Sustained Virological Response to Interferon-α Is Associated with Improved Outcome in HCV-related Cirrhosis: A Retrospective Study

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on behalf of the Italian Association of the Study of the Liver Disease (AISF).

The effect of achieving a sustained virological response (SVR) following interferon-α (IFNα) treatment on the clinical outcomes of patients with HCV-related cirrhosis is unknown. In an attempt to assess the risk of liver-related complications, HCC and liver-related mortality in patients with cirrhosis according to the response to IFNα treatment, a retrospective database was developed including all consecutive patients with HCV-related, histologically proven cirrhosis treated with IFNα monotherapy between January 1992 and December 1997. SVR was an undetectable serum HCV-RNA by PCR 24 weeks after IFNα discontinuation. HCC was assessed by ultrasound every 6 months. Independent predictors of all outcomes were assessed by Cox regression analysis. Of 920 patients, 124 (13.5%) were classified as achieving a SVR. During a mean follow-up of 96.1 months (range: 6-167) the incidence rates per 100 person-years of liver-related complications, HCC and liver-related death were 0, 0.66, and 0.19 among SVR and 1.88, 2.10, and 1.44 among non-SVR ($P < 0.001$ by log-rank test). Multivariate analyses found that non-SVR was associated with a higher risk of liver-related complications (hazard ratio, HR, not applicable), HCC (HR 2.59; 95% CI 1.13-5.97) and liver-related mortality (HR 6.97; 95% CI 1.71-28.42) as compared to SVR. *Conclusion:* Thus, in patients with HCV-related, histologically proven cirrhosis, achievement of a SVR after IFNα therapy was associated with a reduction of liver-related mortality lowering both the risk of complications and HCC development. Irrespective of SVR achievement, all patients should continue surveillance because the risk of occurrence of HCC was not entirely avoided. (HEPATOLOGY 2007;45:579-587.)

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Interferon (IFN)-based treatments are the only therapeutic options for halting progression of chronic HCV infection toward end-stage liver disease, including HCC, because this drug combines antiviral and antiproliferative properties.[1,2] While the anti-HCV therapeutic efficacy of IFN seems to be restricted only to patients who achieve a sustained virological response (SVR), it is unclear whether IFN treatment may attenuate the evolutionary course of chronic HCV infection, regardless of virus eradication.[3] One prospective randomized trial[4] showed reduced HCC incidence in IFN-treated patients with chronic HCV irrespective of virological response, but these results have not been reproduced. A retrospective analysis of a large database in Japan[5] and other small studies[6-8] designed to assess the rate of SVR following IFN therapy have not provided conclusive evidence that the progression of liver disease underling HCV infection was halted in nonresponder patients.

While progression of HCV is likely to be slowed in all patients with chronic HCV and mild liver disease achieving a SVR, this is not the case in patients with concurrent cirrhosis.[5,9-12] Patients with cirrhosis have less chance of clearing serum HCV-RNA with IFN than patients with milder liver disease, possibly because of their more advanced age, changes in the liver microcirculation which prevent optimal interaction between IFN and infected liver cells, and low compliance.[13] Moreover, at this stage of the disease, patients with cirrhosis who achieve a SVR are not entirely protected against the risk of developing HCC. This could be attributed to either the persistence of liver cells committed to develop into cancer, or the persisting carcinogenic effect of extensive liver fibrosis. Not surprisingly, 2 meta-analyses of anti-HCV studies demonstrated a limited reduction in the risk of HCC in cirrhotic patients who successfully responded to IFN therapy versus untreated or nonresponsive patients.[14,15] However, these meta-analyses also demonstrated clinical heterogeneity of the patient populations investigated and substantial differences among treatment centres in the management of patients.

Therefore, to determine the impact of achievement of a SVR on the clinical outcomes of cirrhosis, we conducted a long-term, retrospective, multicenter analysis of prospectively collected data from a cohort of 920 Italian patients with histologically proven HCV cirrhosis, who were treated with IFN and followed up according to standardized criteria.

Patients and Methods

Study Design

From February to December 2002, all tertiary center members of the Italian Association for the Study of the Liver (AISF), were invited to participate in a questionnaire-based investigation aimed at recording data for consecutive patients with HCV-related cirrhosis treated with IFN monotherapy between January 1992 and December 1997.

IFN therapy was offered to all patients with HCV-related cirrhosis. Chronic HCV infection was diagnosed based on serum positivity for both anti-HCV and HCV-RNA, and the diagnosis of complete cirrhosis was required to be certified by histological criteria (Ishak score of 6 or Knodell score of 4). Liver biopsy must have been carried out within 18 months of the start of IFN treatment. Criteria to submit patients to liver biopsy were: serum anti-HCV positivity and increased transaminases in the absence of gastroesophageal varices; past or present episodes of liver decompensation; and common contraindications to liver biopsy or to IFN. In each participating center, an independent pathologist, reviewed and confirmed the diagnosis.

