Tumor Biol. (2015) 36:6255–6263
DOI 10.1007/s13277-015-3311-8

RESEARCH ARTICLE

Vascular invasion in hepatitis B virus-related hepatocellular carcinoma with underlying cirrhosis: possible associations with ascites and hepatitis B viral factors?

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Received: 16 September 2014 / Accepted: 5 March 2015 / Published online: 2 April 2015
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Abstract Vascular invasion is one of the most important prognostic factors for patients with hepatocellular carcinoma (HCC). The objective of the current, retrospective study was to determine the associations of ascites and hepatitis B viral factors (HBeAg and anti-HBe status and HBV DNA levels), as well as tumor-related factors (size, tumor number, grade, and location) with micro- or macroscopic vascular invasion in patients with HCC that developed as a result of hepatitis B virus (HBV)-related cirrhosis. A total of 336 consecutive patients were included. Potential factors associated with micro- or macroscopic vascular invasion were analyzed by logistic regression. Ascites were more commonly detected in patients with micro- or macroscopic vascular invasion, and the presence of ascites was independently associated with vascular invasion. Among patients with mild-to-moderate or severe ascites, the odds ratio for macroscopic vascular invasion was 4.83 (95 % confidence interval [CI] 2.29–10.16) and 11.87 (95 % CI 4.53–31.07), respectively. Similarly, the presence of ascites was associated with microscopic vascular invasion (OR 5.00; 95 % CI 1.23–20.31). In contrast, hepatitis B viral factors were not significantly associated with vascular invasion. The presence of ascites was associated with vascular invasion in patients with HBV-related cirrhotic HCC. Thus, patients with ascites, vascular invasion should be considered and more frequent surveillance should be performed after curative treatment.

Keywords Ascites · Hepatitis B virus · Liver cirrhosis · Hepatocellular carcinoma · Neoplasm invasiveness

Abbreviations

AFP	α-Fetoprotein
Anti-HBe	Antibody to hepatitis B e antigen
CI	Confidence interval
HBeAg	Hepatitis B e antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the fifth most commonly occurring cancer worldwide [1, 2]. China alone accounts for approximately half of the world's cases [3, 4]. The main etiology of HCC in China is endemic chronic hepatitis B virus (HBV) infection, and in over 80 % of patients, the tumor develops from a background of cirrhosis [5–7]. In chronic HBV-infected cirrhotic patients, HCC can arise without warning but progresses rapidly, ultimately with poor prognosis and short life expectancy when HCC is detected at an advanced stage [8, 9].

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Vascular invasion is one of the most important prognostic factors for patients with HCC and may involve the micro- and/or the macrovasculature. It is clear that both micro- and macroscopic vascular invasion are independent predictors of tumor recurrence and poor survival after surgical treatment [10–13]. It has been shown that tumor diameter, number of nodules, histological grading, and α-fetoprotein (AFP) are predictive of macroscopic vascular invasion in cirrhotic HCC, while tumor size and grade are predictive of microscopic vascular invasion in HCC [14–19].

In addition to the 'known' tumor-related factors, the presence of ascites has also been shown to have a prognostic value in both resectable and inoperable cirrhotic HCC [20–22]. In a study that included 105 cirrhotic HCC patients who underwent percutaneous ethanol injection, the presence of ascites was associated with lower survival rates over the 6-year follow-up [23]. Similarly, in another study that included 182 HCC patients with Child's class A or B cirrhosis, the presence of ascites was associated with significantly worse survival after transcatheter arterial chemoembolization [24]. As a result, the presence of ascites is now considered in several prognostic indices to predict survival time of patients with HCC [25, 26]. Despite this, the association between ascites and micro- or macroscopic vascular invasion is unclear.

Chronic HBV infection is a well-recognized risk factor for cirrhosis and hepatocellular carcinoma (HCC). In a study of 203 Chinese patients with small HCC (tumor size ≤3 cm), hepatitis B e antigen (HBeAg) was associated with a greater risk of early recurrence (within 1 year) and poorer 5-year survival after curative resection [27]. Other reports have revealed that HBV DNA levels are correlated with tumor recurrence and survival in HCC after resection or intervention therapies [28, 29]. The measurement of viral load and detection of anti-HBe are also useful for estimating a patient's prognosis after curative resection [30–32]. However, the role of these viral factors in the development of vascular invasion particularly in HBV-related HCC and cirrhosis remain unclear.

