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Effect of the Etiology of Viral Cirrhosis on the Survival of Patients with Hepatocellular Carcinoma

Maria Chiara Cantarini, M.D.,[1] Franco Trevisani, M.D.,[1] Antonio Maria Morselli-Labate, Ph.D.,[2] Gianludovico Rapaccini, M.D.,[3] Fabio Farinati, M.D.,[4] Paolo Del Poggio, M.D.,[5] Maria Anna Di Nolfo, M.D.,[6] Luisa Benvegnù, M.D.,[7] Marco Zoli, M.D.,[1] Franco Borzio, M.D.,[8] and Mauro Bernardi, M.D.[1] for the Italian Liver Cancer (ITA.LI.CA) group.

[1]*Dipartimento di Medicina Interna, Cardioangiologia, Epatologia and* [2]*Dipartimento di Medicina Interna e Gastroenterologia Alma Mater Studiorum-University of Bologna;* [3]*Cattedra di Medicina Interna II, Università Cattolica di Roma;* [4]*Dipartimento di Scienze Chirurgiche e Gastroenterologiche, University of Padova;* [5]*Divisione di Medicina, Ospedale Treviglio-Caravaggio, Treviglio;* [6]*Divisione di Medicina, Ospedale Bolognini, Seriate;* [7]*Dipartimento di Medicina Clinica e Sperimentale, University of Padova, Italy;* [8]*Dipartimento di Medicina, Unità di Gastroenterologia, Ospedale Fatebenefratelli, Milano; Italy*

OBJECTIVES: The aim of this study was to assess whether hepatocellular carcinoma occurring in the setting of hepatitis B or C virus infection has different prognosis.

METHODS: We performed a multicentric case-control study comparing 102 pairs of patients affected by hepatitis B virus- or hepatitis C virus-related hepatocellular carcinoma. Patients were matched for sex (male/female: 84/18 pairs), age, center, and period of enrollment, underlying chronic liver disease (cirrhosis/chronic hepatitis: 97/5 pairs), Child-Pugh class (A/B/C: 70/25/7 pairs), hepatocellular carcinoma stage (nonadvanced/advanced: 50/52 pairs) and, when possible, modality of cancer diagnosis (75 pairs: 47 during and 28 outside surveillance).

RESULTS: In the whole population, patients with hepatitis B tended to have a poor prognosis than those with hepatitis C ($p = 0.160$), and this difference became statistically significant among the patients with an advanced hepatocellular carcinoma ($p = 0.025$). Etiology, Child-Pugh class, gross pathology, and alpha-fetoprotein were the significant independent prognostic factors in the whole population. The distribution of these prognostic factors did not differ between patients with hepatitis B or hepatitis C, both in the whole population and in the subgroup of advanced hepatocellular carcinomas.

CONCLUSION: Hepatitis B virus-related hepatocellular carcinomas have a greater aggressiveness than hepatitis C virus-related tumors, which becomes clinically manifest once they have reached an advanced stage.

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INTRODUCTION

Hepatitis B and C virus (HBV and HCV) infections are well-known risk factors for cirrhosis and hepatocellular carcinoma (HCC) and account for most of the HCC cases worldwide (1). There is increasing evidence that these infections have different pathways of oncogenesis. In HBV infection, besides chronic inflammation, the integration of HBV-DNA into the hepatocyte DNA and the expression of viral proteins which transactivate human oncogenes play a central role in hepatocarcinogenesis (2). Conversely, the chronic inflammatory state induced by viral infection seems to play the main role in the HCV oncogenesis, even though viral proteins have been recently found to exert a direct oncogenic action (3). This may explain the differences seen in gene expression (4), genetic alterations (5), and natural history (6) between HCCs occurring during HBV and HCV infection. According to the genetic alterations, the natural history suggests that HBV has a more direct carcinogenic activity: HBV-related HCCs arise more frequently in a set of less advanced liver disease with respect to HCV-related neoplasms; moreover, although the extent of liver necroinflammation is a risk factor for HCC regardless the etiology, the severity and inflammatory activity of cirrhosis are more important in HCV than in HBsAg carriers (6, 7), in whom viral replication is a major predictor of HCC (8).

Given the distinct oncogenic pathway and natural history, differences in the prognosis of HCC would also be expected according to its etiology. However, despite the abundant literature, this issue remains unsettled because of the controversial results generated by an unbalance between the two populations concerning the main prognostic factors of HCC. In fact, most of the studies concerning either surgical (9–13) or unselected patients (14–17) did not stratify the patients

according to age, liver function, cancer stage, and extrahepatic comorbidity. Even the modality of cancer diagnosis may be important because a regular surveillance more likely detects slow growing tumors (length time bias) (18). The present case-control study aimed to assess the impact of viral infection *per se* on the clinical history of HCC by comparing HBV and HCV Caucasian patients with a tumor superimposed on chronic liver disease (CLD), matched for the known prognostic factors of this cancer.

