Hepatitis B Virus Surface Antigen-Negative and Hepatitis C Virus Antibody-Negative Hepatocellular Carcinoma: Clinical Characteristics, Outcome, and Risk Factors for Early and Late Intrahepatic Recurrence After Resection

Tao Li, MD1; Lun-Xiu Qin, MD2; Xiao Gong, MD3; Jian Zhou, MD2; Hui-Chuan Sun, MD2; Shuang-Jian Qiu, MD2; Qing-Hai Ye, MD2; Lu Wang, MD2; and Jia Fan, MD2

BACKGROUND: Although the incidence of hepatitis B virus surface antigen (HBsAg)-negative/hepatitis C virus antibody (HCVAb)-negative hepatocellular carcinoma (NBNC-HCC) is gradually increasing, it has been mostly ignored in previous studies. The objective of this exploratory study was to investigate the clinicopathologic characteristics and prognostic factors that influence recurrence and survival in patients with NBNC-HCC.

METHODS: A retrospective analysis was performed of 675 patients with NBNC-HCC and 3529 patients with HBsAg-positive/HCVAb-negative HCC (BNC-HCC) who underwent curative resection between 1997 and 2009. Intrahepatic recurrences were classified into early (<1 year) and late (>1 year) recurrences. Multivariate competing risks analyses with Bonferroni correction were used to evaluate independent prognostic factors.

RESULTS: There were no significant differences between the NBNC-HCC and BNC-HCC groups regarding overall survival, cumulative incidence of HCC-specific death, and recurrence. However, the patients with NBNC-HCC were much older (P < .001), were associated less often with cirrhosis or elevated α-fetoprotein levels (P < .001), and had a much lower ratio of men to women (P < .001). NBNC-HCC tumors were larger (P < .001), but were involved less often with vascular invasion (P = .004). Women, serum γ-glutamyl transpeptidase level, tumor size, tumor capsule, and tumor differentiation were identified as independent risk factors for HCC-specific survival in patients with NBNC-HCC. The cumulative incidence of HCC-specific death for women with NBNC-HCC was significantly greater than for men with NBNC-HCC (P < .001). Tumor capsule and vascular invasion were identified as independent risk factors for early recurrence of NBNC-HCC, whereas tumor differentiation was identified as the only significant risk factor for late recurrence.

CONCLUSIONS: Patients who had NBNC-HCC had characteristics and prognostic factors that differed from those in patients who had BNC-HCC. Women with NBNC-HCC should be more closely monitored, and it may be worthwhile to evaluate estrogen administration for the maintenance of sex hormone balance and to improve these poor outcomes.

KEYWORDS: hepatitis, hepatocellular carcinoma, recurrence, risk factors, prognosis.

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 6% of all human cancers. Infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is associated with approximately 85% of HCC worldwide, highlighting the need for programs to prevent infections. Today, greater than 90% of countries worldwide have introduced HBV vaccine into their national infant immunization schedules, thus dramatically decreasing the incidence of HBV-related HCC. For areas in which HCV infection is prevalent, the incidence of HCV-related HCC also is decreasing, whereas HBV surface antigen (HBsAg)-negative/HCV antibody (HCVAb)-negative HCC (NBNC-HCC) is gradually increasing, in line with low-risk areas, including western countries, possibly because of alcohol-related liver disease (ALD) or nonalcoholic steatohepatitis (NASH) associated with obesity.

Although HCC arising in ALD or NASH without HBV or HCV infection has been a rare observation in previous studies, a worldwide increase in alcohol use and the epidemic rise in obesity and diabetes mellitus have made ALD and NASH common causes of HCC throughout the world. A positive association between the incidence of HCC and ALD or NASH has been reported from many countries. These epidemiologic results suggest that both heavy cumulative alcohol intake and NASH are risk factors for the development of HCC, particularly among HBsAg-negative or HCVAb-negative individuals.

Different etiologies of HCC may cause different clinical characteristics and outcomes, thereby requiring different preventive and therapeutic strategies. Although HBsAg-positive or HCVAb-positive HCC has been thoroughly studied, less is known about NBNC-HCC.
investigated in the past decades, little data regarding NBNC-HCC are available in the literature. Furthermore, the differences between NBNC-HCC and HBsAg-positive/HCVAb-negative HCC (BNC-HCC) are unclear. In this study, we retrospectively analyzed data from 675 consecutive patients with NBNC-HCC and 3529 patients with BNC-HCC who underwent curative resection in our institute to investigate the prognostic factors that influenced recurrence and survival and to clarify the differences between these 2 groups.