Some tumor factors, including tumor diameter, number, histological grading, and metastasis, have been shown to be associated with vascular invasion of HCC. Macroscopic vascular invasion is easy to detect using imaging techniques; by contrast, microscopic vascular invasion, the presence of which is associated with high risk of recurrence and poor survival even after liver transplantation and radical resection, is usually identified after surgery. This is also true for some characteristics of tumors such as histological grading. Fine needle aspiration is not regularly performed for patients with HCC owing to the risk of needle track seeding [33–35]. The heterogeneity of the tumor limits its diagnostic accuracy and usefulness [36, 37]. Moreover, most patients with HCC have underlying cirrhosis and poor liver function, which may result in life-threatening complications such as bleeding after biopsy [38]. Therefore, in our study, we first wanted to identify other non-tumor-related factors that were associated with vascular invasion, which might help in the early detection of microvascular invasion before resection or transplantation. Second, we wanted to determine whether HBV could affect the development of vascular invasion in HCC. Thus, we investigated the association between the presences of ascites or HBV viral factors with micro- or macroscopic vascular invasion in chronic HBV-infected HCC patients with underlying cirrhosis.

Patients and methods

Study population

This retrospective cross-sectional study included Chinese patients consecutively admitted to Daping Hospital, Changzheng Hospital, and Changhai Hospital between January 2012 and December 2013 with HBV-related HCC and underlying liver cirrhosis. And there were no obvious differences of clinical index among cohorts.

Inclusion and exclusion criteria

Only patients with HCC who were confirmed to be positive for serum hepatitis B surface antigen were eligible for this study. Underlying cirrhosis was diagnosed on the basis of histopathology or a combination of clinical and biochemical findings, as well as typical features observed using imaging techniques such as ultrasonography, computerized tomography, and resonance imaging. Inclusion of HCC patients was based on either histological examination or a combination of α-fetoprotein (AFP) (>400 ng/mL) and typical imaging features on at least two radiological imaging techniques, including ultrasonography, computerized tomography, resonance imaging, and hepatic angiography with lipiodol [39, 40]. Histopathology was available for 153 patients who underwent tumor resection or liver transplantation. Owing to the risk of needle track seeding and bleeding as well as the cirrhotic background of the livers, fine needle aspiration was not performed on the remaining patients who received intervention therapies such as ablation, ethanol injection, and transcatheter arterial chemoembolization after diagnosis.

Patients co-infected with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus were excluded. Patients with cirrhosis of other etiologies or past history of schistosomiasis were excluded. All patients were diagnosed with HCC for the first time in the hospital. Patients with any suspicious space-occupying lesion on ultrasonography or computer tomography, recurrent HCC, other cancers metastasize to liver, or intervention treatments before admission were excluded.

This study was approved by the institutional review board of Daping Hospital, Changzheng Hospital, and Changhai Hospital and adhered to the principles of the Declaration of Helsinki. All patients provided informed consent.

Data collection

All data for the patients were obtained from electronic medical records and/or hard-copy clinical charts and assessed eligibility for the study.

Demographic characteristics, including gender and age at the diagnosis of HCC, were retrieved from their medical records. In Asia, the infection of HBV is usually acquired perinatally or in early childhood; therefore, the time of HBV infection was considered equivalent to the age of the patients.

The status of serum HBV markers including HBeAg, anti-HBe, and HBV DNA levels (expressed as $\log_{10}$ copies/mL titer) at diagnosis of HCC was collected from the patients' medical records. Serum HBeAg and anti-HBe levels for all patients were measured using a Roche E170 analyzer (Roche Diagnostics, Germany) in our hospital. HBV DNA levels were tested using a 7500 Real-Time PCR System (Applied Biosystems Co., CA, USA), which had a detection limit of 3 $\log_{10}$ copies/mL.

Laboratory tests at diagnosis of HCC, before undergoing any treatment, were considered eligible and performed using conventional methods, including the AFP test (reference range 0–20 ng/mL).

The severity of ascites was stratified as 'none,' 'mild-to-moderate,' or 'severe' based on ultrasonography findings by the same physician at the first admission. The status of ascites was determined before any treatment was given. No paracentesis was performed before admission. No past history of using diuretics was recorded.