The exclusion criteria were: age over 70 years; lack of histological diagnosis of cirrhosis, gastroesophageal varices; previous episodes of decompensation or bleeding; Child class B or C, concurrent HCC or extrahepatic tumors; and subjects coinfected with hepatitis B (HBsAg-positive) or HIV (anti-HIV positive).

Patients received IFN monotherapy 3 times weekly with a single dose ranging from 3MU to 6MU for 1 year. Antiviral therapy was stopped in patients not responding after 6 months of therapy, and these patients did not receive maintenance therapy. All centers were required to have a long-term surveillance follow-up program after stopping IFN. In order to ensure that all eligible, consecutive patients were included in the database, all centers had to guarantee the pre-existing registration of the cohort of patients treated during the study period.

An Excel database including all demographic, virological and clinical data was set-up between February 2002 and December 2002. Main outcomes that occurred up to June 2005 were recorded. This study was approved by the Ethical committees of all participating centers.

Assessment of Response to IFN

A SVR was defined as undetectable serum HCV-RNA 24 weeks after discontinuation of IFN. Serum HCV-RNA was measured annually during follow-up and at the time of the last visit. Patients not fulfilling SVR criteria, including all patients who relapsed after the achievement of the end of treatment response, were classified as non-SVR.

Investigations

Evaluation of the patients involved obtaining a medical history, performing a physical examination and con-

ducted laboratory tests, including a complete blood count, biochemical tests, and markers for viral hepatitis. Serum samples were tested for HBsAg and antibody to hepatitis B core antigen (anti-HBc) by commercially available enzyme-immunoassays (Abbott Laboratories, North Chicago, IL). Anti-HCV was assessed by a second-generation enzyme-linked immunoassay (ELISA, Ortho Diagnostic Systems, Raritan, NJ). Serum samples were tested for HCV-RNA using nested PCR (limit of sensitivity: 50 UI/L). All centers were required to follow the standardized guidelines laboratory procedures of Italian Association for the Study of the Liver (see www.webais-f.org). HCV genotype was typed by Line Probe Assay (InnoLipa, Innogenetics, Zwijndrecht, Belgium) on available frozen stored sera. The amount and duration of alcohol intake were recorded at an interview and confirmed by relatives. Alcohol abuse was defined by a daily intake of more than 40 g of ethanol in women or more than 60 g in men for a minimum of l0 years.

Follow-Up

The length of the study was calculated from the starting date of IFN therapy and ended at death or at the last follow-up visit. Liver function tests and a complete physical examination were performed every 6 months. The alpha-fetoprotein (AFP) assay and ultrasound (US) scan were repeated at 6-month intervals.

Endpoints

Whenever possible, the diagnosis of HCC was made by US-guided fine-needle biopsy, of a node detected by US, using a 21-gauge needle (Tru-Cut, TSK Laboratories, Tokyo, Japan) to obtain samples for histological assessment.[16] Diagnostic criteria for HCC were: (1) histological, based on internationally accepted criteria[17] and (2) clinical, in patients with AFP value greater than 400 ng/mL and evidence of focal liver lesion at imaging techniques. After 2002, the HCC diagnosis was based according to guidelines of European Association for the Study of the Liver (EASL).[18]

Liver-related complications (ascites, upper gastrointestinal bleeding, and hepatic encephalopathy) were considered an endpoint in all patients with or without the occurrence of HCC. In the subjects who developed HCC, liver complications were recorded only when they occurred before tumor development. Ascites was diagnosed by clinical examination and/or US detection. Porto-systemic encephalopathy (PSE) was defined by clinical parameters. The source of gastroesophageal bleeding was confirmed by endoscopy whenever possible. Liver transplantation was considered as liver-related death endpoint.

Sample Size Calculation

The number of patients needed to detect a Hazard Ratio (HR) of 2.0 with $\alpha = 0.05$ and $\beta = 0.20$, in a 5-year follow-up study, was 140 patients in the SVR group and 140 patients in the non-SVR group. This was calculated assuming a SVR rate of 15% and an incidence of HCC development of 0.5 per 100 person/years for SVR subjects and 2.4 per 100 person/years for non-SVR subjects.[19]

Analysis of Heterogeneity

To evaluate treatment centre heterogeneity, we compared the rate of relevant outcomes of the study (achievement of SVR and HCC occurrence) according to the number of enrolled patients in each center ($<$50, 50-100, $>$100).

Statistical Analysis

Continuous variables are reported as mean and standard deviation, and categorical variables as absolute and relative frequencies. The unpaired Student t test was used to compare the mean and the Chi-squared test was applied to categorical variables. The receiver operating characteristic (ROC) curve was used to identify the best cut-off point to categorize continuous variables. Multiple logistic regression analysis was used to identify predicting variables of SVR.