MATERIALS AND METHODS

Study Population

Data from patients with HCC who underwent curative resection at the Liver Cancer Institute of Fudan University between January 1997 and December 2009 were retrieved from a prospectively collected database and were retrospectively analyzed. Patients with NBNC-HCC patients were defined those who had serum that was negative for both HBsAg and HCVAb, whereas patients with BNC-HCC patients were seropositive for HBsAg but negative for HCVAb. Patients who had HCC with occult hepatitis B infection also were included in the BNC-HCC group. Curative resection was defined as complete macroscopic removal of the tumor without exposure of tumor cells on the cut surface with macroscopic tumor clearance confirmed on a computed tomography (CT) scan or magnetic resonance imaging (MRI) study of the liver 1 month after hepatic resection. All pathologic specimens were reviewed by 2 pathologists to confirm the diagnosis of HCC. The histologic grade of tumor differentiation was assigned according to the proposed European Association for the study of the Liver criteria for HCC. Ultrasound-guided fine-needle biopsy sometimes was needed to confirm the diagnosis when imaging was atypical. Extrahepatic recurrence was defined as a new lesion in the remnant liver with typical imaging appearance (tumor demonstrates hepatic arterial enhancement, and, during the portal venous and equilibrium phases, the tumor fades off, whereas the pseudocapsule enhances brightly) on CT/MRI studies and an elevated AFP level according to the proposed European Association for Study of the Liver criteria for HCC. Ultrasound-guided fine-needle biopsy sometimes was needed to confirm the diagnosis when imaging was atypical. Extrahepatic recurrence was defined as a new lesion detected outside the remnant liver. When extrahepatic recurrence was suspected, other malignancies would be ruled out by imaging modalities, serologic tumor markers, and/or pathologic examination.

Intrahepatic recurrences were divided into early and late recurrences using 1 year as the cutoff value, as suggested in the study by Poon et al, and the value of this time cutoff was confirmed by the different predictive factors and prognosis identified for the 2 groups. In this study, we included only the first recurrence: Early recurrence was defined as the first intrahepatic recurrence within 1 year after curative resection, and late recurrence was defined as the first intrahepatic recurrence ≥1 year after curative resection.

Follow-Up

Patients were followed regularly in the outpatient clinic and were monitored prospectively for recurrence according to a standard protocol that included serum AFP measurements and ultrasound or contrast CT studies. Patients were followed every 2 months during the first postoperative year and at least every 3 to 6 months thereafter. AFP measurement and liver ultrasonography were performed during each visit. A CT scan of the abdomen was obtained every 6 months. Bone scans or MRI studies were performed if localized bone pain was reported.

HCC-specific death was defined as death because of tumor progression; whereas non-HCC–specific death was defined as death because of liver failure or complications of portal hypertension or nonliver-related disease, such as myocardial infarction, stroke, renal failure, suicide, and trauma. Intrahepatic recurrence was defined as a new lesion in the remnant liver with typical imaging appearance (tumor demonstrates hepatic arterial enhancement, and, during the portal venous and equilibrium phases, the tumor fades off, whereas the pseudocapsule enhances brightly) on CT/MRI studies and an elevated AFP level according to the proposed European Association for Study of the Liver criteria for HCC. Ultrasound-guided fine-needle biopsy sometimes was needed to confirm the diagnosis when imaging was atypical. Extrahepatic recurrence was defined as a new lesion detected outside the remnant liver. When extrahepatic recurrence was suspected, other malignancies would be ruled out by imaging modalities, serologic tumor markers, and/or pathologic examination.