The Child-Pugh score was calculated based on the severity of ascites (none=1, mild-to-moderate=2, severe=3), encephalopathy (none=1, grades 1 to 2=2, grades 3 to 4=3), serum albumin (>35 g/L=1, 28–35 g/L=2, <28 g/L=3), total serum bilirubin (<35 μmol/L=1, 35–51 μmol/L=2, >51 μmol/L=3), and prolongation of prothrombin time (<4 s=1, 4–6 s=2, >6 s=3). The points were stratified into three grades (A=5–6 points, B=7–9 points, and C=10–15 points).

Tumor size, number, location and presence of vascular invasion (macro- and microscopic invasion), and metastasis were retrieved from medical records. In addition, histological grade was determined for 153 patients by histopathology. Tumor grade was categorized as low, intermediate, or high according to the Edmondson and Steiner scheme. The macroscopic types of HCC were classified as solitary or multiple. In patients with multiple tumors, the largest lesion was used as the index lesion. Macroscopic vascular invasion was defined as gross invasion of the right or left main branches of the portal vein or the hepatic veins detected by imaging techniques and histopathology or in surgery and extracted from the patients' records. In the subgroup of 153 patients for whom histopathology was available, microscopic vascular invasion was defined as the presence of tumor emboli or clusters of cancer cells within the central vein, the portal vein, and/or large capsular vessels [15]. All patients had a chest X-ray, while additional investigations to detect metastases were performed when extrahepatic involvement was suspected.

Statistical analysis

Data entry was performed using EpiData Entry (version 3.02, The EpiData Association, Denmark, Europe), the validity of the data was double-checked by two investigators independently, and discrepancies resolved. Continuous variables were tested for normal distribution and expressed as means (standard deviation) or medians (range) as appropriate. Categorical variables were compared by χ^2 test, and continuous variables were compared by Student's t test, Mann-Whitney U test, or Kruskal-Wallis test, as appropriate. Potential factors associated with micro- or macroscopic vascular invasion were analyzed separately by logistic regression. Twenty-five patients with both major and microscopic vascular invasion were excluded from analyses of microscopic vascular invasion. Collinearity tests were performed before multivariate analysis. All statistical procedures were performed using the Statistical Program for Social Sciences (SPSS version 16.0; SPSS Inc., USA). A two-sided p value of less than 0.05 was considered statistically significant. However, the statistical analysis was carried among those three hospitals, and there were no significant differences (data not shown).

Results

A total of 336 HBV-related cirrhotic patients (mean±standard deviation age, 49.8±9.7 years; males/females, 274/62) fulfilling the inclusion criteria with the diagnosis of HCC were consecutively admitted and included in our study; 153 of them had histopathology. Clinical features and characteristics of tumors with or without macro- or microscopic vascular invasion are presented in Tables 1 and 2, respectively. The overall proportion of patients with macroscopic vascular invasion was 30.7 % (n=103/336). Most of the patients with HCC were male, with a male-to-female ratio of 4:1, although no difference in sex distribution was found among patients with or without vascular invasion. Patients with macrovascular invasion were younger than the patients without macrovascular invasion (47.5±9.2 vs. 50.8±9.7 years; p=0.005).

In the subgroup of 153 patients with histology results, the proportion of patients with micro- or macroscopic vascular invasion was 16.3 % (n=25/153) and 24.2 % (n=37/153), respectively, and 7.8 % (n=12/153) had only pathologically

Table 1 Clinical features and characteristics of tumors of 336 HBV-related cirrhotic HCC patients with or without macroscopic vascular invasion

Characteristics	All patients	Macroscopic vascular invasion		*p* value
		Absent	Present	
	336	233	103	
Male gender	274 (81.5)	187 (80.3)	87 (84.5)	NS
Age	49.8±9.7	50.8±9.7	47.5±9.2	0.004
HBeAg positivity	64 (19.0)	43 (18.5)	21 (20.4)	NS
Anti-HBe positivity	228 (67.9)	159 (68.2)	69 (67.0)	NS
Log_{10} HBV DNA level (copies/mL)[a]	5.17±1.19	5.17±1.20	5.19±1.16	NS
<3	64 (19.0)	45 (19.3)	19 (18.4)	NS
≥3	272 (81.0)	188 (80.7)	84 (81.6)	
Child-Pugh class				<0.001
A	192 (57.1)	155 (66.5)	37 (35.9)	
B	97 (28.9)	48 (20.6)	49 (47.6)	
C	47 (14.0)	30 (12.9)	17 (16.5)	
Presence of ascites				<0.001
None	227 (67.6)	180 (77.3)	47 (45.6)	
Mild to moderate	73 (21.7)	41 (17.6)	32 (31.1)	
Severe	36 (10.7)	12 (5.2)	24 (23.3)	
Tumor size (cm)	5.2 (1.0–18.0)	4.3 (1.0–18.0)	7.7 (1.9–17.5)	<0.001
≤5	161 (47.9)	144 (61.8)	17 (16.5)	<0.001
>5	175 (52.1)	89 (38.2)	86 (83.5)	
Tumor number				<0.001
Solitary	224 (66.7)	172 (73.8)	52 (50.5)	
Multiple	112 (33.3)	61 (26.2)	51 (49.5)	
Metastases				<0.001
Absent	281 (83.6)	213 (91.4)	68 (66.0)	
Present	55 (16.4)	20 (8.6)	35 (34.0)	
AFP				<0.001
≤1000 ng/mL	213 (63.4)	173 (74.2)	40 (38.8)	
>1000 ng/mL	123 (36.6)	60 (25.8)	63 (61.2)	
Serum albumin (g/L)	35.9±5.9	36.3±6.0	35.0±5.3	NS
Prothrombin time (s)	15.6±4.0	15.7±4.1	15.4±3.7	NS