The main outcomes of the study were liver-related complications, HCC incidence and liver-related mortality. Cumulative incidence curves of liver-related complications, HCC and liver mortality according to response to IFN treatment were plotted using the Kaplan-Meier method.[20] The differences between groups were assessed using log-rank tests. The time frame for each outcome was defined as the time from starting IFN treatment until the onset of the event. Data was censored when individuals died from non–liver-related causes, received a liver transplant or were lost during follow-up. The variables which proved to be significant at univariate analysis were tested by the multivariate Cox proportional hazards regression model to assess their independent effect on the development of events during the follow-up.[21] The results were expressed as hazard ratio (HR) and their 95% confidence interval (CI). Data handling and analysis were performed with the Statistical Package for Social Sciences (SPSS 13.0; SPSS Inc., Chicago, IL). All tests were 2-sided and $P < 0.05$ was considered to be statistically significant.

Results

Study Cohort

Twenty-three centers in Italy agreed to participate in the study. Overall, 1,214 patients were registered. Among

Table 1. Baseline Characteristics of 883 Patients with HCV-Related Histologically Proven Cirrhosis Stratified According to Response to IFN

Variable	All Patients	SVR (n = 124)	non-SVR (n = 759)	*P* Value
Age (years) (mean ± SD)	54.7 ± 8.6	52.6 ± 9.6	55.0 ± 8.4	0.004
Gender				
Male	557 (63.1%)	91 (73.4%)	468 (61.7%)	<0.001
Female	326 (36.9%)	33 (26.6%)	291 (38.3%)	
Serum bilirubin (mg/dL)	0.9 ± 0.4	0.9 ± 0.3	0.9 ± 0.3	0.4
Serum albumin (g/L)	40.2 ± 4.8	41.0 ± 5.2	40.2 ± 4.8	0.07
Prothrombin time (%)	86.8 ± 11.1	87.3 ± 11.4	86.7 ± 11.2	0.6
ALT (times ULN)	3.6 ± 2.2	3.8 ± 2.6	3.6 ± 2.2	0.2
Platelets ($\times 10^9$/L)	142.3 ± 42.3	150.0 ± 41.7	141.9 ± 42.1	0.03
Alpha-FP (ng/mL)	16.9 ± 43.8	15.0 ± 52.3	17.2 ± 42.3	0.6
HCV genotype*				
1-4	476 (73.5%)	28 (37.3%)	448 (78.2%)	<0.001
2-3	172 (26.5%)	47 (62.7%)	125 (21.8%)	
Anti-HBc*				
Positive	303 (43.7%)	41 (43.6%)	262 (44.0%)	0.9
Negative	390 (56.3%)	53 (56.4%)	337 (56.0%)	
History of alcohol intake*				
Yes	219 (27.8%)	28 (25.7%)	191 (28.2%)	0.6
No	569 (72.2%)	81 (74.3%)	488 (71.8%)	

Abbreviations: SVR, sustained virological response; ULN, upper limit of normal.
*Data missing.

these, 34 patients with Child B class cirrhosis, 222 with no diagnosis of cirrhosis obtained by liver biopsy and/or gastroesophageal varices and 16 aged >70 years of age, were excluded. Four centres failed to demonstrate the preexisting registration of treated subjects during the study period, and consequently 22 patients were excluded. Thus, 920 fulfilled the inclusion criteria and were considered eligible for the study.

The overall response rate was 124/920 (13.5%) after a course of IFN monotherapy. The SVR rate according to genotype was 6% for genotype 1 patients, 29.1% for genotype 2, 13.6% in genotype 3 and 0% in genotype 4. The mean follow-up was 96.1 ± 38.4 months (103.3 ± 33.5 in SVR versus 94.9 ± 39.0 in non SVR; $P = 0.02$).

During the follow-up period, 167 of 796 initially non-SVR patients were re-treated either with IFN alone (n = 66) or with IFN plus ribavirin (n = 101). Of these, 37 patients who achieved a SVR after re-treatment were excluded since they were no longer comparable with subjects who achieved SVR after the first course of IFN. In contrast, 130 patients who did not achieve a SVR after re-treatment were included in the study cohort since their viral status did not change during the entire study period. Therefore, 883 subjects were considered for the final analysis.

The baseline characteristics of patients according to response to IFN therapy are shown in Table 1. All patients were Caucasians, the mean age was 54.7 ± 8.6 (range 21-70) years; there were 557 (63.1%) males. HCV genotype was available in 648 patients cases (73.4%); genotype 1 was detected in 465 patients, genotype 2 in 150, genotype 3 in 22, and genotype 4 in 11. The mean total received IFN dose was 556.5 ± 379.1 (645.0 ± 348.2 in SVR versus 541.8 ± 382.3 in non-SVR; $P = 0.005$). Platelet count was significantly lower in the non-SVR group, but there were no between-group differences in serum bilirubin, albumin, and prothrombin time.

ROC curve analysis showed that 54 years and a value of platelets of 109,000/mmc were the best cut-off points to discriminate among SVR and non-SVR patients. A significant difference was also observed in the proportion of subjects lost to follow-up between the 2 groups (11.9% in SVR and 19.9% in non-SVR patients, respectively, $P = 0.03$).

