGURE 1. Cumulative probability of (A) worsening of stage of cirrhosis (according to Child's classification), (B) development of hepatocellular carcinoma, and (C) death or liver transplantation in 88 interferon therapy (IFN)-treated and in 101 untreated patients with cirrhosis (by using the Kaplan–Meier method and the log rank test).

TABLE 3
Independent Variables Associated with Worsening of Child's Stage, HCC Development, and Death or OLT during Follow-Up in 189 Patients with Cirrhosis (Cox's Regression and Wald Test)

Variables	Coefficient	Standard error	*P*
Worsening of cirrhosis's stage			
Leukocytes	−0.0005	0.0001	0.001
ALP	0.0074	0.0024	0.003
IFN	−1.1226	0.4532	0.013
Bilirubin	0.0685	0.0309	0.027
Prothrombin time	−0.0309	0.0185	0.096
Development of HCC			
IFN	−1.9556	0.5097	0.001
Albumin	−0.1733	0.0536	0.001
Duration of cirrhosis	0.0240	0.0962	0.012
GGT	0.0037	0.0019	0.057
Prothrombin time	−0.0326	0.0177	0.065
Death or OLT			
IFN	−1.8659	0.6537	0.004
GGT	0.0063	0.0022	0.004
Albumin	−0.2042	0.0723	0.005
Prothrombin time	−0.0555	0.0248	0.025
ALP	0.0058	0.0032	0.070

HCC: hepatocellular carcinoma; OLT: orthoptic liver transplantation; ALP: alkaline phosphatase; IFN: interferon therapy; GGT; γ-glutamil-transpeptidase.

developed HCC, and 21 patients (11.1%) died from liver-related causes (liver failure with HCC, 15 cases; liver failure without HCC, 3 cases; variceal bleeding, 2 cases; haemoperitoneus, 1 case), whereas 2 patients underwent liver transplantation. The frequency of these events in IFN-treated and untreated patients is shown in Table 2. The cumulative probabilities of disease progression ($P < 0.01$), development of HCC ($P < 0.001$), and death or liver transplantation ($P < 0.005$) were significantly higher in untreated patients compared with IFN-treated cases when assessed by the Kaplan–Meier method and the log rank test (Fig. 1).

Multivariate analysis (Cox's regression) was then performed to assess which variables were related independently to the occurrence of the different outcomes during follow-up. Table 3 shows that IFN was detected as an independent variable affecting worsening of cirrhosis, development of HCC, and death or OLT.

To better assess the independent role of IFN in the above-mentioned events, an adjustment for all other independent risk factors identified by multivariate analysis was performed. The estimated cumulative probability of worsening of stage of cirrhosis, of development of HCC, and of death or liver transplantation remained significantly higher in untreated patients compared with treated patients, even after correction for all other independent variables (Fig. 2). These events were not observed during IFN and occurred 1–9 years after withdrawal.

Effects of IFN in Relation to HBV and HCV Infection

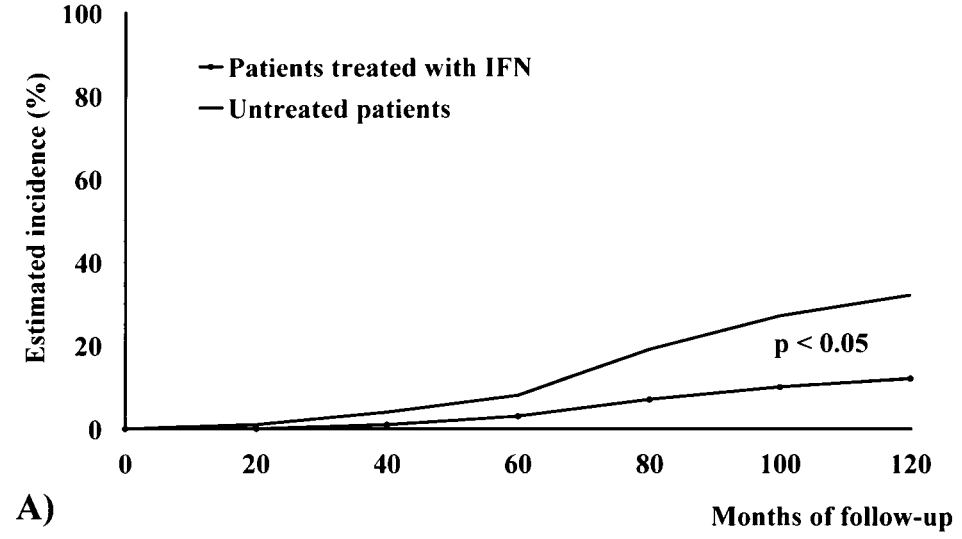
When patients were divided according to the etiology of cirrhosis, the incidence of worsening of stage of cirrhosis, of HCC development, and of death or OLT during follow-up appeared to be affected in a significant manner by IFN only in anti-HCV-positive patients (Table 4). Differences between INF-treated and untreated patients were not significant in HBsAg-positive cases or in patients with HBV and HCV coinfection, most likely as consequence of the small number of patients and events in these subgroups.