Data expressed as number (%), mean±SD, median (range) where appropriate

NS not significant

[a] The mean and standard deviation was based on those patients with detectable HBV DNA

identified vascular invasion. Twenty-five patients had both macro- and microvascular invasion and were not included in the logistic analysis presented in Tables 3 and 4.

Characteristics of HCC in patients with HBV-related cirrhosis and association with vascular invasion

Most HBV-related cirrhotic patients had solitary tumors, which developed more frequently on the right lobule of the cirrhotic liver than on the left lobule, with a right-to-left ratio of 3.55. Tumor-related factors including diameter (>5 cm), metastasis, and AFP >1000 ng/mL were significantly and independently associated with macrovascular invasion, both in univariate and in multivariate analyses, as shown in Tables 1 and 3. Similarly, a high tumor grade and AFP >1000 ng/mL were significantly and independently associated with microvascular invasion, both in univariate and in multivariate analyses, as shown in Tables 2 and 4.

Association of the presence of ascites with vascular invasion

Overall, ascites were more frequently detected in patients with vascular invasion (all $p<0.001$). The presence of ascites was independently correlated with macro- and microscopic vascular invasion both in univariate and in multivariate analyses (Tables 3 and 4). The odds ratio for macroscopic vascular invasion was 4.83 (95 % confidence

Table 2 Clinical features and characteristics of tumors of 153 patients with HCC and HBV-related liver cirrhosis

Characteristics	Patients with histopathology	Both macro- and microscopic	Microscopic vascular invasion		
			Absent	Present	*p* value
	153	25	116	12	
Male gender	127 (83.0)	22 (88.0)	96 (82.8)	9 (75.0)	NS
Age	50.3±9.3	44.6±7.7	51.4±9.4	48.3±8.1	0.004
HBeAg positivity	27 (17.6)	5 (20.0)	20 (17.2)	2 (16.7)	NS
Anti-HBe positivity	107 (71.1)	16 (64.0)	84 (72.4)	7 (58.3)	NS
Log_{10} HBV DNA level (copies/mL)[a]	5.15±1.13	4.92±0.84	5.10±1.13	5.74±0.97	NS
<3	33 (21.6)	4 (16.0)	26 (22.4)	3 (25.0)	NS
≥3	120 (78.4)	21 (84.0)	90 (77.6)	9 (75.0)	
Child-Pugh class					NS
A	100 (65.4)	12 (48.0)	81 (69.8)	7 (58.3)	
B	38 (24.8)	12 (48.0)	23 (19.8)	3 (25.0)	
C	15 (9.8)	1 (4.0)	12 (10.3)	2 (16.7)	
Presence of ascites					0.017
None	113 (73.9)	12 (48.0)	94 (81.0)	7 (58.3)	
Mild to moderate	26 (17.0)	7 (28.0)	17 (14.7)	2 (16.7)	
Severe	14 (9.2)	6 (24.0)	5 (4.3)	3 (25.0)	
Tumor size (cm)	4.5 (1.3–16.8)	7.5 (2.5–15.0)	4.0 (1.3–16.8)	4.3 (2.2–10.0)	NS
≤5	88 (57.5)	6 (24.0)	75 (64.7)	7 (58.3)	NS
>5	65 (42.5)	19 (76.0)	41 (35.3)	5 (41.7)	
Tumor number					NS
Solitary	117 (76.5)	14 (56.0)	94 (81.0)	9 (75.0)	
Multiple	36 (23.5)	11 (44.0)	22 (19.0)	3 (25.0)	
Histological grade					0.005
Low or intermediate (grades I, II)	142 (92.8)	22 (88.0)	111 (95.7)	9 (75.0)	
High (grades III, IV)	11 (7.2)	3 (12.0)	5 (4.3)	3 (25.0)	
AFP					0.036
≤1000 ng/mL	103 (67.3)	7 (28.0)	90 (77.6)	6 (50.0)	
>1000 ng/mL	50 (32.7)	18 (72.0)	26 (22.4)	6 (50.0)	