Multiple logistic regression analysis found that achievement of a SVR was associated with genotype 2 and 3 (RR 6.26; 95% CI 3.74-10.50) and age below 54 years (RR 1.87; 95% CI 1.12-3.12), while baseline platelet count had no impact on response to IFN (RR 1.99; 95% CI 0.91-4.38).

Outcomes of the Study

1. Liver-related complications. The incidence rates of liver-related complications per 100 person-years of follow-up was 0 in SVR patients and 1.88 (95% CI: 1.54-2.27) in non-SVR patients (Table 2). The Kaplan Meyer curves of incidence of liver-related complications according to response to IFN are shown in Fig. 1 ($P < 0.01$ by log-rank test). Ascites was the most common complication (78 cases, 66.7%), followed by bleeding from gastroesophageal varices (26 cases, 22.2%) and hepatic encephalopathy (13 cases, 11.1%). By Cox multiple re-

Table 2. Number and Rate of Events Developed During Follow-up in 883 Patients with HCV-Related Histologically Proven Cirrhosis Stratified According to Response to IFN

Strata	Number of Patients	Person- Years	Number of Event	Rate/100 Person Years (95% CI)	Rate Ratio (95% CI)	*P* Value*
Liver-related complications**						
non-SVR	759	5,703	107	1.88 (1.54–2.27)	n.a.	<0.001
SVR	124	1,061	0	0 (0–0.35)		
HCC						
non-SVR	759	5,805	122	2.10 (1.75–2.51)	3.12 (1.42–6.86)	<0.001
SVR	124	1,055	7	0.66 (0.27–1.87)		
Liver-related mortality**						
non-SVR	728	5,781	83	1.44 (0.14–1.78)	7.59 (1.84–31.29)	<0.001
SVR	120	1,019	2	0.19 (0.02–0.71)		
Non liver-related mortality						
non-SVR	759	6,004	31	0.52 (0.35–0.73)	1.28 (0.44–3.68)	0.2
SVR	124	1,077	4	0.37 (0.1–0.96)		

Abbreviations: SVR, sustained virological response; n.a., not applicable.
*By log-rank test. **Patients who died of non liver-related causes were excluded.

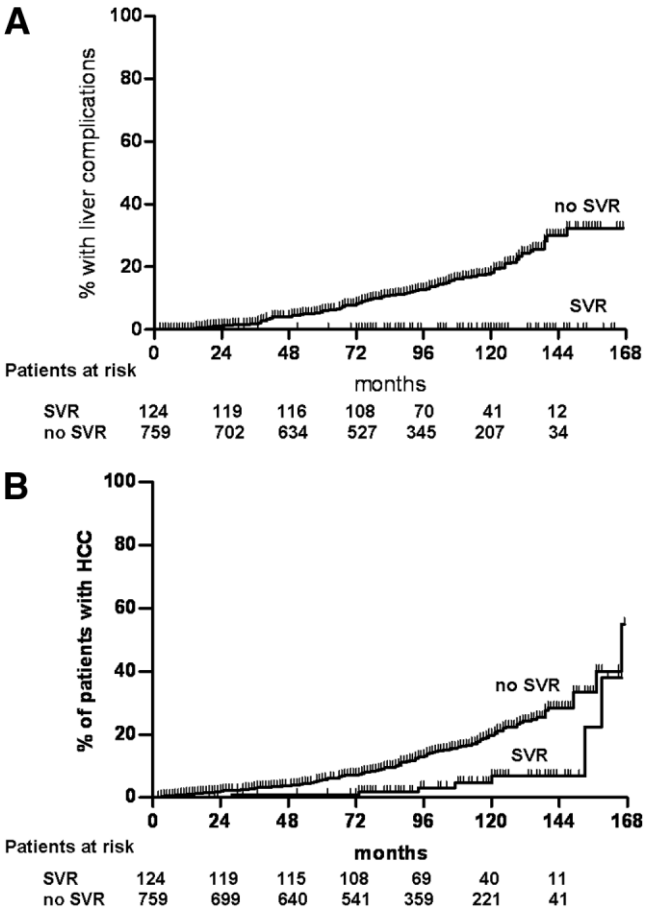

Fig. 1. Cumulative incidence of liver-related complications (A) and HCC (B) in 883 patients with HCV-related histologically proven cirrhosis stratified according to response to IFN ($P = 0.001$ by log-rank test). SVR: sustained virological response.

gression analysis the two variables significantly associated with liver-related complications were the non achievement of SVR (crude and adjusted HR not applicable because the value was zero in one cell of the contingency table) and a low platelet values (Table 3).

