A 6-month versus a 12-month surveillance for hepatocellular carcinoma in 559 hemophiliacs infected with the hepatitis C virus

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Hepatocellular carcinoma (HCC) is an increasingly frequent cause of mortality in hemophiliacs with chronic viral hepatitis. Early diagnosis of the tumor at an initial stage is known to improve the outcome of HCC treatment. Because all HCC cases detected in a previous study based upon annual ultrasound (US) surveillance of hemophiliacs with elevated alanine aminotransferase levels were multinodular, this study was designed to evaluate if a more intense surveillance with US and alphafetoprotein (AFP) serum levels of all the patients infected with the hepatitis C virus (HCV) improved the identification of single nodule tumors. A multicenter cohort of 559 HCV-infected hemophiliacs was divided into 2 arms, one followed up at 6-month intervals and one at 12-month intervals depending on the choice and available facilities of each treatment center. During a 6-year surveillance period, HCC was diagnosed in 5 (2.4%) of 210 patients in the 6-month group and in 3 (0.9%) of 349 patients in the 12-month group. The overall incidence rate of HCC was 239 per 100 000 per year (397 per 100 000 per year in the 6-month group and 143 per 100 000 per year in the 12-month group; differences not statistically significant). By multivariate analysis, HCC risk was increased 12.9-fold with alcohol intake more than 80 g/d and 15.2-fold with AFP levels higher than 11 ng/mL. Liver-related death occurred in 8 cases (1.4%), including 3 with HCC. Still alive and tumor free after 24 to 34 months from diagnosis are 3 patients with multinodular tumors treated with repeat chemoembolization followed by orthotopic liver transplantation. In conclusion, 6-month surveillance with US did not increase the chances of detection of single nodule tumors, but it is reasonable to assume that successful treatment of multinodular tumors based upon debulking with chemoembolization and liver transplantation was facilitated by this approach. (Blood. 2003;102:78-82)

Introduction

Hepatocellular carcinoma (HCC) is an increasingly frequent cause of mortality in patients with inherited bleeding disorders who developed chronic viral hepatitis after replacement therapy with coagulation factor concentrates infected with the hepatitis C virus (HCV) or hepatitis B virus (HBV).[1-3] Because the outcome of HCC treatment largely depends on the evolutionary stage of the tumor, the identification of early tumors through surveillance of infected patients is the best practical approach for improving the management of HCC.[4] In 1992, 11 Italian hemophilia centers started a 4-year surveillance program in 385 patients with persistently elevated alanine aminotransferase (ALT) levels by means of abdominal ultrasound (US) examination and serum alphafetoprotein (AFP) determination carried out at 12-month intervals. Because their tumor was multinodular and/or invasive, 6 patients with HCC identified during surveillance were not eligible for such curative treatments as liver resection or liver transplantation.[2] Orthotopic liver transplantation is the only therapeutic option for patients with multiple HCC nodules, but on an empiric basis it is currently recommended only to patients with a maximum of 3 nodules smaller than 3 cm in volume and no vascular invasion or extrahepatic localizations.[5-8] To test the hypothesis that a more intense surveillance of patients at risk of HCC might help to identify more patients with curable tumors, a larger cohort of 559 unselected patients with inherited coagulation disorders and HCV infection, with normal or elevated ALT levels, was recruited by 11 Italian hemophilia centers in 1996 and followed up at 6- or 12-month intervals for a period of 6 years.

Patients and methods

Enrollment and follow-up

On the basis of the results of the previous study of hemophiliacs with elevated ALT levels analyzed by hepatic US examination and AFP levels, which was carried out by 11 Italian centers at yearly intervals,[2] a 6-month interval surveillance was advised for all hemophiliacs with HCV infection. The protocol was approved by the institutional review board of the coordinating institution (Milan Maggiore Hospital). From

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A complete list of the members of the Study Group of the Association of Italian Hemophilia Centers appears in the "Appendix."