PATIENTS AND METHODS

Patients

SELECTION, EXCLUSION, AND MATCHING CRITERIA. Among 1,237 patients with HCC seen consecutively from January 1986 to December 2001 in seven clinical Institutions, we retrospectively selected those in whom HCC was superimposed on CLD due to HBV or HCV infection, and data on modality of HCC diagnosis, cancer stage, liver function, treatment applied, and follow-up were reported in the clinical records. Exclusion criteria were: non-viral CLD, coinfections (HBV/hepatitis delta or HBV/HCV), and concomitant alcohol abuse. A total of 699 patients fulfilled these criteria: 133 (19%) were HBsAg+ (HBV cases) and 566 (81%) were anti-HCV+ (HCV cases). Among them, we were able to match 102 HBV patients (cases) with 102 HCV individuals (controls) according to the following criteria: sex (84 males and 18 females), age (within 7 yr [median age: 62 yr, range: 43–82]), center and period (within 7 yr) of enrollment, underlying CLD (97 pairs [96.1%] had cirrhosis and 5 pairs [3.9%] chronic hepatitis), Child-Pugh class (19) (A: 70 [68.6%], B: 25 [24.5%], C: 7 [6.9%]), HCC stage according to the Milano criteria (20) (nonadvanced: 50 [49.0%], advanced: 52 [51.0%]), and, when possible, modality of HCC diagnosis (75 pairs [73.5%]: 47 [46.1%] on surveillance, 28 [27.4%] outside surveillance). As expected, the median age did not differ between HBV and HCV patients (63.5 [range: 46–82] *vs* 62.0 [43–78], $p = 0.420$) as well as the percentage of cases recruited in each quinquennium of the study (1986–1990: 11.8% *vs* 5.9%; 1991–1995: 33.3% *vs* 37.2%; 1996–2001: 54.9% *vs* 56.9%, $p = 0.323$)

Diagnosis of cirrhosis was supported by biopsy in 76 patients (39 HVB and 37 HCV cases), and was based on the presence of clinical and laboratory features of portal hypertension (oesophageal varices at endoscopy and/or collateral circulation at ultrasonography [US]) in the remainder. The severity of liver cirrhosis was assessed according to Child-Pugh classification.

Diagnosis of non cirrhotic chronic hepatitis was always based on liver histology.

Diagnosis of HCC was corroborated by histological or cytological findings in 110 patients (53 HBV and 57 HCV cases), while it was based on unequivocal clinical and imaging data and on the follow-up in the remainder.

HCC stage was defined according to Milano criteria (20) as "nonadvanced" (when the cancer was solitary $\leq$5 cm or plurifocal $\leq$3 lesions, with the largest diameter $\leq$3 cm, without evidence of vascular invasion and distant metastases) and "advanced," when the tumor exceeded these limits. Cancer stage was assessed with both abdominal US and CT scan features and, when appropriate, by angiography and magnetic resonance. To detect metastases, all patients underwent chest X-ray and abdominal US. Bone scintigraphy and CT scans of chest and brain were performed when clinically indicated.

The macroscopic types of HCC were classified as solitary, paucifocal ($\leq$3 nodules), multifocal (>3 nodules), diffuse, and massive type (21). When possible (95 HBV and 96 HCV cases), patients were also restaged according to the CLIP system (22).

Modality of cancer diagnosis was defined under "surveillance," when HCC was detected during regular follow-up based on semiannual or annual US and alpha-fetoprotein (AFP) determination, "incidental," when an asymptomatic neoplasm was discovered outside any surveillance program or during diagnostic procedures performed for extrahepatic diseases, "symptomatic," when HCC was diagnosed because of symptom appearance.

The criteria used to allocate patients to the different *therapeutic options* (orthotopic liver transplantation [OLT], hepatic resection, percutaneous ethanol injection [PEI], radiofrequency ablation [RF], and transarterial hepatic chemoembolization [TACE]) have been reported in detail elsewhere (21).

LABORATORY. Liver function tests (serum alanine aminotranspherase [ALT], expressed as multiples of the upper limit of reference range [ULRR], bilirubin, albumin, and prothrombin activity [PT]) and serum alpha-fetoprotein (AFP: normal values: $\leq$20 ng/mL) were determined by conventional methods. Hepatitis B virus markers were tested by radioimmunoassay or enzyme-linked immunosorbent assay and antibody anti-hepatitis C virus by ELISA I (up to April 1991), II, and III generation, using commercial kits.