2. HCC Incidence. The incidence rates of HCC per 100 person-years of follow-up were 0.66 (95% CI: 0.27-1.37) for SVR and 2.10 (95% CI: 1.75-2.51) for non-SVR patients (Table 2). Thus, patients who did not achieve a SVR had 2.59-fold higher rate of HCC than SVR patients. The Kaplan Meyer curves of HCC development in the 2 groups of patients are shown in Fig. 1 ($P < 0.01$ by log-rank test). In multivariate analysis, age older than 54 years, male gender, low platelet count, and non-SVR status were independent predictors of the likelihood of HCC occurrence (Table 3).

3. Mortality. The incidence rates of liver-related mortality per 100 person-years of follow-up were 0.19 (95% CI: 0.02-0.71) in SVR and 1.44 (95% CI: 0.14-1.78) in non-SVR patients (Table 2). The Kaplan Meyer curves of liver-related mortality according to response to IFN are shown in Fig. 2 ($P < 0.001$ by log-rank test). By Cox-regression model, the HR of liver-related death was 6.97-fold higher in non-SVR patients (Table 3). In contrast, the incidence rates for non-liver related mortality per 100 person/years of follow-up were similar in SVR and non-SVR patients (Table 2).

Analysis of Heterogeneity

The results of analysis of heterogeneity across treatment centres according to the rate of SVR are summarized in Table 4. No significant differences were observed between the centres in the rate of HCC development both in patients with or without SVR (Fig. 3).

Table 3. Univariate and Multivariate Analysis of Factors Associated with Liver-related Complications, HCC, and Liver-related Mortality

Variable	Strata	Crude HR (95% CI)	Adjusted HR (95% CI)	*P* Value
Liver-related complications				
Age (years)	>54 vs ≤54	1.09 (0.76–1.58)		
Sex	male vs female	0.75 (0.52–1.07)		
Platelets ($\times 10^9$/L)	≤109.0 vs >109.0	2.95 (2.03–4.29)	2.59 (1.71–3.95)	<0.001
HCV Genotype	1–4 vs 2–3	0.9 (0.58–1.40)		
SVR	no vs yes	n.a.	n.a.	
HCC				
Age (years)	>54 vs ≤54	2.35 (1.59–3.49)	2.52 (1.62–3.93)	<0.001
Sex	male vs female	1.76 (1.19–2.61)	3.03 (1.87–4.92)	<0.001
Platelets ($\times 10^9$/L)	≤109.0 vs >109.0	1.77 (1.20–2.60)	1.64 (1.06–2.52)	0.025
HCV Genotype	1–4 vs 2–3	1.19 (0.77–1.87)		
SVR	no vs yes	3.41 (1.59–7.31)	2.59 (1–13–5.97)	0.025
Liver-related mortality				
Age (years)	>54 vs ≤54	1.65 (1.05–2.61)	1.53 (0.97–2.42)	0.07
Sex	male vs female	1.14 (0.73–1.78)		
Platelets ($\times 10^9$/L)	≤109.0 vs >109.0	1.81 (1.13–2.89)	1.63 (1.03–2.61)	0.04
HCV Genotype	1–4 vs 2–3	0.83 (0.47–1.46)		
SVR	no vs yes	7.72 (1.9–31.37)	6.97 (1.7–28.42)	0.007

Abbreviations: HR, hazard ratio; n.a., not applicable, see text; SVR, sustained virological response.

Discussion

This study shows, in a large Caucasian population, a significant reduction in the rates of liver-related complications, HCC and liver-related mortality in patients with HCV-related cirrhosis who achieved a SVR following IFN monotherapy. During a mean follow-up period of 8 years, the risk of HCC was 2.6-fold higher and that of liver-related deaths was approximately 7-fold higher, respectively, in those failing to clear HCV after IFN therapy.

This analysis included the largest ever cohort of patients with histologically-proven cirrhosis. Patients were subjected to prospective follow-up. The study was adequately powered to exclude over-fitting biases and the study population is likely to accurately represent Italian patients with HCV-related cirrhosis, since treatment centres throughout the country participated. A limited number of patients were lost to follow-up in both treatment groups, and the sensitivity of the study was increased by the longer follow-up observed in SVR patients, which reduced the underestimation of outcomes in this population at lower risk of events.

The study enrollment period (1992-1997) was selected because, at that time, IFN was the only available antiviral drug to treat HCV infection; ribavirin was later introduced into clinical practice. The influence of HCV genotypes on IFN response was unknown at that time. Thus, there was greater likelihood of including patients who had received homogenous treatment.

We planned a delay of at least 6 years between the last enrollment (end of 1998) and the end of follow-up (June 2005), to guarantee the observation of a large number of outcomes in a population with a low risk of events. The reliability of HCV-RNA tests were ensured by the use of annual viral assessments over the entire study period.