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**Statistical Analysis**

The chi-square test or the Fisher exact probability test was used to evaluate categoric variables, and the Student t test was used to evaluate continuous variables. The cumulative overall survival rate was calculated using the Kaplan-Meier method and was compared using the log-rank test. Overall survival was calculated from the date of resection to the date of death regardless of the cause of death. HCC-specific survival was defined as the time from the date of resection to the date death from HCC, and the cumulative incidence of HCC-specific death was estimated given non-HCC death as a competing risk. Recurrence-free survival was calculated from the date of resection to the date when tumor recurrence was diagnosed or from the date of resection to the last visit if recurrence was not diagnosed, and the patients were censored at the date of death or the date of last follow-up. The cumulative incidence of recurrence was estimated by considering nonrecurrence death as a competing risk. A competing risks regression model with Bonferroni correction was used to determine independent factors for survival and recurrence based on the variables that were selected in univariate analysis.

Statistical analyses were performed using the SPSS statistical software package (version 13.0; SPSS Inc., Chicago, Ill). Statistical analyses with competing risks were carried out using Stata (version 11.0; Stata Corp., College Station, Tex). A Bonferroni-adjusted P value was the normal P value multiplied by the number of outcomes being tested. If the resulting adjusted P value was > 1.0, then it was be rounded down to 1.0. Two-tailed P values <.05 were considered statistically significant.

**RESULTS**

**Baseline Characteristics of the NBNC-HCC and BNC-HCC Groups**

In total, 4204 patients with HCC who underwent curative resection were included in this study. Among them, 675 patients had NBNC-HCC, and the other 3529 patients had BNC-HCC. The clinicopathologic characteristics of these 2 groups are compared in Table 1. Compared with the BNC-HCC group, patients in the NBNC-HCC group were much older (P <.001), were associated less with cirrhosis or elevated AFP levels (P <.001), and had a much lower ratio of men to women (P <.001). Tumors in the NBNC-HCC group were larger (P <.001), were usually single and without a tumor capsule (P <.001), and less often had vascular invasion (P =.004). There were no significant differences between the 2 groups regarding other factors.

| Variable                  | NBNC-HCC, n = 675 | BNC-HCC, n = 3529 | P     |
|---------------------------|-------------------|-------------------|-------|
| Sex                       |                   |                   | .001  |
| Men                       | 521 (77)          | 3070 (87)         |       |
| Women                     | 154 (23)          | 459 (13)          |       |
| Age, y                    |                   |                   | .001  |
| ≤50                       | 181 (27)          | 1866 (53)         |       |
| >50                       | 494 (73)          | 1663 (47)         |       |
| AFP, ng/mL                |                   |                   | .001  |
| ≤20                       | 371 (55)          | 1091 (31)         |       |
| >20                       | 304 (45)          | 2438 (69)         |       |
| ALT, U/L                  |                   |                   | .337  |
| ≤75                       | 593 (88)          | 3052 (86)         |       |
| >75                       | 82 (12)           | 477 (14)          |       |
| GGT, U/L                  |                   |                   | .144  |
| ≤50                       | 264 (39)          | 1276 (36)         |       |
| >50                       | 411 (61)          | 2253 (64)         |       |
| ALB, g/L                  |                   |                   | .466  |
| <35                       | 31 (5)            | 186 (5)           |       |
| ≥35                       | 644 (95)          | 3343 (95)         |       |
| Child-Pugh score          |                   |                   | .162  |
| A                         | 616 (91)          | 3275 (93)         |       |
| B                         | 59 (9)            | 254 (7)           |       |
| Cirrhosis                 |                   |                   | .001  |
| Yes                       | 367 (54)          | 3144 (89)         |       |
| No                        | 308 (46)          | 385 (11)          |       |
| Tumor size, cm            |                   |                   | .001  |
| ≤5                        | 263 (39)          | 1825 (52)         |       |
| >5                        | 412 (61)          | 1704 (48)         |       |
| No. of tumors             |                   |                   | .001  |
| Single                    | 599 (89)          | 2888 (82)         |       |
| Multiple                  | 76 (11)           | 641 (18)          |       |
| Tumor capsule             |                   |                   | .001  |
| Yes                       | 290 (43)          | 1783 (51)         |       |
| No                        | 385 (57)          | 1736 (49)         |       |
| Vascular invasion         |                   |                   | .004  |
| Yes                       | 189 (28)          | 1187 (34)         |       |
| No                        | 486 (72)          | 2342 (66)         |       |
| Tumor differentiation*    |                   |                   | .563  |
| I-II                      | 451 (67)          | 2398 (68)         |       |
| III-IV                    | 224 (33)          | 1131 (32)         |       |
| Preoperative TACE         |                   |                   | .298  |
| No                        | 644 (95)          | 3332 (94)         |       |
| Yes                       | 31 (5)            | 197 (6)           |       |
| Postoperative TACE        |                   |                   | .362  |
| No                        | 402 (60)          | 2035 (58)         |       |
| Yes                       | 273 (40)          | 1494 (42)         |       |