Data expressed as number (%), mean (SD), median (range) where appropriate

NS not significant

[a] The mean and standard deviation was based on those patients with detectable HBV DNA

Table 3 Multivariate analysis of factors predictive of macroscopic vascular invasion in HBV-related cirrhotic patients with hepatocellular carcinoma (n=336)

Characteristics	Odds ratio	95 % CI	*p* value
Tumor size (>5 cm)	8.52	4.23–17.13	<0.001
Metastases	5.23	2.40–11.37	<0.001
Presence of ascites			<0.001
None	1 (reference)		
Mild to moderate	4.83	2.29–10.16	
Severe	11.87	4.53–31.07	
AFP >1000 ng/mL	3.77	2.05–6.90	<0.001

interval [CI] 2.29–10.16) among patients with mild-to-moderate ascites and 11.87 (95 % CI 4.53–31.07) among patients with severe ascites. Similarly, the odds

Table 4 Multivariate analysis of factors predictive of microscopic vascular invasion in a subgroup of HBV-related cirrhotic patients with hepatocellular carcinoma (n=128)

Characteristics	Odds ratio	95 % CI	*p* value
High-grade tumor	11.83	2.00–70.10	0.006
Presence of ascites	5.00	1.23–20.31	0.024
AFP >1000 ng/mL	4.88	1.26–18.84	0.021

The 25 patients with histopathology but had micro- and macroscopic vascular invasion were not included in this table

ratio for microvascular invasion was 5.00 (95 % CI 1.23–20.31).

The levels of serum albumin and prothrombin time, two markers of the severity of underlying cirrhosis, were not significantly different between patients with and without macroscopic vascular invasion (serum albumin, $p=0.07$; prothrombin time 0.49); similar results were obtained for patients with and without microscopic vascular invasion ($p=0.59$ and 0.86, respectively).

Association of hepatitis B viral factors and macro- or microscopic vascular invasion

Of the patients with hepatocellular carcinoma and HBV-related cirrhosis in our study, approximately 70 % were positive for serum anti-HBe, and over 80 % had detectable HBV DNA (≥3 $\log_{10}$ copies/mL). Of the patients who were positive for serum HBeAg, the presence of micro- or macroscopic vascular invasion as well as other tumor-related characteristics, including diameter, number, location, metastases, and histological grade, were not significantly different from those in HBeAg negative ones. This was also true for patients positive for anti-HBe or who had detectable HBV DNA levels, when compared with those who were negative for anti-HBe or without/undetectable HBV DNA levels <3 $\log_{10}$ copies/mL. The patients with vascular invasion had higher, albeit not statistically significant, median HBV DNA than patients without vascular invasion. Hepatitis B viral factors, including serum HBeAg and anti-HBe status as well as HBV DNA levels, were not significantly associated with micro- or macroscopic vascular invasion in multivariate analyses.

Discussion

For HBV-related HCC patients with underlying cirrhosis, liver transplantation is an effective simultaneous therapeutic option for cirrhosis, as well as for HCC, that may offer a chance for disease-free survival. For many years, researchers have attempted to expand the Milan criteria to identify more patients that might benefit from transplantation [41, 42]. However, many studies confirmed that patients with macro- or microvascular invasion had elevated risk of postoperative recurrence and decreased long-term survival rate [10, 43, 44]. Thus, the presence of vascular invasion is a key predictor of prognosis in patients with HCC [43–45]. In this article, our study of 336 HCC patients with HBV-related cirrhosis attempted to define some of the predictive factors that might correlate with micro- or macroscopic vascular invasion in this specific population. Every patient included in our study underwent ultrasonography (a gold standard method to evaluate the severity of ascites) at the first admission, and all patients were diagnosed with HCC for the first time in the hospital. And cohorts showed no obvious differences among the three hospitals. Consistent with previous studies [15, 19, 43, 46], we found that tumor-related factors including diameter (>5 cm), metastasis, and AFP >1000 ng/mL were significantly associated with macrovascular invasion, while a high tumor grade and AFP >1000 ng/mL were associated with microvascular invasion. Moreover, we also explored the possibility that the presence of ascites or hepatitis B viral factors was correlated with micro- and macrovascular invasion, in HCC patients with HBV-related cirrhosis, which has rarely been reported before now.