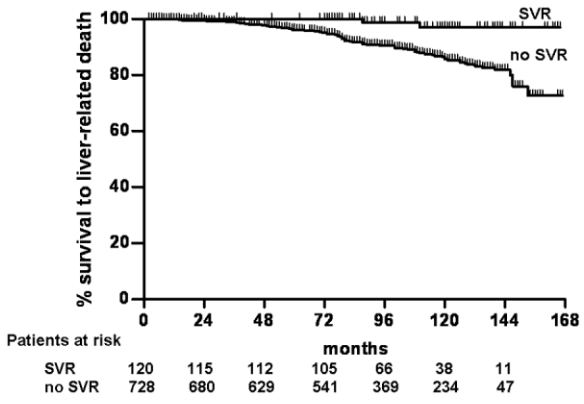

Fig. 2. Cumulative incidence of liver-related mortality in 848 patients with HCV-related histologically-proven cirrhosis stratified according to response to IFN ($P = 0.001$ by log-rank test). Patients died for non–liver-related causes were excluded. SVR, sustained virological response.

Table 4. Rate of Sustained Virological Response (SVR) According to Number of Patients Enrolled at Each Center (Group A: < 50; Group B: 50–100; Group C > 100)

	Group A (n = 307)	Group B (n = 256)	Group C (n = 320)	*P* Value
SVR	42 (13.7%)	34 (13.3%)	48 (15.0%)	0.8
no SVR	265 (86.3%)	222 (86.7%)	272 (85.0%)	

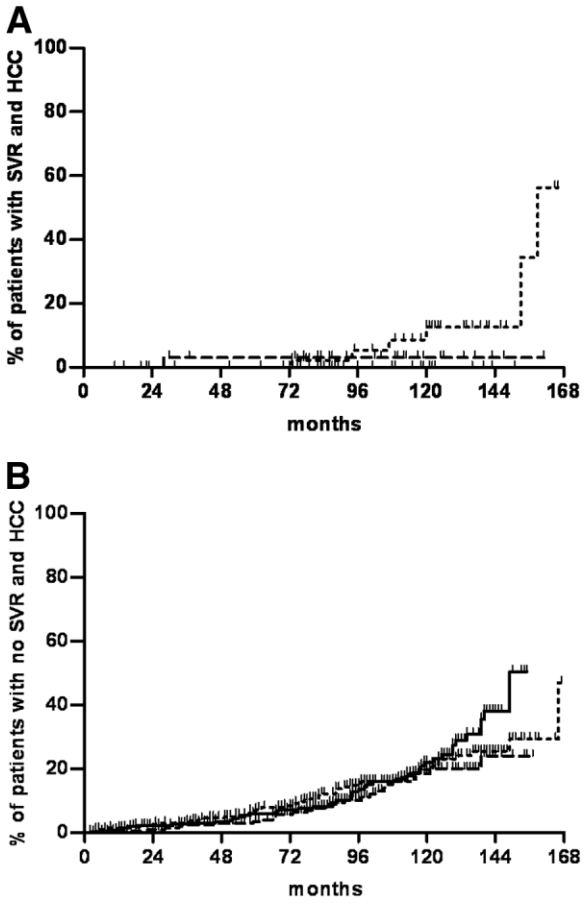

Fig. 3. Cumulative incidence of HCC in (A) sustained virological response (SVR) and (B) no sustained virological response in 883 patients with HCV-related histologically proven cirrhosis stratified according to number of patients enrolled at each centre (straight line: < 50; broken line: 50-100; dotted line > 100). (P = n.s. by log-rank test).

Previous studies aimed at assessing the anti-HCV activity of IFN were not able to demonstrate reduced liver decompensation rates in IFN recipients.[22,23] The only study suggesting a cause-effect relationship between IFN, HCV eradication and the prevention of clinical decompensation did not conduct separate analyses for patients with cirrhosis versus those with chronic hepatitis.[24] Indeed, it is expected that IFN therapy might also lower the risk of HCC in patients with chronic HCV infection, since this drug combines antiviral and antiproliferative properties. Although IFN therapy prevented HCC in selected, it is not clear whether or not the achievement of a SVR might lower the risk of liver cancer in patients with complete cirrhosis.[9] A retrospective analysis and a prospective study of patients followed up for 6.8 years conducted in Japan showed that HCC risk was reduced, but not abolished, in patients with cirrhosis.[5,25] However, the overall reduction in HCC risk in SVR cirrhotic patients was limited as it did not exceed 20% with respect to untreated or non-responder patients.[14,15]

A clear-cut relationship between SVR and drop of HCC risk in Caucasian patients with cirrhosis has not been established. A multicentre retrospective study in Europe showed a slight reduction in HCC risk in patients with HCV-related cirrhosis treated with IFN as compared to untreated ones. The difference, however, disappeared when patients were analysed on the basis of the liver disease severity at entry.[22] Conversely, our database provides evidence that HCC risk was reduced, even though not abolished, among Caucasian patients with cirrhosis who successfully responded to IFN therapy.