Finally, the *causes of death* were also analyzed in the two groups.

Putative Prognostic Factors

Viral etiology, sex, age, Child-Pugh class, AFP levels, ALT levels, cancer stage, gross pathology types, median diameter, vascular invasion, metastases, modality of diagnosis, comorbidity, CLIP score, and treatments were tested as putative prognostic factors at both the univariate and multivariate analysis.

Main Outcome Measures

Survival was calculated from the time of cancer diagnosis to death or with values censored at the date of the last follow-up visit until 48 months after diagnosis. The analysis was conducted until the fourth year because a 4-yr survival can be considered a "long-term" survival for unselected HCC patients. Survival of HBV and HCV patients was compared in both the overall population and subgroups stratified according to the demographic and the main clinical matching criteria: sex, age (median), Child-Pugh class, and cancer stage.

Statistical Analysis

Median, range, and frequencies were used as descriptive statistics. Contingency tables were analyzed by the Fisher exact test, the Pearson chi-square test, and the Mantel-Haenszel test for linear association, as appropriate. The Mann-Whitney test was applied to continuous variables. Survival estimates were calculated according to the Kaplan-Meier method and were compared between the two groups by univariate and multivariate forward step-wise Cox analysis. The hazard ratios and their 95% confidence intervals (95% CI) were also calculated. All statistical analyses were performed using the SPSS 8.0 statistical package (Chicago, IL). Two-tailed p values less than 0.05 were considered statistically significant.

The study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the senior staffs of the participating institutions.

RESULTS

Whole Population

AFP levels were within the reference range (0–20 ng/mL) in 46.1% of cases and reached diagnostic levels (>200 ng/mL (23)) only in 22.0% of them.

Most tumors were monofocal (46.6%) or paucifocal (24.5%), and the median tumor diameter (of the largest lesion when the tumor was multifocal) was 3.2 cm (range: 0.8–17). Both macroscopic vascular invasion (9.3%) and metastases (4.4%) were uncommon.

Extrahepatic comorbidities were present in 29.4% of patients.

Among the therapeutic approaches offered to patients, TACE (35.8%) or percutaneous ablative techniques (24.4%) were those most commonly applied, and only 15.4% of patients were amenable to surgical procedures.

As expected (21), most deaths (74.3%) were attributable to cancer progression.

Comparison Between HBV and HCV Patients (Table 1)

CLINICAL FEATURES. Among the analyzed parameters only ALT levels significantly differed, being lower in HBV patients than in the counterpart. Despite the fact that the modality of HCC diagnosis was not a mandatory matching criterion, it was well balanced between groups, most patients having cancer diagnosis during surveillance. The CLIP score too did not differ between HBV and HCV carriers.

SURVIVAL. The median follow-up was 19.5 (range 1–126) months for HBV patients and 23.0 (1–90) months for HCV patients ($p = 0.069$). The survival rate in the two groups is reported in Figure 1. Although HBV patients tended to have a worse survival, the difference did not reach statistical significance (hazard ratio: 1.27; 95% CI: 0.91–1.76; $p = 0.160$). The survival rates at 1, 2, 3, and 4 yr in HBV and HCV patients were 67.3%, 43.6%, 25.9%, and 20.9% *versus* 82.9%, 53.8%, 32.3%, and 21.8%, respectively.

The comparison of the survival between HBV and HCV patients (hazard ratio and 95% CI) according to the demographic characteristics, Child-Pugh class and cancer stage is summarized in Figure 2.

When patients were stratified according to sex, the survival did not differ between HBV and HCV either in males (hazard ratio: 1.34; 95% CI: 0.93–1.91; $p = 0.116$) or in females (hazard ratio: 1.00; 95% CI: 0.45–2.24; $p = 0.994$). When patients were classified according to median age, no differences were observed between HBV and HCV individuals aged ≤62 yr (hazard ratio: 1.03; 95% CI: 0.63–1.67, $p = 0.906$) while, among older patients, HBV individuals tended to have a poorer prognosis (hazard ratio: 1.55; 95% CI: 0.99–2.44, $p = 0.055$).

No differences in survival emerged according to Child-Pugh class (class A: hazard ratio: 1.23; 95% CI: 0.81–1.87, $p = 0.327$; class B: hazard ratio: 1.50; 95% CI: 0.82–2.76, $p = 0.188$; class C: hazard ratio: 1.65; 95% CI: 0.52–5.29, $p = 0.396$).