Abbreviations: AFP, α-fetoprotein; ALT, alanine aminotransferase; BNC-HCC, HBsAg-positive/HCVAb-negative hepatocellular carcinoma; GGT, γ-glutamyltransferase; NBNC-HCC, HBsAg-negative/HCVAb-negative hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

*Tumors were graded according to the Edmondson classification.
Follow-Up and Prognosis of the NBNC-HCC and BNC-HCC Groups
The mean follow-up was 28 months (range, 1-134 months) for the NBNC-HCC group and 27 months (range, 1-152 months) for the BNC-HCC group. The 1-year, 3-year, and 5-year overall survival rates for the NBNC-HCC group were 77.7%, 57%, and 48.8%, respectively, and did not differ significantly from the rates for the BNC-HCC group (79.8%, 54.7%, and 43.9%, respectively; P = .544) (Fig. 1a).

Multivariate Analyses of Prognostic Factors for Hepatocellular Carcinoma-Specific Survival in the NBNC-HCC and BNC-HCC Groups
The independent prognostic factors that were related to HCC-specific survival in multivariate analysis using the competing risks regression model with Bonferroni correction are listed in Table 2. Multivariate analysis indicated that being a woman (P = .008; hazard ratio [HR], 1.75; 95% confidence interval [CI], 1.28-2.41), GGT level (P < .001; HR, 1.89; 95% CI, 1.40-2.54), tumor size (P = .040; HR, 1.55; 95% CI, 1.14-2.11), tumor capsule (P < .001; HR, 2.07; 95% CI, 1.54-2.79), and tumor differentiation (P < .001; HR, 1.72; 95% CI, 1.31-2.27) were independent risk factors for HCC-specific survival in patients with NBNC-HCC; whereas GGT level (P < .001; HR, 1.52; 95% CI, 1.29-1.78), tumor size (P < .001; HR, 1.48; 95% CI, 1.28-1.71), and vascular invasion (P < .001; HR, 1.77; 95% CI, 1.53-2.04) were independent risk factors for HCC-specific survival in patients with BNC-HCC.

Prognosis of Patients With Intrahepatic, Recurrent NBNC-HCC
Of the 675 patients with NBNC-HCC, 189 patients (28%) developed intrahepatic recurrences, 21 patients (3%) developed extrahepatic recurrence, and 15 patients (2%) developed both intrahepatic and extrahepatic recurrences. Of the 189 patients who had intrahepatic recurrences, 5 developed recurrence within 1 month and were excluded from further analysis of intrahepatic recurrence, because previous studies regarded recurrences that developed within 1 month as residual disease. Patients who had extrahepatic recurrences also were excluded, because the prognosis and characteristics of patients with intrahepatic and extrahepatic recurrences differ significantly. The 1-year, 3-year, and 5-year cumulative incidence of intrahepatic recurrence in the NBNC-HCC group were 19%, 33.1%, and 39.3%, respectively, and did not differ significantly from those in the BNC-HCC group (20.5%, 35.8%, and 42.9%, respectively; P = .186) (Fig. 1b).

Of the 189 patients who had intrahepatic recurrences, 107 (56.6%) were early recurrences, and the median time to recurrence was 4 months. The other 82 recurrences developed after 12 months, and the median time to recurrence was 27 months. For the BNC-HCC group, 692 of 1214 recurrences (57%) were early recurrences, which was not significantly different from the NBNC-HCC group (P = .937).

The overall survival rates for patients with recurrent NBNC-HCC at 1 year, 3 years, and 5 years were 72.7%, 44.6%, and 35.8%, respectively, which was significantly worse compared with the nonrecurrent group (77.9%, 62.5%, and 54.4%, respectively; P < .001). Better overall survival was observed in the late-recurrence group (100%, 73.5%, and 61.2% at 1 year, 3 years, and 5 years, respectively) compared with the early recurrence group (52.6%, 18.9%, and 10.5%, respectively; P < .001).

