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Patterns of hepatocellular carcinoma development in hepatitis B virus and hepatitis C virus related cirrhosis

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Abstract

To compare incidence, risk factors and morphologic pattern of hepatocellular carcinoma (HCC) development in hepatitis B virus (HBV) and hepatitis C virus (HCV) related cirrhosis, 401 patients were followed prospectively by periodic ultrasound examination for 14–189 months (mean: 84.8 ± 36.7). During follow-up, 77 (19.2%) patients developed HCC, with 5 and 10 year cumulative incidence of 10 and 27.5%, respectively. The risk of HCC was significantly higher in HBV and HCV co-infected patients ($P = 0.014$) compared to those with single HBsAg or anti-HCV (antibodies to hepatitis C virus) positivity. In anti-HCV positive cases the annual risk of HCC increased from 2% in the first 5 year period to 4% in the third 5 year period, while it decreased from 2 to 0% in the same time periods in the HBsAg positive group. By Cox's regression, age above 59 years ($P = 0.001$), male sex ($P = 0.09$), longer duration ($P = 0.04$) and more advanced stage ($P = 0.01$) of cirrhosis, lower platelets count ($P = 0.001$) and higher ALT levels were significant risk factors for HCC in anti-HCV positive patients, while only high α-fetoprotein (AFP) levels during follow-up ($P = 0.04$) was a significant risk factor for HCC in HBsAg positive cases. The pattern of HCC was nodular in 63 (81.8%) patients and infiltrating in 14 (18.2%), and the former type was associated with older age ($P = 0.0001$), longer duration ($P = 0.002$) and more advanced stage ($P = 0.0001$) of cirrhosis but not with the viral etiology of disease. In contrast, development of infiltrating HCC was unrelated to age and disease duration and stage, and was associated with male sex ($P = 0.01$), HBV infection ($P = 0.06$) and HBV and HCV co-infection ($P = 0.0001$). Our results indicate different incidence profile, risk factors and patterns of morphogenesis of HCC development in HBV and HCV associated cirrhosis, suggesting different mechanisms of carcinogenesis.

Keywords: Hepatocellular carcinoma (HCC); Cirrhosis; Hepatitis B virus (HBV); Hepatitis C virus (HCV)

1. Introduction

Mortality rate for hepatocellular carcinoma (HCC) has significantly increased during the past two decades worldwide (Taylor-Robinson et al., 1997; Stroffolini et al., 1998; El-Serag and Mason, 1999). Prospective surveillance studies conducted in different countries indicate that HCC represents one of the major complications and cause of death in patients with cirrhosis, with an annual incidence varying from 1 to 6% (Zaman et al.,

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1985 Colombo et al., 1991; Ikeda et al., 1993; Kato et al., 1994; Benvegnù et al., 1994; Cottone et al., 1994; Oka et al., 1994; Pateron et al., 1994; Borzio et al., 1995). In recent years we have observed a change in the presenting features of HCC in cirrhotic patients, with an increasing prevalence of females, most likely reflecting the effect of hepatitis C over hepatitis B, a higher number of cases diagnosed in the phase of well compensated cirrhosis and a prevalence reduction of HBsAg positive cases with a parallel increase in HCV related cases. These changes are most likely the consequence of a cohort effect of infected with HCV in the 1960s–1970s, as well as of improved sensitivity of diagnostic techniques leading to earlier detection of small, subclinical HCC lesions during ecographic screening of cirrhotic patients. Chronic hepatitis type B and type C are considered the major causes of cirrhosis and of HCC (Beasley et al., 1981; Tsukuma et al., 1993; Ikeda et al., 1993; Benvegnù et al., 1994; Fattovich et al., 1995; Benvegnù and Alberti, 1996; Ikeda et al., 1998), although it is still not determined whether any difference exists in pathogenetic mechanisms and patterns of presentation between hepatitis B virus (HBV) and HCV-induced HCC (Bréchot et al., 1980; Shafritz et al., 1981; Bréchot, 1996; Trevisani et al., 1996; Cacciola et al., 1999). Several longitudinal studies have identified two main patterns of tumor development (Shinagawa et al., 1984; Sheu et al., 1985; Ebara et al., 1986; Okazaki et al., 1989; Colombo et al., 1991; Oka et al., 1994; Cottone et al., 1994; Borzio et al., 1995). Most patients present with single or, more rarely, multiple expanding, encapsulated nodules, while a minority show a more aggressive, infiltrating form of HCC.