The occurrence of HCC in patients with cirrhosis achieving a SVR, however, is not unprecedented.[6,9,26] Cirrhosis is the main independent risk factor for HCC development,[9,27] and residual viremia, i.e., HCV-RNA not detected by the currently available commercial PCR assays, may explain the risk of HCC in these patients.[28] The finding that male subjects aged >54 years have a higher risk of developing HCC suggests that host-related factors and/or the duration of the disease might enhance the risk of HCC. Therefore, our results support the need of screening/surveillance programs despite viral eradication in patients with cirrhosis as recently stated.[29]

Our data suggest that the reduction of liver-related mortality among SVR patients resulted from the combined efficacy of IFN against decompensation or bleeding and HCC, because these complications are the leading causes of death in patients with early cirrhosis due to HCV.[30,31] Data indicate that IFN may prevent the worsening of the disease by suppressing HCV-related liver inflammation which favors hepatocellular failure and the progression of portal hypertension, and by modulating several cell factors involved in neoplastic transformation, such as RNA-dependent PKR and 2′,5′ oligo adenyl synthetase.[32] In murine models, IFN inhibits liver cell proliferation and stimulates apoptosis of preneoplastic hepatocytes.[33,34] Our findings, which show that the rates of non–liver related mortality were similar in SVR as in non-SVR patients, confirm that IFN activity was specifically addressed toward lowering liver-related mortality among SVR patients.

This study may be affected by some methodological weaknesses. First, in cohort studies with no control group, prognostic factors other than treatment might hamper the validity of the obtained results. However, given that we only compared patients who received treatment, both groups (SVR and non-SVR) were likely to have been exposed to similar selecting factors. The enrollment of homogeneous patients with a diagnosis of cirrhosis obtained by liver biopsy and treated with similar schedules of antiviral therapy helped to lower both the hidden differences in terms of the severity of the disease and avoid the con-

founding effect of inclusion of patients with chronic hepatitis without cirrhosis, as observed in previous reports.[5,6]

Also, we performed univariate and multivariate analyses to determine whether the different outcomes among SVR and non-SVR patients could have been influenced by different baseline disease severity. Statistical analyses showed that the platelet count cut-off point of 109,000/mmc emerged as an independent predictor of decompensation as well as HCC and liver-related mortality. This indicates that the degree of portal hypertension, of which the platelets are a reliable surrogate marker, as described,[35] is a powerful indicator of cirrhosis outcome. Despite the fact that the platelet count was significantly lower in the non-SVR group, its value did not influence the impact of SVR as an independent predictor of all outcomes in multivariate analysis. Hence, the risk that the better outcomes observed in the SVR group were attributable to the presence of less severe disease at entry was reduced.

The lack of the heterogeneity between centers as evaluated by main outcomes of the study (rate of SVR, incidence of HCC) indicated that all participating centers had similar competence and satisfactory skills in the management of these patients. Furthermore, when we assessed the association between the response to treatment and the clinical outcomes, all factors identified as being independent predictors of response to IFN (i.e., HCV genotype) were taken into account as potential confounding variables. Of interest, platelet count was not associated with the likelihood of achieving SVR. This suggests that hematological side effects and adherence to therapy may be similar in the 2 groups, thus reducing the probability that premature treatment discontinuation was more frequent in the non-SVR group.

The retrospective nature of this study may have represented a potential source of selection bias. However, the rates of SVR in our study is consistent with those obtained in similar patients using this schedule of treatment,[36] and might be justified by the sole inclusion of a cohort of patients with "early" stage of cirrhosis with moderate of portal hypertension and, of a high proportion of patients infected by "easy-to-treat" genotype 2.[37]

In conclusion, our data show that, in patients with HCV-related histologically proven cirrhosis, the achievement of a SVR following IFN treatment was associated with a reduction in liver-related mortality and with a lower risk of liver-related complications and occurrence of HCC.