Instead, when patients were stratified according to cancer stage, in the 50 pairs with early HCC the survival did not differ between HBV and HCV cases (hazard ratio: 1.05; 95% CI: 0.62–1.77, $p = 0.863$; Figs. 2 and 3) while HBV infection was associated with a worse survival (hazard ratio: 1.62; 95% CI: 1.06–2.48, $p = 0.025$) among the 52 pairs with advanced HCC (Figs. 2 and 4). In this subgroup, the survival rates at 1, 2, 3, and 4 yr of HBV and HCV patients were 47.1%, 32.8%, 9.7%, and 4.9% *versus* 74.9%, 42.6%, 24.7%, and 12.5%, respectively. Interestingly, this result was mostly due to a lower survival of HBV patients who underwent palliative therapy as compared to their HCV counterpart, since among the 66 patients with advanced HCC treated with radical procedures or TACE the survival did not significantly differ (hazard ratio 1.61; 95% CI: 0.94–2.75; $p = 0.082$).

Putative Prognostic Factors (Table 2)

The univariate Cox regression analysis showed that age, Child-Pugh class, AFP, cancer stage, gross pathology and diameter of HCC, vascular invasion, modality of diagnosis, treatments, and CLIP score were predictors of survival. Multivariate Cox regression analysis identified etiology, Child-Pugh class, AFP, and gross pathology as independent prognostic factors.

The distribution of the independent prognostic factors according to the etiology did not differ in the whole population (Table 1) as well as in patients with advanced HCC (AFP: $p = 0.221$, gross pathology: $p = 0.427$). In the latter group, the multivariate analysis removed from the model the Child-Pugh class ($p = 0.121$), while the etiology maintained its prognostic value indicating that the survival adjusted for the others independent factors was lower in HBV patients than in HCV patients (Table 3). Finally, an additional multivariate analysis including all the matching variables was performed in this group. Among them, etiology (HBV: hazard ratio 1.75, 95% CI 1.14–2.68, $p = 0.011$) and Child-Pugh class (class B: hazard ratio 1.41, 95% CI 0.87–2.29, $p = 0.167$; class C:

Table 1. Demographic and Clinical Features of Patients According to the Etiology of Cirrhosis

Variable	HBV ($n = 102$)	HCV ($n = 102$)	p Value
AFP levels[1]			0.451[a]
≤20 ng/mL	47 (49.5%)	41 (42.7%)	
21–200 ng/mL	28 (29.5%)	33 (34.4%)	
>200 ng/mL	20 (21.1%)	22 (22.9%)	
ALT levels[2]			0.043[b]
(ULRR; median, range)	1.43 (0.75–10.00)	2.00 (0.09–11.41)	
Gross pathology types			0.524[c]
Monofocal	47 (46.1%)	48 (47.1%)	
Paucifocal	27 (26.5%)	23 (22.5%)	
Multifocal	14 (13.7%)	22 (21.6%)	
Diffuse	9 (8.8%)	6 (5.9%)	
Massive	5 (4.9%)	3 (2.9%)	
Cancer diameter (cm; median, range)[3]	3.45 (0.8–17)	3 (1–10.8)	0.287[b]
Vascular invasion			0.336[d]
Absent	95 (93.1%)	90 (88.2%)	
Portal	7 (6.9%)	12 (11.8%)	
Metastases			1.000[d]
Absent	98 (96.1%)	97 (95.1%)	
Present	4 (3.9%)	5 (4.9%)	
Modality of cancer diagnosis			0.595[c]
Surveillance	53 (52.0%)	60 (58.8%)	
Casual	27 (26.5%)	22 (21.6%)	
Symptoms	22 (21.6%)	20 (19.6%)	
Comorbidity			0.282[d]
Absent	76 (74.5%)	68 (66.7%)	
Present	26 (25.5%)	34 (33.3%)	
Therapy[4]			0.782[c]
OLT	5 (5.0%)	4 (4.0%)	
Resection	13 (13.0%)	9 (8.9%)	
PEI/RF	22 (22.0%)	27 (26.7%)	
TACE	34 (34.0%)	38 (37.6%)	
Palliation or none	26 (26.0%)	23 (22.8%)	
CLIP score[5]			0.615[a]
0	31 (32.6%)	28 (29.4%)	
1	29 (30.5%)	38 (39.6%)	
2	17 (17.9%)	17 (17.7%)	
3	11 (11.6%)	7 (7.3%)	
4–6	7 (7.4%)	6 (6.2%)	
Causes of death[6]			0.308[c]
HCC progression	47 (70.1%)	44 (78.6%)	
Liver failure	12 (17.9%)	7 (12.5%)	
Bleeding	5 (7.5 %)	1 (1.8%)	
Sepsis	1 (1.5 %)	0	
Other	2 (3.0%)	4 (7.1%)	