In a prospective surveillance program conducted in 401 patients with liver cirrhosis, we have compared HCC development in HBV and HCV related disease by assessing: (1) overall incidence and annual rate over long-term follow-up; (2) associated risk factors; and (3) morphologic patterns of tumor appearance.

2. Patients and methods

2.1. Patients

Since 1986 we have been conducting a prospective surveillance program in patients with cirrhosis with periodical (every 6 months) clinical assessment and US examination of the liver, in order to allow early detection of HCC and to monitor the natural course of the liver disease, onset of complications and long-term outcomes. The criteria for inclusion in the cohort were: (1) presence of cirrhosis, diagnosed by histologic or clinical findings (presence of irregular margins at US, portal hypertension with laboratory evidence of chronic liver disease), (2) presence of stage A or B disease, according to Child–Pugh classification (Pugh et al., 1973); and (3) absence of clinical and ultrasonographic evidence of liver cancer at entry with α-fetoprotein (AFP) levels < 200 ng/ml. Using these criteria a total of 401 consecutive patients with cirrhosis, seen in our Department between 1986 and 1997, were included in this study. There were 247 (61.6%) males and 154 (38.4%) females and mean age at inclusion was 58.0 ± 9.3 years (range 25–81 years). Three hundred and thirty nine (84.5%) patients had a Child–Pugh stage A and 62 (15.5%) a stage B cirrhosis. Cirrhosis was diagnosed by liver biopsy in 358 (89.3%) patients and based on clinical criteria in 43 (10.7%) cases. In 207 (51.6%) patients cirrhosis was found at inclusion, while in the remaining cases known disease duration was 4.4 ± 3.6 years (range 1–18 years). Two hundred and eighty-four (70.8%) patients were anti-HCV (antibodies to hepatitis C virus) positive, 50 (12.5%) were HBsAg positive and 19 (4.7%) were both HBsAg and anti-HCV positive. Eighty-five (21.2%) patients had a history of alcohol abuse. Of the remaining cases 8 (2.2%) had primary biliary cirrhosis, 5 (1.3%) autoimmune and 7 (1.9%) cryptogenic disease.

2.2. Methods

All patients were followed prospectively with periodical (every 6 months) ultrasound examina-

tion of the liver, clinical and laboratory evaluation, including serum alanine–aminotransferase (ALT) and serum AFP levels. Serum HBV and HCV markers (hepatitis B surface antigen (HBsAg) and anti-HCV) were also tested at inclusion and during follow-up in all cases, partially by retrospective analysis of stored samples.

Abdominal ultrasound examination was performed with a high resolution real-time instrument (AUC 940, Ansaldo, Hitachi Medica Corporation, Tokyo, Japan) with a 3.5 MHz convex transducer. In all patients, US evaluation was always carried out by the same operator (L.B.), with standardized criteria. Fine needle biopsy, when necessary, was done under sonographic guidance using 22-gauge thin needle (Ecojekt, modified Chiba needle, Hospital Service, Hakko Shoji, Japan).

HCC was diagnosed by US assisted fine needle biopsy of focal lesion of the liver as they became detectable during follow-up. Microbiopsy specimens were fixed in 10% formalin and stained with ematoxylin and eosin. Presence of HCC was established according to internationally accepted criteria (Peters, 1976). Upper abdominal computed tomography (CT) was performed in all patients with focal lesions of the liver detectable and/or with increased levels of AFP (above 200 ng/ml) or peripheral portal thrombosis during follow-up.

2.3. Serologic testing

Anti-HCV was determined by second generation enzyme-linked immunosorbent assay (Ortho Diagnostic System, Raritan, NJ) and by second generation recombinant immunoblotting assay (Chiron Corporation, Emeryville, CA). Hepatitis B surface antigen was detected by commercially available kits (Abbott Diagnostics, North Chicago, IL).