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January 1996 to December 2001, 6 Italian centers decided to adopt the more intense surveillance regimen recommended (including the measurement of AFP serum levels and hepatic US examination), whereas the remaining 5 centers decided, for reasons of cost and available facilities, to continue to follow-up with their patients with annual visits. A larger cohort of patients was eligible for this study as the presence of serum HCV-RNA as a marker of chronic infection with HCV was the only inclusion criterion. The duration of HCV infection was calculated assuming that the first infusion with blood, plasma, or non–virus-inactivated concentrates had transmitted HCV.[9,10] At enrollment, all patients underwent a full clinical examination and routine liver chemistries. Patients were also tested for serum markers of HBV and HCV (hepatitis B surface antigen and anti-HCV, second-generation enzyme-linked immunosorbent assay [ELISA]; Ortho Diagnostics, Raritan, NJ), anti-HIV (ELISA; Ortho Diagnostics), HBV-DNA (HBV bDNA; Bayer, Tarrytown, NY), HCV genotype (Inno-LIPa; Innogenetics, Ghent, Belgium), and AFP (ELISA; Abbott Laboratories, Chicago, IL). Abdominal US was carried out using conventional real-time equipment.

Diagnosis of cirrhosis and HCC

Cirrhosis was diagnosed on the basis of clinical signs of portal hypertension (platelets $< 100 \times 10^9$/L, albumin < 3.5 g/L, and serum cholinesterase activity < 4.5 U/L), endoscopic signs (oesophageal varices and portal hypertensive gastropathy), and US findings (irregular margins of the liver, dilated portal vein axis, and splenomegaly). The clinical stage of the disease was classified according to the Child-Pugh score.[11]

HCC was diagnosed on the basis of the abdominal US identification of a focal lesion in a patient with serum AFP level exceeding 400 ng/mL. In patients with a focal lesion at abdominal US and serum AFP level lower than 400 ng/mL, HCC was diagnosed by demonstrating arterial hypervascularization of the nodule by biphasic or triphasic spiral computerized tomography scan or magnetic resonance.[4] A liver biopsy with an echo-guided thin needle (21-gauge Tru-cut needle) was carried out only for diagnostic purposes in patients with a nodule not fulfilling the above-described diagnostic criteria.

To assess the number of tumor nodules, size, and extrahepatic spread, patients with HCC were further investigated with chest x-ray, spiral abdominal computerized tomography, and bone scintiscan. Disease was classified as mononodular or multinodular on the basis of spiral computerized tomography scan. To calculate tumor size, the 2 main diameters of the lesion(s) were measured.

Treatment options for HCC

The following therapeutic algorithm was developed: orthotopic liver transplantation was the first-line treatment for HIV-seronegative patients who had cirrhosis, were younger than 60 years, and had a single tumor smaller than 5 cm in diameter or no more than 3 tumors smaller than 3 cm (Milan criteria).[4] Transplantation was also considered for patients with more than 3 nodules showing no vascular invasion at diagnosis and during the waiting period. Transcatheter arterial chemoembolization (TACE) was used as a debulking therapy in patients listed for liver transplantation. Patients who had a single tumor nodule but no cirrhosis or who did not fit the general criteria for transplantation were considered for hepatic resection or percutaneous interstitial ablation. Patients with large or inoperable tumors were considered for TACE. Patients ineligible for any of the aforementioned treatments were given palliative treatments.

Statistical analysis

Continuous variables were expressed as medians and ranges and were compared using the Mann-Whitney *U* test. The chi-squared test or the Fisher exact test were used to compare proportions. Unconditional logistic regression was used to calculate the crude odds ratios (ORs) of HCC according to the following: age older than 62 years (95th percentile of the control distribution), age at the time of HCV infection older than 40 years (95th percentile of the control distribution), AFP levels higher than 11 ng/mL at entry (95th percentile of the control distribution), ALT levels higher than 176 IU/L at entry (90th percentile of the control distribution), and alcohol intake more than 8 g/d. These variables were also included in a multivariate model to assess the effect of each one adjusted for the other variables.