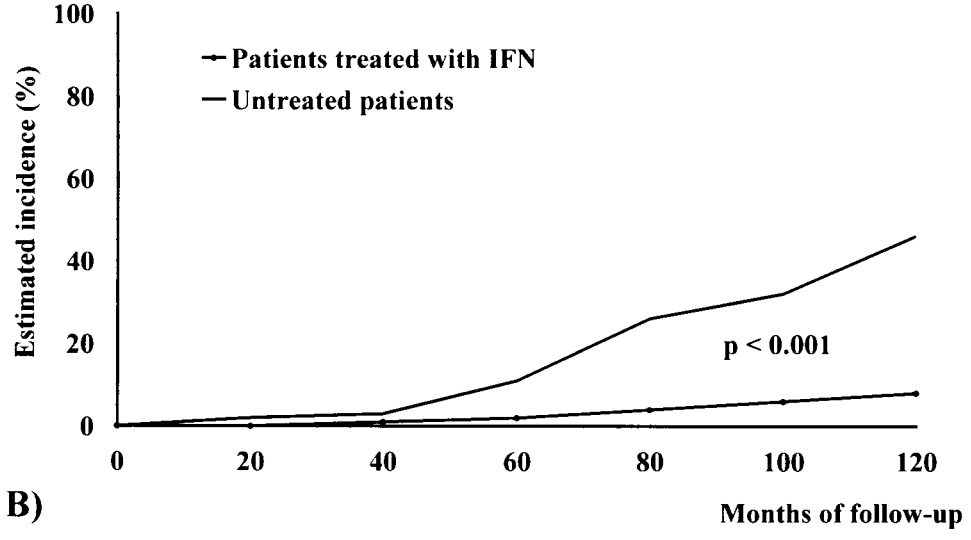
Outcome in Treated Patients in Relation to Dose and Duration of Treatment and Type of Response

Thirty-three patients (37.5%) received a total dose of interferon lower than 300 MU, and 55 patients (62.5%) received a total dose higher than 300 MU. Forty patients (45.5%) were treated for 6 months or less, whereas the remaining 48 patients (54.5%) received IFN for 6–12 months. Table 5 provides a summary, showing that no significant differences in the rates of unfavorable events were found in relation to the total dose of interferon administered. patients who had been treated for more than 6 months showed a significant lower incidence of worsening of stage of cirrhosis ($P < 0.05$), whereas the rates of HCC and of death or OLT were not affected by the duration of treatment.

Thirteen patients who did not normalize ALT during a first cycle of interferon were retreated with a second cycle, and all of them behaved again as nonresponders who did not show normalization of ALT. Twelve of them remained with a stable disease without worsening of cirrhosis Child stage or development of HCC during follow-up. One patient died from variceal bleeding.

A complete and sustained biochemical response to IFN, with long lasting normalization of ALT, was observed in 17 of the 88 patients treated (19.3%). The corresponding rates in HBsAg-positive, HBsAg-positive, and anti-HCV-positive, and anti-HCV-positive patients were 20% (2 of 10 patients), 33% (1 of 3 patients), and 18.7% (14 of 75 patients), respectively. In HBsAg-positive and HBsAg/anti-HCV-positive patients, biochemical response to IFN was accompanied by a persistent suppression of viral replication (disappearance of HBV DNA and of HCV RNA from serum), whereas among anti-HCV-positive cases, 6 of 14 patients who showed a persistent ALT normalization after IFN remained positive for HCV-RNA in serum.