Multivariate Analyses of Predictive Factors for Patients With Early and Late Recurrences of NBNC-HCC and BNC-HCC
The results from multivariate analyses of predictive factors using a competing risks regression model for early and late intrahepatic recurrence are provided in Table 3 with P values adjusted by Bonferroni correction. Multivariate analysis revealed that tumor capsule (P = .036; HR, 1.83; 95% CI, 1.16-2.89) and vascular invasion (P = .020; HR, 1.80; 95% CI, 1.20-2.70) were independent risk factors for early intrahepatic recurrence in patients with NBNC-HCC; whereas GGT level (P = .004; HR, 1.37; 95% CI, 1.14-1.65), tumor size (P < .001; HR, 1.51; 95% CI, 1.28-1.78), and vascular invasion (P = .024; HR, 1.79; 95% CI, 1.19-2.72) were independent risk factors for early intrahepatic recurrence in patients with BNC-HCC. Only tumor differentiation (P = .057; HR, 1.52; 95% CI, 0.98-2.35) had marginal significance for late intrahepatic recurrence in patients with NBNC-HCC, whereas tumor size (P = .040; HR, 1.52; 95% CI, 0.98-2.35) was the only independent risk factor identified for late intrahepatic recurrence in patients with BNC-HCC.
incidence of intrahepatic recurrence in the BNC-HCC group (women: 20.2%, 35.7%, and 41%, respectively; men: 20.6%, 35.8%, and 43.2%, respectively; $P = .310$) (Fig. 1c) and the NBNC-HCC group (women: 23.2%, 35.8%, and 41.8%, respectively; men: 17.8%, 32.3%, and 38.9%, respectively; $P = .587$) (Fig. 1d).

In the BNC-HCC group, women had a better prognosis, with a median survival of 73 months versus only 39 months for men ($P < .001$). The 1-year, 3-year, and 5-year overall survival rates among men with BNC-HCC were 77.2%, 51.5%, and 39.9%, respectively, which not only was significantly worse than the rates among women
with BNC-HCC (79.8%, 64.1%, and 55.7%, respectively; \( P < .001 \)), but also was significantly worse than the rates among men with NBNC-HCC (79.5%, 59.8%, and 51.2%, respectively; \( P = .011 \)). When non-HCC death was considered as a competing risk, the 1-year, 3-year, and 5-year cumulative incidence of HCC-specific death for men with BNC-HCC (18.3%, 38.9%, and 49.2%, respectively) also was significantly greater than the rate among women with BNC-HCC (16.9%, 30.9%, and 38.6%, respectively; \( P < .001 \)) (Fig. 1e) and among women with NBNC-HCC (16.6%, 32.3%, and 38%, respectively; \( P < .001 \)).

The median survival of women with NBNC-HCC was 22 months, which was significantly shorter than that of men (65 months; \( P < .001 \)). The overall survival rate among women with NBNC-HCC at 1 year, 3 year, and 5 years was 65%, 42.8%, and 34.2%, respectively, which not only was significantly worse than the rate among men with NBNC-HCC (79.5%, 59.8%, and 51.2%, respectively; \( P < .001 \)), but also was significantly worse than the rate among women with BNC-HCC (79.8%, 64.1%, and 55.7%, respectively; \( P < .001 \)). When non-HCC death was considered as a competing risk, the 1-year, 3-year, and 5-year cumulative incidence of HCC-specific death among women with NBNC-HCC (29.6%, 48.7%, and 58%, respectively) also was significantly greater than the incidence among men with NBNC-HCC (16.6%, 32.3%, and 38%, respectively; \( P < .001 \)) (Fig. 1f) and among women with BNC-HCC (16.9%, 30.9%, and 38.6%, respectively; \( P < .001 \)).

Comparison of Characteristics Between Women and Men With NBNC-HCC

The clinicopathologic characteristics of men and women with NBNC-HCC are compared further in Table 4. Women were associated less with elevated serum levels of AFP (\( P < .001 \)), GGT (\( P = .016 \)), and cirrhosis (\( P < .001 \)). Although tumors among women were much larger (\( P = .024 \)) and were associated less with capsule formation (\( P = .001 \)), they also were involved less with vascular invasion (\( P < .001 \)). There were no significant differences between men and women regarding other factors.