[a]Mantel-Haenszel linear-by-linear association.
[b]Mann-Whitney U-test.
[c]Pearson chi-square test.
[d]Fisher's exact test.
[1]Data available in 95 HBV pts and 96 HCV pts (93.6% of the whole population).
[2]Data available in 96 HBV pts and 98 HCV pts (95.1% of the whole population).
[3]Data available in 92 HBV pts and 94 HCV pts (91.2% of the whole population).
[4]Data available in 100 HBV pts and 101 HCV pts (98.5% of the whole population).
[5]Data available in 95 HBV pts and 96 HCV pts (93.6% of the whole population).
[6]Data available in 67 HBV pts and 56 HCV pts (60.3% of the whole population).
ULRR = multiples of the upper limit of the reference range; PEI = percutaneous ethanol injection; RF = radiofrequency ablation; TACE = transarterial chemoembolization.

hazard ratio 5.50, 95% CI 2.46–12.13, $p<0.001$) were the independent predictors of survival.

DISCUSSION

Although a number of studies have focused on this issue, whether HBV- and HCV-related HCCs have a different prognosis remains controversial. Investigations aimed to analyze the outcome of HCC patients treated with resection report that HBsAg carriers had either the same (9, 10) or a better (12, 13) or a worse prognosis than their counterpart (11). This conflict of results is likely generated by the fact that the main prognostic factors of HCC were not well balanced among patient groups. The same methodological limitation explains

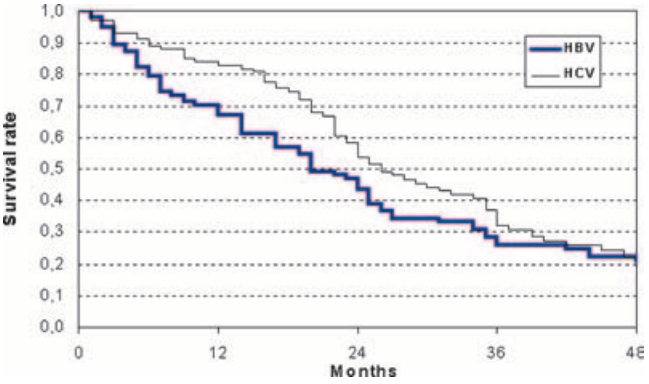

Figure 1. Survival according to the viral etiology of chronic liver disease. In HBV patients the survival tended to be lower than in HCV patients but the difference did not reach statistical significance ($p = 0.160$).

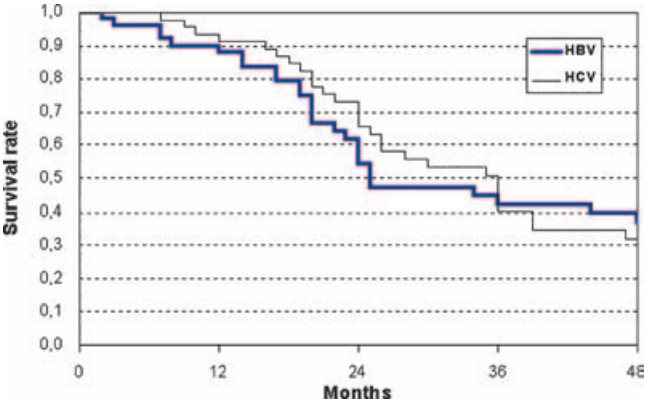

Figure 3. Survival according to the viral etiology of chronic liver disease in patients with early hepatocellular carcinoma. No difference was observed between HBV and HCV patients ($p = 0.863$).

the divergent conclusions achieved even in unselected patients (14–17).

To the best of our knowledge, only one Japanese study stratified patients according to some predictors of survival (15) and showed a different prognosis only in Child-Pugh class A patients, according to the cancer stage and treatment: the prognosis of stage I–II HCCs treated with hepatic resection was better in HBV than in HCV patients, while the opposite result was seen for advanced HCCs treated with TACE. However, even these results are not conclusive because the stratification reduced the sample size of each group to a few cases and the identity between groups was not guaranteed. Some confounding factors, such as demographic features, the motivation for detecting HCC, comorbid illnesses, and period of enrollment were not taken into account.