2.4. Statistical analysis

The Kaplan–Meier's product limit survival analysis was performed to evaluate the cumulative incidence of HCC during follow-up, and the Mantel–Cox log-rank test was used to estimate the cumulative probability of HCC in relation to etiology of cirrhosis and to compare the cumulative probability of developing nodular or infiltrating HCC in the different etiologic strata. Multivariate analysis by the Cox's proportional hazards regression model was performed separately in HBsAg positive and in anti-HCV positive patients to estimate (with 95% confidence intervals) the independent role of age, sex, duration and stage of cirrhosis, alcohol abuse, base-line platelet count, and ALT and AFP behavior during follow-up. Univariate analysis by the Student's t-test and the Pearson's χ^2-test (or the Fisher exact test when appropriate), with 95% confidence intervals, were used to compare age and duration and stage of cirrhosis at tumor diagnosis, as well as ALT and AFP behavior during follow-up and etiological factors of liver disease in relation to the pattern of HCC. The Cox's regression analysis was also used to evaluate the risk of developing nodular or infiltrative/diffuse HCC in relation to age, sex, duration and stage of cirrhosis, and ALT and AFP behavior during follow-up, and P-values were calculated by Wald's test. A P-value of less than 0.05 for univariate analysis and less than 0.1 for multivariate analysis was considered statistically significant. Data analysis was performed with the BMDP statistical package (Brown et al., 1990).

3. Results

3.1. Incidence of HCC in HBV and in HCV associated cirrhosis

During a mean follow-up period of 84.8 ± 36.7 (14–189) months, 77 (19.2%) patients developed HCC, with an overall cumulative incidence of 10, 27.5 and 45% at 5, 10 and 15 years, respectively (Fig. 1). By the Kaplan–Meier method and the log-rank test, the cumulative probability of HCC development was significantly higher in HBV and HCV co-infected patients ($P = 0.014$) compared to those with single HBsAg or anti-HCV positivity (Fig. 2). By the 10th year of follow-up, co-infected patients had 60% risk of HCC compared to 20–25% risk in those with HBV or HCV alone. No significant difference in the overall risk of

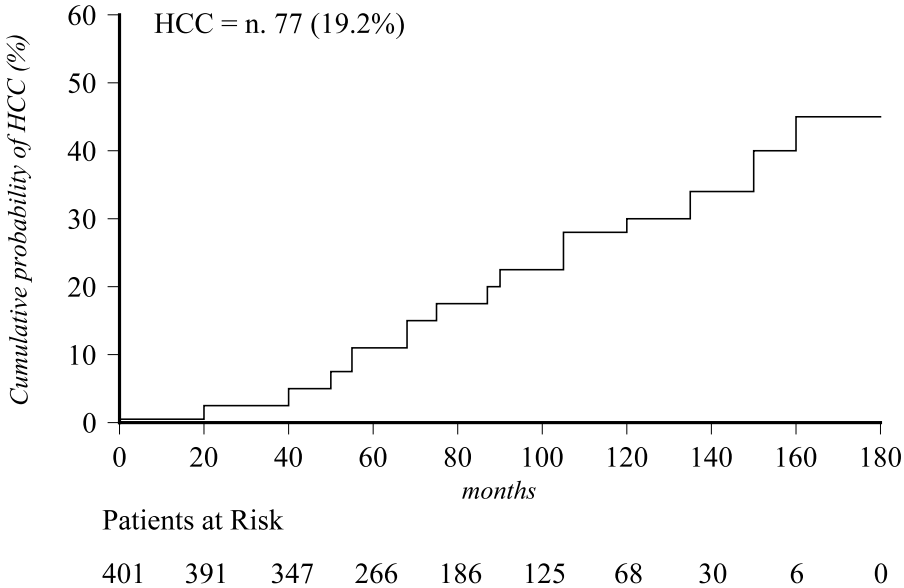

Fig. 1. Cumulative incidence of HCC in 401 patients with cirrhosis by the Kaplan–Meier analysis.

HCC was observed between HBV and HCV related cirrhosis, while the annual rate of HCC development assessed over a 15 year period of follow-up showed an opposite trend in HCV related cirrhosis compared to HBV related disease. In the anti-HCV positive patients the annual incidence of HCC raised from 2% in the first 5 year period to 4% in the third 5 year period, while decreased from 2 to 0% in the HBsAg positive group. This different trend correlated with different ALT behavior during follow-up, as the percentage of patients with normal ALT was constant over time in anti-HCV positive cases, while increased from 34 to 67% in HBsAg positive patients (Fig. 3).