Results

Patient characteristics

Of the 2926 patients with inherited bleeding disorders attending the 11 participating centers, 643 (22%) met the inclusion criteria and 559 (19%) entered and completed the 6-year surveillance program. These included 301 patients from the previous study.[2] The 84 patients not compliant to the study included 66 who did not accept to undergo surveillance, 7 lost to follow-up, and 11 who missed 2 consecutive screening time points. The epidemiologic and clinical characteristics of these patients were comparable with those of the patients enrolled in the study. The main clinical and laboratory features of the 559 patients at entry are shown in Table 1. As expected, most patients were males with hemophilia A or B and circulated genotype 1a or 1b of HCV. Approximately one fourth had HIV coinfection. There were 66 patients (12%) with cirrhosis: 48 (73%) had Child-Pugh A, and 18 (27%) had Child-Pugh B. Cirrhosis was more prevalent in patients coinfected with HIV than in anti-HIV seronegatives (25/127, 20% vs 41/432, 9%; $P = .002$.). There were 509 patients (91%) with AFP levels within the normal limits (7-11 ng/mL across all 11 centers); 444 patients (79%) had either

Table 1. Epidemiologic and clinical features of 559 patients with inherited coagulopathies at study entry

	No.	Percent
Patients (males)	559 (547)	98
Age, y*	33 (8-82)	
Type of coagulopathy		
Hemophilia A		
Severe	301	54
Moderate/mild	157	28
Hemophilia B		
Severe	37	7
Moderate/mild	22	4
Others	42	7
Duration of infection, y*	20 (8-59)	NA
Age at infection, y*	9 (0-62)	NA
AFP level, ng/mL*	2.3 (0.1-82.4)	NA
ALT level, IU/L*	66 (5-645)	NA
HCV type		
1a	207	37
1b	167	30
2	43	8
3	50	9
4	10	2
Mixed	14	2
ND	68	12
Cirrhosis	66	12
HBV infected	27	5
HIV infected	127	23

NA indicates not applicable; ND, not done.
*Median (range).

Table 2. Main features and outcome of 8 patients with HCC

	Case 1*	Case 2	Case 3†	Case 4	Case 5	Case 6	Case 7	Case 8
Hemophilia type, severity‡	A, s	A, m	B, m	A, m	A, m	A, m	A, m	B, s
Age,§ y	52	69	55	68	57	51	53	57
HCV type	1b	1b	1a	1a	1b	NA	1b	1a
Child-Pugh score	A	A	B	B	A	B	B	A
Surveillance intervals, mo	12	6	12	12	6	6	6	6
AFP level at diagnosis, ng/mL	11	5	86	42	162	5	52	22
Number of nodules	1	3	> 10	5	6	7	5	3
Tumor size, mm	23	20-30	5-70	18-30	6-40	10-40	8-40	10-20
Vascular invasion	No	Yes	Yes	Yes	No	Yes	No	No
Treatment	TACE, OLT	TACE	TACE	TACE	TACE, OLT‖	None	TACE, OLT	TACE¶
Survival, mo	24	5	7	6	24	2	34	15
Outcome	Alive	Alive	Dead	Dead	Alive	Dead	Alive	Alive

NA indicates not available; OLT, orthotopic liver transplantation.
*HBV coinfected.
†HIV coinfected.
‡s indicates severe; m, mild.
§Age at the time of HCC diagnosis
‖From living related donor.
¶Listed for OLT.

persistently or intermittently elevated serum ALT levels. There were no statistically significant differences in ALT and AFP levels between anti–HIV-positive and anti–HIV-negative patients. Either before or during the study, 121 anti–HIV-negative patients (22%) were treated with interferon alpha alone or in combination with ribavirin; 33 (6%) achieved a sustained virologic response, defined as the absence of viremia for at least 6 months after treatment interruption.

Liver-related deaths and HCC

During the 6-year follow-up, 21 patients (4%) died: 6 (5%) were HIV seropositive, and 15 (3%) were HIV seronegative. Among the former, 2 died of HIV-related events, 2 of liver failure (1 with HCC), and 2 of causes unrelated to liver or HIV. Among the 15 anti–HIV negative patients, 6 died of liver failure (2 with HCC) and 9 of causes unrelated to the liver. Overall, 8 hemophiliacs (1.4%) died of liver-related disease.

HCC developed in 8 patients (1.4%) with an incidence rate of 239 cases per 100 000 per year. Table 2 summarizes the clinical features and outcome of HCC patients, all with clinically documented cirrhosis. Multinodular HCC was detected in 7 patients (87%), 5 among those under 6-month surveillance and 2 among those under 12-month surveillance (5/210, 2.4% vs 2/349, 0.6%; not significant). The only patient with a single nodule at diagnosis (case 1) belonged to the 12-month group. At diagnosis, 5 HCC patients (63%) had serum AFP levels higher than the upper normal limit but less than 400 ng/mL. Overall, 7 patients underwent TACE and 1 was left untreated. The patient with the single tumor nodule and 2 patients with multinodular tumor (cases 5 and 7) were given 1 to 4 courses of TACE followed by liver transplantation. HCV recurred in all patients who received transplants who, however, are HCC free and alive for 14, 22, and 24 months after liver transplantation.