Our case-control study provides a further insight into this topic, using a design where the main prognostic factors of HCC were well balanced between HBV and HCV patients. In order to match the patients for HCC staging, we utilized the Milano criteria, originally proposed and almost universally adopted to select candidates for liver transplantation. These criteria offer a clear-cut prediction of life expectancy not only of patients transplanted but also of those treated with percutaneous ablative procedures (24). Moreover, they showed a robust prognostic validity even in unselected (21) and elderly (25) HCC patients. Finally, in our study this stratification yielded a distribution of gross pathologic types of HCC (an independent predictor of survival in the present study) that was similar between HBV and HCV patients. We also matched patients for center and period of enrollment, to minimize biases due to different diagnostic and therapeutic techniques. Finally, our patient groups were well balanced also for the modality of diagnosis, in order to avoid the length time bias, which confers a better prognosis to tumors diagnosed during surveillance since this practice is more likely to detect a slow than a rapid growing tumor (18).

A potential limitation of our study may derive from the lack of match for cancer differentiation, due to the fact that in half of our cases the HCC diagnosis was based on clinical data. However, the prognostic impact of histology is not essential when liver function, AFP level, and tumor extension are considered, as confirmed by several prognostic systems

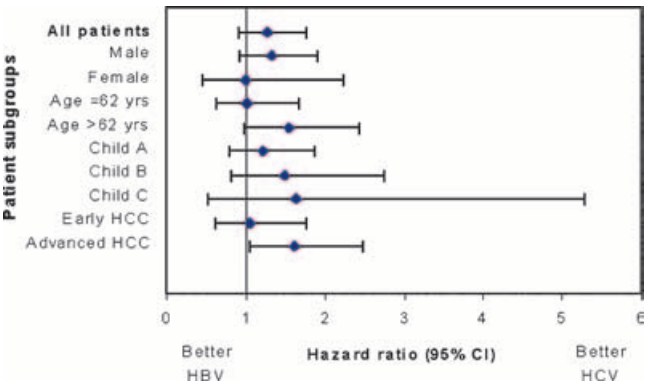

Figure 2. Prognostic value (hazard ratio and 95% CI) of viral etiology in patients with hepatocellular carcinoma stratified according to demographic data, Child-Pugh class and cancer stage.

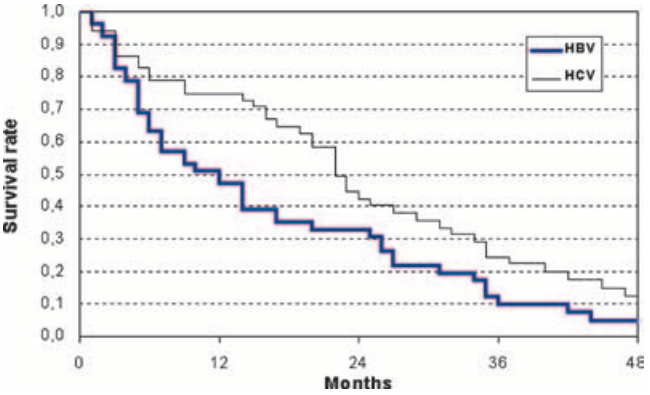

Figure 4. Survival according to the viral etiology of chronic liver disease in patients with advanced hepatocellular carcinoma. HBV patients had a lower survival than HCV patients ($p = 0.025$).

Table 2. Prognostic Factors in the Whole Population of Patients

	Univariate analysis, hazard ratio (95% CI)	*p* Value	Multivariate analysis, hazard ratio (95% CI)	*p* Value
Etiology	1.27 (0.91–1.76)	0.160	1.51 (1.00–2.29)	0.048
Age	1.03 (1.01–1.05)	0.014		
Sex	1.17 (0.76–1.82)	0.476		
Comorbidity	1.30 (0.91–1.86)	0.157		
Child-Pugh class		<0.001[a]		<0.001[a]
B	1.49 (1.03–2.15) [b]	0.034[b]	1.53 (0.98–1.37)[b]	0.588[b]
C	6.10 (3.28–11.35) [b]	<0.001[b]	8.65 (3.56–20.98)[b]	<0.001[b]
AFP		<0.001[a]		0.006[a]
21–200 ng/mL	1.12 (0.75–1.68)[c]	0.566[c]	1.50 (0.94–2.38)[c]	0.091[c]
>200 ng/mL	2.29 (1.49–3.51)[c]	<0.001[c]	2.43 (1.43–4.14)[c]	0.001[c]
ALT	1.02 (0.93–1.13)	0.629		
Cancer stage	2.33 (1.66–3.26)	<0.001		
Gross pathology types		<0.001[a]		<0.001[a]
Paucifocal	0.95 (0.61–1.48)[d]	0.813[d]	0.76 (0.46–1.27)[d]	0.297[d]
Multifocal	2.09 (1.36–3.23)[d]	<0.001[d]	2.24 (1.39–3.60)[d]	<0.001[d]
Diffuse	20.26 (10.04–40.92)[d]	<0.001[d]	38.32 (7.9–185.7)[d]	<0.001[d]
Massive	9.35 (4.27–20.47)[d]	<0.001[d]	11.03 (2.51–48.5)[d]	0.001[d]
Cancer diameter	1.18 (1.09–1.27)	<0.001		
Vascular invasion	2.86 (1.72–4.77)	<0.001		
Metastases	2.04 (1.00–4.17)	0.051		
Modality of diagnosis		<0.001[a]		
Casual	2.04 (1.36–3.04)[e]	<0.00[e]		
Symptoms	2.94 (1.97–4.40)[e]	<0.001[e]		
Treatments		<0.00[a]		
Resection	1.74 (0.57–5.34)[f]	0.333[f]		
PEI/RF	1.88 (0.66–5.37)[f]	0.238[f]		
TACE	2.76 (1.00–7.64)[f]	0.051[f]		
Palliation/none	5.74 (2.05–16.08)[f]	<0.001[f]		
CLIP score	1.91 (1.61–2.27)[g]	<0.001[g]		