References

1. EASL International Consensus Conference on Hepatitis C. J Hepatol 1999;30:956-961.
2. National Institutes of Health Consensus Development Conference Statement: Management of Hepatitis C. HEPATOLOGY 2002;36:S1-S19.
3. Heathcote EJ. Prevention of hepatitis C virus-related hepatocellular carcinoma. Gastroenterology 2004;127:S294-S302.
4. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051-1055.
5. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non cirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med 1999;131:174-181.
6. Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. HEPATOLOGY 1998;27:1394-1402.
7. Gramenzi A, Andreone P, Fiorino S, Cammà C, Giunta M, Magalotti D, et al. Impact of interferon therapy on the natural history of hepatitis C virus related cirrhosis. Gut 2001;48:843-848
8. Mazzella G, Accogli E, Sottili S, Festi D, Orsini M, Salzetta A, et al. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. J Hepatol 1996;24:141-147.
9. Makiyama A, Itoh Y, Kasahara A, Imai Y, Kawata S, Yoshioka K, et al. Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. Cancer 2004;101:1612-1622.
10. Veldt BJ, Saracco G, Boyer N, Camma C, Bellobuono A, Hopf U, et al: Long-term clinical outcome of chronic hepatitis patients C who develop with sustained virological response to Interferon monotherapy. Gut 2004; 53:1504-1508.
11. Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, et al. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C, but not significantly in an advanced stage: a retrospective study in 1148 patients. J Hepatol 1999;30:653-659.
12. Iwasaki Y, Takaguchi K, Ikeda H, Makino Y, Araki Y, Ando M, et al. Risk factors for hepatocellular carcinoma in hepatitis C patients whit sustained virologic response to interferon therapy. Liver Int 2004;24:603-610.
13. Wright TL. Treatment of patients with hepatitis C and cirrhosis. HEPATOLOGY 2002;36(Suppl. 1):S185-S194.
14. Cammà C, Giunta M, Andreone P, Craxì A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol 2001;34:593-602.
15. Papatheodoridis GV, Papadimitropoulos VC, Hadziyannis SG. Effect of interferon therapy on the development of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a meta-analysis. Aliment Pharmacol Ther 2001;15:689-698.
16. Borzio M, Borzio F, Macchi R, Croce AM, Bruno S, Ferrari A, et al. The evaluation of fine-needle procedures for the diagnosis of focal liver lesions in cirrhosis. J Hepatol 1994;20:117-121.
17. Kondo F, Ebara M, Sugiura N, Wada K, Kita K, Hirooka N, et al. Histological features and clinical course of large regenerative nodules: evaluation of their precancerous potentiality. HEPATOLOGY 1990;12:592-598.
18. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al.; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001; 35:421-430.
19. Fattovich G, Giustina G, Degos F, Diodati G, Tremolada F, Nevens F, et al. Effectiveness of interferon alpha on incidence of hepatocellular carcinoma and decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol 1997;27:201-205.
20. Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. J Am Stat Assoc 1958;53:457-481.
21. Cox DR. Regression models and life table. J R Stat Soc 1972;34:187-220.
22. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997;112:463-472.
23. Benvegnù L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, et a. Concurrent hepatitis B and C virus infection and risk of

hepatocellular carcinoma in cirrhosis. A prospective study. Cancer 1994; 74:2442-2448.
24. Coverdale SA, Khan MH, Byth K, Lin R, Weltman M, George J, et al. Effects of interferon treatment response on liver complications of chronic hepatitis C: 9-year follow-up study. Am J Gastroenterol 2004;99:636-644.
25. Shiratori Y, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, et al. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. Ann Intern Med 2005;142:105-114.
26. Bruno S, Borzio M, Battezzati PM. Interferon-alpha in chronic hepatitis C. Ann Intern Med 1998;128:956.
27. Simonetti RG, Camma C, Fiorello F, Cottone M, Rapicetta M, Marino L, et al. Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients whit cirrhosis: a case-control study. Ann Intern Med 1992;116:97-102.
28. Gerotto M, Dal Pero F, Bortoletto G, Ferrari A, Pistis R, Sebastiani G, et al. Hepatitis C minimal residual viremia (MRV) detected by TMA at the end of Peg-IFN plus ribavirin therapy predicts post-treatment relapse. J Hepatol 2006;44:83-87.
29. Bruix J, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. HEPATOLOGY 2005;42:1208-1236.
30. Benvegnù L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. Gut 2004;53:744-749.
31. Sangiovanni A, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, et al. The natural history of compensated cirrhosis due to hepatitis C virus: a 17-year cohort study of 214 patients. HEPATOLOGY 2006;43:1303-1310.
32. Ichikawa T, Nakao K, Nakata K, Yamashita M, Hamasaki K, Shigeno M, et al. Involvement of IL-1 beta and IL-10 in IFN-alpha-mediated antiviral gene induction in human hepatoma cells. Biochem Biophys Res Commun 2002;294:414-422.
33. Nakaji M, Yano Y, Ninomiya T, Seo Y, Hamano K, Yoon S, et al. IFN-alpha prevents the growth of pre-neoplastic lesions and inhibits the development of hepatocellular carcinoma in the rat. Carcinogenesis 2004;25: 389-397.
34. Merle P, Barraud L, Lefrancois L, Chevallier M, Guerret S, Maisonnas M, et al. Long-term high-dose interferon-alpha therapy delays Hepadnavirus-related hepatocarcinogenesis in X/myc transgenic mice. Oncogene 2003; 22:2762-71.
35. Degos F, Christidis C, Ganne-Carrie N, Farmachidi JP, Degott C, Guettier C, et al. Hepatitis C virus related cirrhosis: time to occurrence of hepatocellular carcinoma and death. Gut 2000;47:131-136.
36. Valla DC, Chevallier M, Marcellin P, Payen JL, Trepo C, Fonck M, et al: Treatment of hepatitis C virus-related cirrhosis: a randomized, controlled trial of interferon alfa-2b versus no treatment. HEPATOLOGY 1999;29: 1870-1875.
37. Dienstag JL, McHutchison JG. American Gastroenterological Association medical position statement on the management of hepatitis C. Gastroenterology 2006;130:225-230.