**DISCUSSION**

Although HBV and HCV have been associated with poorer survival compared with other etiologies in several studies of HCC, in our current study, there were no significant differences between the BNC-HCC and...
NBNC-HCC groups regarding overall survival, the cumulative incidence of HCC-specific death, and recurrence. However, patients with NBNC-HCC were significantly older and were associated less frequently with cirrhosis or elevated AFP levels, which may have been the result of less viral impairment of the liver. Our study also demonstrated that the majority of tumors in the NBNC-HCC group were less involved with vascular invasion and usually were solitary and unencapsulated, in contrast to tumors of BNC-HCC group, which tended to be multiple and encapsulated. These results were consistent with previous studies indicating that capsule formation is more common in HCC that develops secondary to HBV infection than HCC that develops secondary to ALD. This is reasonable because BNC-HCC is associated more with cirrhosis, which is known to predispose patients to multicentric hepatocarcinogenesis and the formation of fibrous septa, and capsule formation is considered a characteristic of HCC that develops in the cirrhotic liver. Therefore, capsule formation is more frequent in patients who have HCC with HBV infection than in those without HBV infection. Furthermore, although we cannot rule out the possibility of decreased screening among patients with NBNC-HCC, less capsule formation may be another important reason for the larger tumor sizes observed in patients with NBNC-HCC, and it has been suggested that capsule formation is part of the defense mechanisms against the growth of HCC.

Previous studies of HCC have revealed several risk factors related to postoperative outcomes. In our study, although, when competing risks were considered, patients with NBNC-HCC and patients with BNC-HCC share some prognostic factors for HCC-specific survival, such as GGT level and tumor size, vascular invasion has prognostic significance only for patients with BNC-HCC, whereas tumor capsule and tumor differentiation have prognostic significance only for patients with NBNC-HCC. Furthermore, although women with NBNC-HCC had some characteristics that different from those in men with NBNC-HCC, such as normal AFP ranges, larger tumor size, and less vascular invasion or capsule formation, being a woman remained an independent prognostic

Table 3. Multivariate Analysis of Risk Factors for Early and Late Intrahepatic Recurrence in Patients With Hepatitis B Virus Surface Antigen (HBsAg)-Negative/Hepatitis C Virus Antibody (HVCAb)-Negative Hepatocellular Carcinoma and Patients With HBsAg-Positive/HCVAb-Negative Hepatocellular Carcinoma Using a Competing Risks Regression Model

| Variable                  | NBNC-HCC         | BNC-HCC          |         |               |          |               |          |               |
|---------------------------|------------------|------------------|---------|---------------|------------------|---------|---------------|------------------|
|                           | HR               | 95% CI           | P       | HR            | 95% CI           | P       |
| Early recurrence          |                  |                  |         |               |                  |         |               |                  |
| GGT, U/L                  |                  |                  |         |               |                  |         |               |                  |
| ≤50                       | 1.00             | 1.00             | 1.00    | 1.00          | 1.00             | 1.00    |
| >50                       | 1.28             | 0.83-1.97        | 1.00    | 1.37          | 1.14-1.65        | .004    |
| Tumor size, cm            |                  |                  |         |               |                  |         |               |                  |
| ≤5                        | 1.00             | 1.00             | 1.00    | 1.00          | 1.00             | 1.00    |
| >5                        | 1.00             | 0.64-1.55        | 1.00    | 1.51          | 1.28-1.78        | < .001  |
| Tumor capsule             |                  |                  |         |               |                  |         |               |                  |
| Yes                       | 1.00             | 1.00             | 1.00    | 1.00          | 1.00             | 1.00    |
| No                        | 1.83             | 1.16-2.89        | .036    | 1.09          | 0.93-1.28        | 1.000   |
| Vascular invasion         |                  |                  |         |               |                  |         |               |                  |
| No                        | 1.00             | 1.00             | 1.00    | 1.00          | 1.00             | 1.00    |
| Yes                       | 1.80             | 1.20-2.70        | .020    | 1.79          | 1.19-2.72        | .024    |
| Late recurrence           |                  |                  |         |               |                  |         |               |                  |
| Tumor differentiationc    |                  |                  |         |               |                  |         |               |                  |
| I-II                      | 1.00             | 1.00             | 1.00    | 1.00          | 1.00             | 1.00    |
| III-IV                    | 1.52             | 0.98-2.35        | .057    | 1.11          | 0.91-1.34        | 1.000   |
| Tumor size, cm            |                  |                  |         |               |                  |         |               |                  |
| ≤5                        | 1.00             | 1.00             | 1.00    | 1.00          | 1.00             | 1.00    |
| >5                        | 1.09             | 0.69-1.71        | 1.00    | 1.25          | 1.03-1.51        | .040    |