[a] *p* Value for the multiple degree of freedom test.
[b] Hazard ratios and *p* values *versus* Child-Pugh class A.
[c] Hazard ratios and *p* values *versus* AFP levels ≤20 ng/mL.
[d] Hazard ratios and *p* values *versus* monofocal type.
[e] Hazard ratios and *p* values *versus* surveillance.
[f] Hazard ratios and *p* values *versus* orthotopic liver transplantation.
[g] Hazard ratio and *p* values for one incremental unit of the score.
PEI: percutaneous ethanol injection; RF: radiofrequency ablation; TACE: transarterial chemoembolization.

(26). Interestingly, in our series the CLIP system was not retained by the Cox model as independent predictor, suggesting that its performance could be improved by including a more detailed stratification of patients for some factors. Based on our results, it is likely that AFP and gross pathology need more break points than those adopted by the CLIP system (400 ng/mL and 50% of the liver volume on imaging), particularly in order to predict the outcome of early HCCs (27).

In line with Tanabe's results (15), we found that stratification for cancer stage is crucial in detecting a different prognosis according to viral infection in a Caucasian population too (Fig. 4). When HCC was detected at an early stage, meeting the Milano criteria, HBV and HCV individuals shared the same prognosis while, when cancer stage exceeded these criteria, HBV patients had a worse prognosis. This result cannot be attributable to: (a) an unbalanced distribution of the independent prognostic factors because, in this subgroup too, they did not differ between HBV and HCV patients; (b) an underestimation of HCC gross pathology in HBV patient, because the diagnostic accuracy of imaging techniques for HCC staging is not affected by the etiology. Therefore, as most patients died due to HCC progression in both groups, it can be surmised that HBV-related carcinogenesis gives HCC an accelerated clinical course. If so, an intriguing question is: why does the clinical history of viral HCC only diverge

Table 3. Independent Prognostic Factors in Patients with Advanced Hepatocellular Carcinoma

	Multivariate analysis	
	Hazard ratio (95% CI)	*p* Value
Etiology	1.72 (1.04–2.83)	0.034
AFP		0.014[a]
21–200 ng/mL	1.31 (0.71–2.41)[b]	0.390[b]
>200 ng/mL	2.30 (1.29–4.10)[b]	0.005[b]
Gross pathology types		<0.001[a]
Paucifocal	0.81 (0.38–1.72)[c]	0.581[c]
Multifocal	1.93 (0.99–3.73)[c]	0.050[c]
Diffuse	15.33 (6.04–38.90)[c]	<0.001[c]
Massive	5.85 (2.17–15.8)[c]	<0.001[c]

[a] *p* Value for the multiple degree of freedom test.
[b] Hazard ratios and *p* values *versus* AFP levels ≤20 ng/mL.
[c] Hazard ratios and *p* values *versus* monofocal type.

after the tumor has reached a given stage? So far, the molecular mechanisms of hepatocarcinogenesis according to the etiology remain largely unknown but some molecular findings support the concept that the cell signalling pathways may be differently affected by HBV and HCV infections, and could help to built some hypotheses to explain the clinical stage-related divergence. One of these findings concerns the role of estrogen receptors. HBV-related HCCs express variant α-estrogen receptors (α-vERs) more frequently than HCV-related tumors (28, 29). The presence of α-vERs, which lose the hormone dependence maintaining constitutive transcriptional activity, marks the tumors with a rapid growth rate (28–30) and, among patients unsuitable for radical treatments, is the strongest predictor of mortality (29). However, the appearance of α-vERs occurs gradually during the progression from CLD toward HCC and the α-ER pattern in dysplastic foci is similar to that of HCC, suggesting an early occurrence of ER mutation in hepatic oncogenesis (28). Therefore, the greater propensity to develop an altered α-Er status by HBV-related tumors could justify their greater aggressiveness but does not explain why a different prognosis only becomes manifest for advanced cancers. More recently, it has been reported that also the mutation of β-ER is more common in HBV- than in HCV-related cancers (31) and, at least in experimental animals, β-vERs appear in the late stages of tumor progression (32). Hence, the mutation of β-ERs could account for a growing biological aggressiveness as the tumor expands, particularly in HBV infected patients. This hypothesis, however, needs to be supported by further investigations on the timing of β-vER mutations in HCC patients.