The 8 patients who developed HCC were older (from 51 to 69 years); they also were older at the time of HCV infection and more often had cirrhosis, alcohol abuse, and higher AFP and ALT levels than the 551 patients who remained HCC-free during surveillance (Table 3).

Risk factors for HCC development

All HCC patients had cirrhosis, compared with 24 of the 104 age-matched patients who remained HCC free during surveillance

Table 3. Comparison between 8 patients who developed HCC and 551 who remained tumor-free during surveillance

Features	Patients who developed HCC, n = 8	Patients who did not develop HCC, n = 551	*P*
Hemophilia A (%)	6 (75)	452 (82)	2
Hemophilia B (%)	2 (25)	57 (10)	NS
Other coagulopathies (%)	0	42 (8)	NA
Age, y (range)	56 (51-69)*	39 (14-88)†	< .001
Age at infection, y (range)	33 (18-55)	9 (0-62)	< .001
Duration of infection, y (range)	21 (13-36)	26 (14-65)	NS
Cirrhosis (%)	8 (100)	58 (11)	< .001
Alcohol intake, more than 80 g/d (%)	3 (37)	35 (6)	< .05
AFP level at entry, ng/mL (range)	6 (3-26)	2 (0.1-82)	< .005
ALT level at entry, IU/L (range)	127 (53-206)	65 (5-645)	< .05
HBV infected (%)	1 (12)	26 (5)	NS
HIV infected (%)	1 (12)	126 (23)	NS
Interferon treatment (%)	0	121 (22)	NS

NS indicates not significant; NA, not applicable.
*Age at the time of HCC diagnosis.
†Age at the end of the 6-year surveillance.

Table 4. Univariate (crude OR) and multivariate analysis (adjusted OR) of the features associated with the risk of developing hepatocellular carcinoma

Variables	Crude OR (95% CI)	Adjusted OR (95% CI)*
Age, older than 62 y	7.3 (1.4-38.2)	1.8 (0.1-36.9)
Age at HCV infection, older than 40 y	6.9 (1.3-35.9)	3.4 (0.2-69.9)
AFP level at entry, higher than 11 ng/mL	11.6 (2.6-51.5)	15.2 (2.7-85.7)
ALT level at entry, higher than 176 IU/L	1.3 (0.1-10.7)	1.2 (0.1-11.8)
Alcohol intake, more than 80 g/d	8.8 (2.0-38.5)	12.9 (2.4-68.7)

*For each variable, ORs are adjusted for the remaining 4 variables considered.

($P < .0001$). By univariate analysis, AFP levels higher than 11 ng/mL at entry, alcohol intake more than 80 g/d, age older than 62 years, and age at HCV infection older than 40 years were significantly associated with an increased risk of HCC (Table 4). By multivariate analysis, the risk of HCC was 15.2-fold (confidence interval [CI], 2.7-85.7) in patients with AFP levels higher than 11 ng/mL at entry and 12.9-fold (CI, 2.4-68.7) in patients with alcohol intake more than 80 g/d (Table 4).

Discussion

In a previous study we demonstrated that annual surveillance by hepatic US examination of hemophilic patients with chronic viral hepatitis and persistently elevated ALT values led to the identification of tumors that were already multinodular in virtually all patients.[2] This study, carried out in a larger cohort of HCV-infected hemophiliacs, including patients with normal or intermittently elevated ALT levels not included in the previous study, demonstrated that stricter surveillance for 6 years at 6-month intervals did not increase the rate of detection of small tumors, with multinodular tumors detected in 5 (2.4%) of 210 patients in the 6-month group and in 2 (0.6%) of 349 in the 12-month group. None of these tumors were discovered independently of serial screening with AFP levels and hepatic US examination.