Abbreviations: BNC-HCC, HBsAg-positive/HCVAb-negative hepatocellular carcinoma; CI, confidence interval; HR, hazard ratio; NBNC-HCC, HBsAg-negative/HCVAb-negative hepatocellular carcinoma.

Only statistically significant factors are listed.

P values were adjusted using Bonferroni correction.

Tumors were graded according to the Edmondson classification.
factor for HCC-specific survival, and the prognosis for women with NBNC-HCC was significantly worse than that for men with NBNC-HCC. These results were in sharp contrast to our current study of BNC-HCC as well as previous HCC studies, which indicated that being a man was an independent risk factor for survival and that women had better survival than men. It has been suggested that this sex disparity can be attributed to both the androgen and estrogen sex hormone pathways, with distinct roles for each sex.

HCC is generally considered an androgen-dependent tumor. However, the hepatocarcinogenesis action of testosterone ultimately is mediated through the androgen receptors (AR), the specific knockout of which significantly reduced tumorigenicity in HCC mouse models. Different from androgen, the role of estrogen in carcinogenesis can be either tumor-protective or tumor-promoting, depending on the different carcinogenic mechanisms mediated by estrogen pathways. Although it has been suggested that the long-term use of oral contraceptives induces hepatic adenoma and focal nodular hyperplasia, distinct profiles of genetic aberrations between HCC and focal nodular hyperplasia adenoma imply the biphasic effects of estrogen in hepatocarcinogenesis. Today, both epidemiologic and animal studies have proven that estrogen plays a protective effect in women with HCC, and estrogen-mediated reduction of interleukin-6 production by Kupffer cells is considered the main mechanism. Increased interleukin-6 levels intensify the local inflammatory response, which not only induces compensatory hepatocyte proliferation and facilitates malignant transformation, but also promotes HCC progression by up-regulating the expression of vascular adhesion molecules.

However, in our study, the patients with NBNC-HCC were much older than the patients with BNC-HCC, and the majority of women with NBNC-HCC were older than a menopausal age of 50 years. Decline in ovarian function with menopause inevitably will cause the increase in interleukin-6, thus the tumor-protective effect of estrogen for HCC will be greatly attenuated in most women with NBNC-HCC. Therefore, the suppression of interleukin-6 by estrogen treatment, such as transdermal estradiol in postmenopausal women, may provide an important strategy for improving the prognosis for patients with NBNC-HCC. Furthermore, the tumor-promoting effect of androgen also will decrease in patients with NBNC-HCC, because testosterone levels begin to decline steadily by about 2% per year after age 40 years. Therefore, decreases in the stimulatory effects of androgen and the protective effects of estrogen were responsible for the better survival of men and the worse survival of women in the NBNC-HCC group. For the same reason, it is not surprising that, in our study, the dominance of men was more evident in the BNC-HCC group than in the NBNC-HCC group, with a ratio of men to women of 6.6:1.0 for the BNC-HCC group and 3.4:1.0 for the NBNC-HCC group.

Recently, an interaction between chronic viral infection and the androgen signaling pathway has been established. The transcriptional activity of AR can be augmented by the HBV X and HCV core oncoproteins, providing a synergism between androgen and chronic viral infection in HCC development. Consequently, NBNC-HCC will have a lower content of AR compared with BNC-HCC. This difference indicates that NBNC-HCC is less androgen-dependent because of less AR content and also may contribute to the better survival of men with NBNC-HCC.