HBV-related tumors have a distinct pattern of genetic mutations with greater chromosome instability than HCCs with other etiologies, and a higher prevalence of loss of heterozygosity (LOH) is correlated with tumor aggressiveness (5, 33). However, in the Laurent-Puig's study (5) the LOH on chromosomes 6q and 9p, considered an independent genomic predictor of poor prognosis, was not more common in HBV patients (5). Conversely, Okabe *et al*. (33) found that the LOH on 13q and 16q, which were associated to undifferentiated tumors, vascular invasion and intrahepatic metastasis, were more common in HBV patients. Iizuka *et al*. (4), by comparing HBV- and HCV-related HCCs, found that several genes promoting signal transduction, transcription and metastasis were upregulated in the former. Finally, the HBV attitude toward a multicentric and aggressive hepatocarcinogenesis is suggested by two findings: the more frequent policlonality of multiple nodules (34) and the higher incidence of infiltrating HCC in patients under periodic surveillance (35).

Therefore, it can be hypothesized that the biological aggressiveness of HBV-related HCCs is basically greater but, in the early tumor stage, the applicability of radical or effective treatments—which completely or largely destroy a small tumor mass—can restrain the difference in tumor progression and prognosis between HBV and HCV patients. Conversely, palliative therapies utilized in a more advanced stage, allow the HBV-related cancer to manifest its more aggressive attitude. In agreement with this hypothesis, the survival of patients with an advanced HCC receiving radical treatment or TACE was not affected by tumor etiology. Alternatively, the different mutational changes occurring at late stages of tumor growth (31, 36) may generate the diverse behavior of advanced HCC.

In conclusion, our study shows that the stratification for HCC stage seems crucial in detecting a different prognosis according to the viral infection. In fact, the greater aggressiveness of HBV-related tumors becomes clinically evident once they have reached an advanced stage, not suitable to ablative procedures or TACE. In a time where new promising anticancer drugs (*i.e.*, anti-angiogenic, anti-Raf-kinase molecules) are coming up to the scene of advanced HCC, our result highlights a prognostic aspect that should be considered in order to evaluate the effectiveness of these drugs in virus-related HCCs.

APPENDIX

Other members of the ITA.LI.CA group

Dipartimento di Medicina Interna, Cardioangiologia, Epatologia, Semeiotica Medica, Università di Bologna: Pietro Andreone, Chiara Bendini, Maurizio Biselli, Paolo Caraceni, Carmela Cursaro, Marco Domenicali, Annagiulia Gramenzi, Silvia Li Bassi, Donatella Magalotti, Giulia Magini, Federica Mirici Cappa, Valentina Santi, Andrea Zambruni; *Cattedra di Medicina Interna II, Università Cattolica del Sacro Cuore di Roma*: Marco Covino, Giovanni Gasbarrini; *Cattedra di Malattie dell'Apparato Digerente, Università di Padova*: Massimo De Giorgio, Simona Gianni, Michela Rinaldi; *Dipartimento di Medicina Clinica e Sperimentale, Clinica Medica V, Università di Padova*: Alfredo Alberti, Angelo Gatta, Maurizio Gios; *Dipartimento di Discipline Chirurgiche, Rianimatorie e dei Trapianti, Università di Bologna*: Gian Luca Grazi, Bruno Nardo, Matteo Ravaioli; *Divisione di Medicina, Ospedale Treviglio-Caravaggio, Treviglio*: Lodovico Gilardoni, Mario Mattiello; *Divisione di Medicina, Ospedale Bolognini, Seriate*: Maria Di Marco, Elena Vavassori; *Dipartimento di Scienze Radiologiche ed Istocitopatologiche, Università di Bologna*: Cristina Rossi.

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Reprint requests and correspondence: Franco Trevisani, M.D., Dipartimento di Medicina Interna, Cardioangiologia, Epatologia, Semeiotica Medica, via Albertoni, 15, 40138 Bologna, Italy.

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