3.2. Risk factors for HCC in HBV and HCV related cirrhosis

Eight (16%) out of the 50 HBsAg positive and 53 (18.7%) out of the 284 anti-HCV positive patients developed HCC during follow-up. By multivariate analysis (Cox's proportional hazards regression), age above 59 years, male sex, longer duration and more advanced stage of cirrhosis, lower platelet count and high or fluctuating levels of ALT during follow-up were significant risk factors for HCC in anti-HCV positive cirrhotic patients, while none of these variables were significant risk factors for HCC in HBsAg positive patients. In this latter group only presence of high or fluctuating serum AFP levels during follow-up was a significant risk factor for tumor development (Table 1).

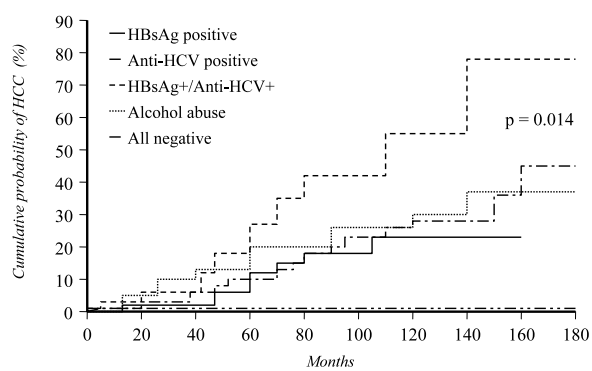

Fig. 2. Cumulative probability of HCC development in relation to etiology of liver disease (Kaplan–Meier method and log-rank test).

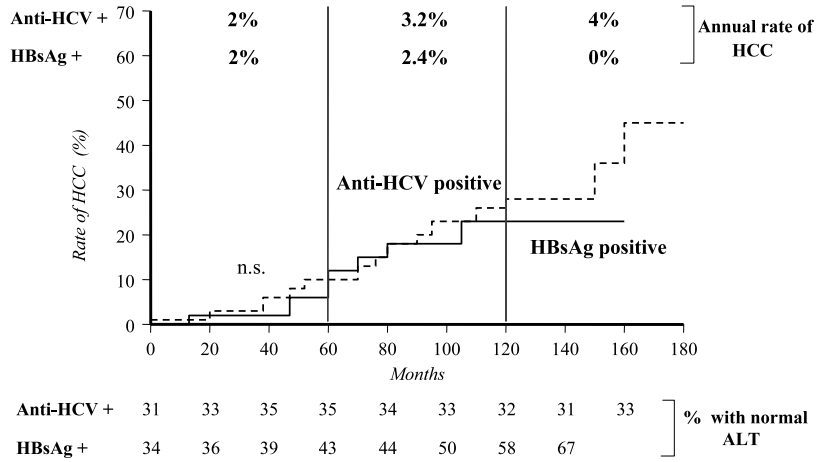

Fig. 3. Comparison of overall and annual incidence of HCC in HBV and HCV related cirrhosis and relation to rate of ALT behavior during follow-up.