A possible explanation for the high rates of multinodular HCC in hemophilic patients is that the tumor may originate as multiple distinct HCC clones generated by different HCV infections acquired through multiple infusions with virus-infected concentrates.[12,13] HCV per se might account for the high rates of multinodular cancer observed in hemophilic patients, as HCV is more involved in multicentric liver carcinogenesis than HBV,[14-18] and the presence of persistent inflammation and increased liver cell proliferation turnover is unequivocally an important factor in the occurrence of multicentric liver carcinogenesis.[19,20] Another possible explanation for the frequent occurrence of multinodular tumors in hemophiliacs could be the frequent coexistence of HCV and HBV infection.[21-24] In a previous study, we demonstrated high rates of occult HBV infection in multitransfused hemophilic patients with chronic non-A, non-B hepatitis,[24] and a recent study in HIV-infected hemophiliacs showed that the risk of HCV-related end-stage liver disease was markedly increased by HBV coinfection.[25]

Orthotopic liver transplantation is the only realistic therapeutic option for multinodular HCC developing in cirrhotic livers.[4] Because tumor recurrence is the prominent cause of death in HCC patients treated with transplantation, patients with large tumors have no indication for transplantation because tumors are less differentiated, more invasive, and recur more often after operation than small tumors.[26] Thus, current recommendations are to restrict transplantation to patients with a single 5-cm or smaller nodule or with a maximum of 3 nodules smaller than 3 cm in diameter and no signs of vascular invasion or extrahepatic metastasis, with 5-year survivals of approximately 75%.[5-8] In the study by Mazzaferro et al,[5] patients with advanced tumors exceeding the Milan criteria showed 59% actuarial survival at 4 years. Comparable rates of survivals were reported in 24 patients with solitary tumor 6.5 cm or smaller or 3 nodules with the largest lesion 4.5 cm or smaller and total tumor diameter 8 cm or smaller.[27] Criteria of patient selection for transplantation can be safely and effectively expanded also to patients with 3 nodules 5 cm or smaller or 5 nodules 3 cm or smaller with no radiologic sign of vascular invasion or disease progression following treatment with chemoembolization.[28] These data are corroborated by the observation that our patients with multinodular HCC who showed no signs of disease progression or vascular invasion during debulking treatment with chemoembolization are still alive and HCC free 14 to 24 months after transplantation. In the study by Yao et al,[27] in patients with tumors exceeding the Milan criteria, recurrence was detected in 8 patients (11%) 2 to 22 months after transplantation.[27]

In conclusion, the results of this study would encourage 6-month surveillance with US and AFP in hemophilic patients with chronic HCV infection because this approach allows identification of patients with multinodular disease still eligible for radical therapies with transplantation. It must be considered, however, that our study was not randomized and that the choice of surveillance intervals depended on convenience and facilities available to each center. Hence, factors other than surveillance frequency may have contributed to the lack of an HCC diagnosis amenable to radical treatment. Consistently with studies in nonhemophilic patients[29-31] this study found that hemophilic patients with chronic hepatitis C and a history of alcohol abuse, high levels of serum AFP, or cirrhosis were at especially high risk of developing HCC. These predictors of tumor development might be helpful in identifying hemophilic patients who are at greater risk of developing cancer and therefore require strict surveillance with US to detect cancer at a stage when a transplantation could potentially be performed (ie, before vascular invasion by HCC occurs).

Appendix

The following colleagues participated in this study by the Study Group of the Association of the Italian Hemophilia Centers: R. Musso, Division of Hematology and Hemophilia Center, Ferrarotto Hospital, Catania; M. Morfini, Department of Hematology and Hemophilia Center, Careggi Hospital, Florence; G. Gamba, Hemophilia Center, Policlinic San Matteo Hospital, Pavia; G. Muleo, Department of Hematology and Hemophilia Center, Pugliese Hospital, Catanzaro; A. R. Tagliaferri, Fifth Division of Medicine and Hemophilia Center, Policlinic Hospital, Parma; F. Baudo, Department of Hematology and Hemophilia Center, Niguarda Hospital, Milan; E. Boeri, Division of Pediatrics, Gaslini Hospital, Genoa; G. Rossetti, Hemophilia Center, S. Chiara Hospital, Trento; A. Ghirarduzzi, First Division of Internal Medicine and Hemophilia Center, Santa Maria Nuova Hospital, Reggio Emilia, Italy.

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