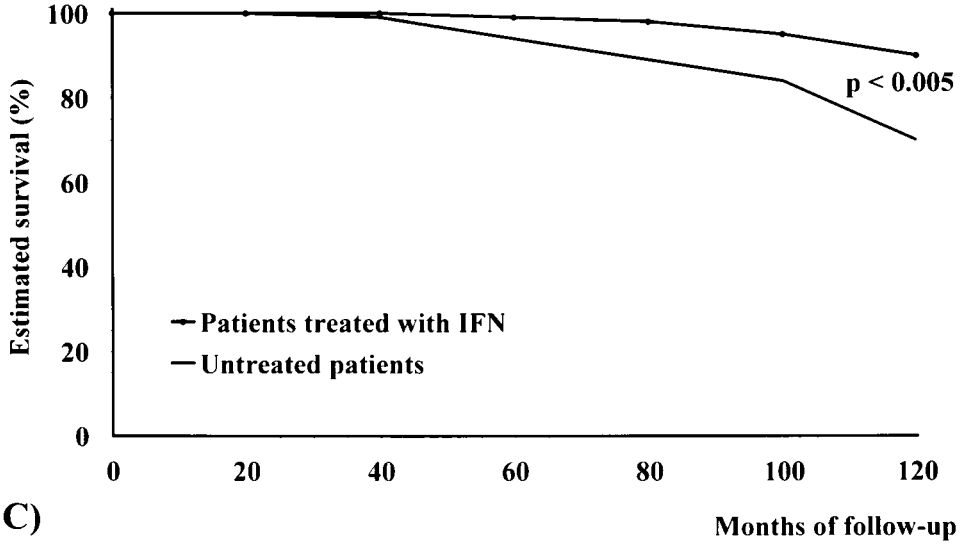


FIGURE 2. Estimated cumulative probability of (A) worsening of Child's stage in relation to interferon therapy (INF) corrected by bilirubin, leukocytes, alkaline phosphatase (ALP), and prothrombin time (PT); (B) development of hepatocellular carcinoma in relation to interferon therapy corrected by albumin, disease duration, PT and γ-glutamil transpeptidase (GGT); (C) death or liver transplantation in relation to interferon therapy corrected by albumin. PT, GGT, and ALP in 189 patients with cirrhosis (by using the Cox's proportional hazard model and the Wald test).

TABLE 4
Effects of IFN on Worsening of Child's Stage, HCC Development, and Death or OLT in HBV$^+$ and HCV$^+$ Cirrhotic Patients

Etiology/events	No. treated with IFN (%)	*P**	No. untreated (%)
HBsAg$^+$ (n = 28)			
Worsening of cirrhosis	1 of 10 (10)	n.s.	4 of 18 (22)
Development of HCC	0 of 10 —	n.s.	4 of 18 (22)
Death of OLT	0 of 10 —	n.s.	3 of 18 (17)
Anti-HCV$^+$ (n = 152)			
Worsening of cirrhosis	5 of 75 (7)	<0.01	16 of 77 (21)
Development of HCC	4 of 75 (5)	<0.001	20 of 77 (26)
Death or OLT	3 of 75 (4)	<0.001	15 of 77 (19)
HBsAg$^+$/anti-HCV$^+$ (n = 9)			
Worsening of cirrhosis	1 of 3 (33)	n.s.	2 of 6 (33)
Development of HCC	1 of 3 (33)	n.s.	3 of 6 (50)
Death or OLT	0 of 3 (0)	n.s.	2 of 6 (33)

HCC: hepatocellular carcinoma; OLT: orthotopic liver transplantation; IFN: interferon therapy; HBV: hepatitis B virus; HCV: hepatitis C virus; HBsAg: hepatitis B surface antigen; n.s.: not significant.

Outcomes in relation to biochemical response to interferon are described in Table 5. Stage progression of cirrhosis and death or liver transplantation were observed only in patients without a sustained response to IFN, whereas HCC development occurred with similar rates in sustained and nonsustained responders.