3.3. Morphologic pattern of tumor development during follow-up

Two different macroscopic patterns of HCC development were observed on the basis of US or CT findings (tumor margin, presence or absence of peri-nodular capsule) (Ohto et al., 1987; Itai, 1987; Yamashita et al., 1993): (1) tumor arising as a small, capsulated nodule, with well-defined margins and expansive growth (nodular type) and, (2) tumor presenting as a spreading mass not clearly defined, with ill-defined margins and infiltrative growth (infiltrating type). During follow-up, HCC developed as nodular type in 63 (81.8%) patients, 51 with a single nodule and 12 with two nodules, while in the remaining 14 (18.2%) cases the tumor developed as an aggressive and infiltrating mass. Patients with nodular HCC had a mean age at the time of tumor development significantly higher than that of patients with infiltrating HCC (66.6 vs. 60.6 years, 95% C.I.: 64.8–68.4 and 56.4–64.8 years, $P = 0.012$), while there were no significant differences between the two groups as to gender, known duration of chronic liver disease, stage of cirrhosis and etiologic factors. On the other hand, high or fluctuating levels of ALT during follow-up were significantly more frequent in nodular HCC than in the infiltrating type ($P = 0.02$), while there was no significant difference in AFP levels. Risk factors for nodular and for infiltrating HCC identified by multivariate analysis are described in Table 2. Older age, longer duration and more advanced stage of cirrhosis were all significantly associated with increased risk of developing nodular but not infiltrating HCC. On the other hand, male sex HBsAg positivity and dual HBsAg and anti-HCV positivity were significant risk factors for development of infiltrating but not nodular HCC. Thus, development of nodular HCC was associated with disease stage and duration, independently of etiology, while development of infiltrating HCC was associated with etiology independently of disease stage and duration. Fig. 4 describes the cumulative probability (Kaplan–Meier method and log-rank test) of developing nodular or infiltrative/diffuse HCC according to etiologic factors. This analysis confirmed the higher risk for infiltrative/diffuse but not for nodular HCC in patients with HBV infection and with HBV/HCV coinfection.

4. Discussion

In this study we have evaluated the incidence, the associated risk factors and the morphologic pattern of appearance of HCC in HBV- and HCV-associated cirrhosis over a long-term obser-

vation period. Our data indicate an increasing incidence time trend of HCC in the whole cirrhotic population during follow-up, rising from a mean of 2% in the first 5 years, to a mean of 3.5% over the subsequent 10 year period. Patients with dual HBV and HCV infection showed the highest incidence of HCC development while patients with single HBV or HCV infection showed similar overall cumulative incidence. However, some interesting differences were observed between these two etiologic groups when the annual incidence of HCC was assessed over time. In HCV related cirrhosis the annual risk increased from 2% during the earliest years of observation to 4% during later follow-up, while decreasing from 2 to 0% in the HBsAg positive group, and this opposite trend correlated with a different behavior of ALT in the two groups. In fact the probability of normalizing ALT on long-term follow-up was higher in the HBV positive patients compared to the HCV positive group, indicating that the reduction in the annual HCC risk observed in patients with long-standing HBV related cirrhosis was most likely a consequence of the large number of patients who entered on inactive phase of liver disease during prolonged follow-up. Other interesting differences were observed when risk factors for HCC in HCV and HBV positive patients were compared by multivariate analysis. In the former, tumor appearance was significantly associated with dura-

Table 1
Risk factors for HCC development in HBV and HCV-related cirrhosis by the Cox proportional hazards regression analysis

Variable	HBsAg positive			Anti-HCV positive		
	Pts. at risk (n. 50)	Exposure Coefficient	*P*	Pts. at risk (n. 284)	Exposure Coefficient	*P*
Age						
<59 years	33	–		128	1	
>59 years	17	–	–	156	1.0624	0.002
Sex						
Males	42	–		156	1	
Females	8	–	–	128	0.5955	0.09
Duration of cirrhosis						
<1 year	26	–		150	1	
≥1 year	24	–	–	134	1.1281	0.004
Child's Pugh stage						
A	34	–		209	1	
B or worsened	16	–	–	75	1.2731	0.01
Alcohol abuse[a]						
Negative	41	–		242	–	
Positive	9	–	–	42	–	–
Platelet count						
$>130\times10^9$/l	27	–		130	0.9917	
$\leq130\times10^9$/l	23	–	–	154	1	0.001
ALT during follow-up						
Normal or normalized	26	–		96	1	
High or fluctuating[b]	24	–	–	188	1.6846	0.06
AFP during follow-up						
<20 ng/ml	44	1		213	–	
High or fluctuating[b]	6	5.2041	0.04	71	–	–

[a] Above 80 g/die for men and above 50 g/die for women.
[b] Values persistently high or with continuous fluctuation during the whole follow-up period.