Currently, the high incidence of intrahepatic recurrence is the main cause for the dismal outcome of patients with HCC. In our series, there were no significant differences between the NBNC-HCC and BNC-HCC groups regarding recurrence rates; however, the potential for lead-time bias may have affected the recurrence rates, because screening intervals may have varied between patients. The prognosis for patients with nonrecurrent
NBNC-HCC was significantly better than that for patients who had recurrences. In addition, for patients who developed recurrences, a better prognosis was observed in the late recurrence group compared with the early recurrence group. These results were comparable to previous studies in patients with recurrent HCC in which most recurrences developed within 1 year after hepatectomy.12 In our study, the majority of intrahepatic recurrences of NBNC-HCC and BNC-HCC also were early recurrences, implicating a need for strict surveillance during first year after resection.

The identification of risk factors that predict early and late intrahepatic recurrence could help to clarify the preventive and therapeutic strategies for recurrence. It has been concluded that only early intrahepatic recurrences of HCC are associated with tumor factors because of suggestions that intrahepatic metastasis is the main mechanism for early recurrences; whereas only nontumorous liver status has been linked to late recurrences because of its multicentric occurrence origin.12,38 In our study, when competing risks were considered, vascular invasion was identified as an independent risk factor for early recurrence of both NBNC-HCC and BNC-HCC, whereas absence of tumor capsule had prognostic significance only for the early recurrence of NBNC-HCC. This indicates that early recurrence of NBNC-HCC also may originate from intrahepatic metastasis, because intrahepatic metastasis starts with intracapsular invasion, progresses to extracapsular invasion, to intravascular invasion, then finally to intrahepatic metastasis.12,39

It has been suggested that only cirrhosis, but not any of the initial tumor factors, is associated with late recurrence after resection of HCC.12 However, those results were drawn from an analysis of patients with HCC without considering the competing risks, and nearly 90% of the patients had hepatitis-related disease and usually had cirrhosis.40 Premalignant lesions frequently are present in cirrhotic livers and could may rise to multicentric occurrence because of high hepatocellular proliferation,41 thereby promoting late recurrences. In contrast, only half of our patients with NBNC-HCC had disease associated with cirrhosis, and their risk of late recurrence depended only on the grade of differentiation of the primary tumor when competing risks were considered. An alternative interpretation of the current results is that late recurrence of NBNC-HCC also may originate from intrahepatic metastasis rather than multicentric occurrence, which is influenced only by the host status and not by any of the initial tumor factors. If this is true, then effective strategies for the prevention of late recurrences of HCC through the suppression of multicentric occurrence may not work in patients with NBNC-HCC. However, further studies based on genetic analysis will be required to provide direct evidence regarding the origins of recurrent NBNC-HCC.

Our current study demonstrates the differences regarding clinicopathologic characteristics and risk factors for prognosis and recurrence between the NBNC-HCC and BNC-HCC groups. Because being a woman is an independent risk factor for poor survival among patients with NBNC-HCC, women with NBNC-HCC should be monitored more closely after surgery, and postoperative imaging surveillance should be intensified, because the majority of patients with NBNC-HCC had negative AFP status. Although adjuvant chemolipiodolization cannot prevent a late recurrence of multicentric occurrence origin, it has been indicated for patients who have a high risk of early recurrence for its efficiency in suppressing intrahepatic metastasis.38 Therefore, adjuvant chemolipiodolization also may be effective in suppressing late recurrences of NBNC-HCC, because it is believed that such recurrences originate from intrahepatic metastasis.

A limitation of the current study is related to its retrospective nature. There is the potential for bias in the patient population, because only patients who underwent curative resection were included. Thus, our findings may not be applicable to patients who were not able to undergo or complete curative resection. Furthermore, as an exploratory study with very little available information on NBNC-HCC from previous studies, extensive preliminary work needs to be done to gain a better comprehension of NBNC-HCC. The origin of intrahepatic recurrence in NBNC-HCC should be confirmed by further studies based on genetic analysis, and the feasibility of preventing or treating NBNC-HCC by estrogen administration is worth evaluating, because sex hormone imbalance is more prevalent in patients with NBNC-HCC; however, such treatment should be under surveillance to avoid the increased risk of other tumors in women. In addition, the different clinical characteristics and outcomes in patients with NBNC-HCC arising from different etiologies, such as ALD or NASH, should be investigated in future studies, because the clinical features of ALD-HCC, NASH-HCC, and unknown HCC are clearly different.42 Clarifying these problems will contribute to the proper strategies needed for the prevention and management of different types of NBNC-HCC.

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