Outcomes in Anti-HCV-Positive Patients Treated with IFN in Relation to the HCV Genotype

Of 78 anti-HCV-positive patients treated with IFN, 11 patients (14.1%) were HCV RNA negative, 39 patients (50%) were infected by HCV-1b, and 28 patients (35.9%) were infected by HCV-2. Rates of disease progression, HCC development, and death or OLT showed no significant differences in relation to HCV genotype, the corresponding figures being 8%, 5%, and 2.5% in HCV-1b-infected patients and 7%, 7%, and 7% in HCV-2-infected cases.

DISCUSSION

Patients with well-compensated liver cirrhosis due to chronic HBV or HCV infection often have ongoing histologic activity that contributes to the further progression of liver damage. The natural course of the disease is often unpredictable, and although there are patients who remain in a compensated stage without major complication for decades,[31,34] others may progress to liver decompensation or HCC in a rather short period.[35] The use of IFN in viral cirrhosis is controversial with respect to its cost effectiveness.

Recent studies have reported conflicting results on whether this treatment may delay the development of HCC. A significant reduction in the incidence of HCC during 2–7 year follow-up has been reported by Nishiguchi et al.[30] in patients with hepatitis C and compensated cirrhosis who had been treated with interferon compared with untreated patients (4% versus 38%; $P = 0.002$). In that study, the effect on tumor development was independent of the biochemical and virological response achieved with IFN. A statistically significant effect of IFN in reducing progression to HCC in HCV-related cirrhosis was observed also by Mazzella et al.,[29] however, it was demonstrable only in patients who showed a complete response to interferon. In contrast to these reports, a retrospective analysis of two large cohorts of European patients with compensated cirrhosis type B or type C did not detect any significant difference in the 5-year risk of developing HCC between interferon-treated and untreated patients.[31,34] In our retrospective analysis of a large cohort of patients presenting with Child's Stage A viral cirrhosis, treatment with interferon was found to be an independent variable associated with a reduced risk of disease progression and of HCC development, and this effect was statistically significant in patients with cirrhosis due to chronic HCV infection. Although the untreated patients had a more advanced stage of liver disease when they were included in this study, the results of the multivariate analysis suggest that IFN had indeed reduced the progression of liver disease and development of HCC, independent of the other relevant variables. It is noteworthy that the duration of therapy and the response achieved appeared to have influenced the progression of Child's stage, which was reduced significantly in patients who were treated for more than 6 months and in patients who developed a sustained response after therapy, whereas no such effect was seen with regard to the development of HCC, which was reduced significantly in treated patients, independent of the dose and duration of treatment and of the type of response. These observations suggest that IFN could reduce the risk of HCC development by its antiproliferative effect, independent of its antiviral and antiinflammatory properties.

Although the results of our study enforce the hypothesis that IFN may be beneficial for patients with Child's Stage A cirrhosis due to HCV, the retrospective nature of this analysis should be a caveat against overinterpretation of the results. Indeed, prospective randomized trials should reproduce these findings in a large number of patients before a definitive conclu-

TABLE 5
Outcome of Cirrhosis in IFN-Treated Patients According to the Dose of Interferon and the Type of Response to Therapy

Measure	No. cases (%)	Worsening of cirrhosis (%)	Development of HCC (%)	Death or OLT (%)
Total dose of interferon				
≤300 MU	33 (37.5)	4 (12)	3 (9)	1 (3)
P[a]		n.s.	n.s.	n.s.
>300 MU	55 (62.5)	3 (5)	2 (4)	2 (4)
Duration of therapy				
≤6 months	40 (45.4)	6 (15)	3 (7.5)	1 (2.5)
P[a]		<0.05	n.s.	n.s.
>6 months	48 (54.6)	1 (2)	2 (4)	2 (4)
Response to IFN				
LTR	17 (19.3)	0 —	1 (5.8)	0 —
P[a]		n.s.	n.s.	n.s.
No LTR	71 (80.6)	7 (10)	4 (5.6)	3 (4)

HCC: hepatocellular carcinoma; OLT: orthotopic liver transplantation; IFN: interferon therapy; LTR: long term responders; n.s.: not significant.
[a] Fisher's exact test.

sion on the long term effects of IFN in viral cirrhosis can be established.

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