Table 2
Risk factors for nodular and for infiltrating HCC in 401 patients with cirrhosis by the Cox proportional hazards regression analysis and Wald test

Variable	Pts. at risk	Nodular HCC		Infiltrating HCC	
		Exposure coefficient	*P*	Exposure coefficient	*P*
Age					
<59 years	208	1		–	
>59 years	193	1.0655	0.0001	–	–
Sex					
Males	247	–		7.4012	
Females	154	–	–	1	0.01
Duration of cirrhosis					
Diagnosis at inclusion	207	1		–	
≤3 years	102	1.1141		–	–
>3 years	92	2.2282	0.002		
Child's Pugh stage					
A	279	1		–	
B or worsened	122	3.1332	0.0001	–	–
Alcohol abuse[a]					
Negative	370	–		–	
Positive	31	–	–	–	–
Anti-HCV					
Negative	117	–		–	
Positive	284	–	–	–	–
HBsAg					
Negative	351	–		1	
Positive	50	–	–	6.4401	0.06
Anti-HCV and HBsAg					
Negative	382	–		1	
Positive	19	–	–	11.1921	0.0001
ALT during follow-up					
Normal or normalized	167	1		–	
High or fluctuating[b]	234	1.6062	0.0001	–	–
AFP during follow-up					
<20 ng/ml	321	–		–	
High or fluctuating[b]	80	–	–	–	–

[a] Above 80 g/die for men and above 50 g/die for women.
[b] Values persistently high or with continuous fluctuation during the whole follow-up period.

tion and stage of cirrhosis and with the presence of continuous biochemical activity while in the HBV positive group HCC development was independent from such stage-related variables, while correlated only with the profile of AFP, suggesting that liver cell dysplasia might have been more pronounced in this group. Two main distinct patterns of HCC development were observed. The majority of patients developed a small, well-defined and capsulated nodule of HCC, while a smaller subgroup presented with a much more aggressive and infiltrating form of HCC. The nodular type, but not the infiltrating type of tumor, was associated with long-standing and persistently histologically active cirrhosis. On the other hand, the nodular type of HCC was not

associated with the etiology of liver disease, while infiltrating HCC was strongly and significantly influenced by etiology, being six times higher in HBsAg positive compared to HBsAg negative patients, and 11 times higher in the presence of HBV and HCV coinfection. Thus, on the basis of these findings, different mechanisms of liver carcinogenesis might operate in HCV related and in HBV related cirrhosis. In patients with hepatitis C, development of HCC seems to follow the natural history of long-standing chronic liver disease, and may be related to the appearance of foci of neoplastic cells within the cirrhotic regenerative nodules, with a stronger effect of the cirrhotic process per se and a less important effect of the underlying virus infection. On the other hand, development of HCC in HBV infected cases appeared to be independent of the duration and stage of cirrhosis, and often occurred not as a well-defined nodule but as a more diffuse and infiltrating tumor suggesting a more direct role of the virus.

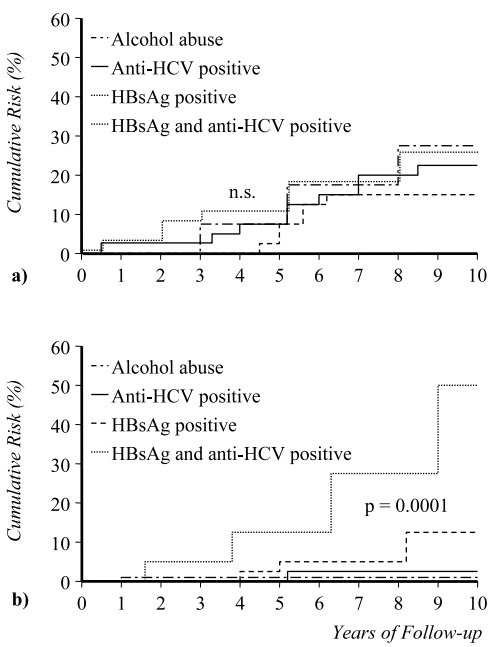

Fig. 4. Cumulative risk of nodular (a) and infiltrating (b) HCC in patients with cirrhosis according to etiology of liver disease (Kaplan–Meier method and log-rank test).

In conclusion, our data indicated that although the overall risk of HCC is similar in HBV and HCV associated cirrhosis, distinct features in tumor development and in morphogenesis patterns can be identified, suggesting that different mechanisms of carcinogenesis might be involved.

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