Ascites is the most common complication of cirrhosis. It has long been considered that the presence of ascites is a marker of portal hypertension and an advanced stage of underlying cirrhosis [47, 48]. In the current study, the presence of ascites was related to micro- and macrovascular invasion. Specially, in HBV-related HCC and cirrhosis with macroscopic vascular invasion, the more frequent development of ascites might be primarily a result of the occupation effect of the tumor, leading to the development of portal hypertension or exacerbation of preexisting portal hypertension and hepatic failure. A recent report supported that the presence of ascites, higher serum bilirubin and α-fetoprotein levels, lower serum albumin level, and worse performance status, as well as other factors (such as younger age) may increase the risk of macrovascular invasion and decrease long-term survival in HCC patients with curative or noncurative treatments [49]. Patients with macroscopic vascular invasion in our study did have worse liver function than those without, as reflected by the higher percentage of patients with Child-Pugh scores in class B or class C ($p<0.001$) among patients with vascular invasion compared with patients without vascular invasion. However, the serum albumin level and prothrombin time, two factors thought to be associated with the severity of underlying cirrhosis, were not significantly different between the two groups. This suggests that ascites are more closely related to vascular invasion in HBV-related cirrhotic patients with HCC than the severity of cirrhosis.

In addition, many cirrhosis patients who develop HCC do not experience hepatic decompensation before or at the time of diagnosis of liver cancer [50]; therefore, the presence of ascites might be used as a potential marker of intrahepatic vascular invasion in HCC. Several studies have reported that the presence of ascites is associated with poor prognosis and short survival time as well as intrahepatic and distant tumor recurrence after intervention therapies in HCC [51, 52]. One of the reasons for this might be its association with vascular invasion. It is known that portal hypertension, splanchnic vasodilatation, and renal sodium retention are fundamental in the pathophysiology of ascites formation in patients with cirrhosis [47, 48, 53, 54]. However, in the present study, we could not identify the characteristics of the ascites or monitor changes in portal vein pressure. Whereas, we proposed that in our study,

they were closely correlated. In addition, it is reported that ascites was correlated with vascular endothelial growth factor (VEGF) in ovarian cancer [55, 56], which may also provide some clues to elucidate the molecular mechanism underlying this association. Despite these, future studies may also need to focus on further exploring these findings in terms of tumor pathology and liver function.

The role of hepatitis B viral factors, including serum HBeAg and anti-HBe status as well as HBV DNA levels, in the risk of vascular invasion was also studied. Although HBV is known to be a carcinogenic factor and is associated with poor prognosis and tumor recurrence both in resectable and in inoperable HCC patients [28, 31], none of these hepatitis B viral factors achieved statistical significance in multivariate or in univariate analyses of vascular invasion in HCC. Thus, as for vascular invasion after the development of HCC, it appears that the characteristics of the tumor itself, such as its diameter, number of nodules, or histological grading, are more important than the etiology-related factors.

Due to the retrospective nature of the study, our study might not be able to reveal the full landscape about the occurrence of vascular invasion in cirrhotic HCC patients; in addition, the number of patients involved in our study, specifically patients with microscopic vascular invasion, was relatively small. Therefore, further studies are needed to confirm these findings. In spite of these limitations, we found some interesting factors that may help to better understand the nature of progression of the disease, which should be specially focused on and further testified in the future cohort study.

In conclusion, aside from characteristics of the tumor itself, the presence of ascites is independently associated with vascular invasion in HBV-related cirrhotic HCC patients, whereas hepatitis B viral factors might be less relevant. In patients with HCC resulting from HBV-related cirrhosis and who are found to have ascites, an increased risk of vascular invasion should be recognized. Surveillance should be performed on a more frequent basis during and after curative treatment such as liver transplantation to monitor the potential development of recurrence.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90. doi:10.3322/caac.20107.
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64:9–29. doi:10.3322/caac.21208.
3. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132:2557–76. doi:10.1053/j.gastro.2007.04.061.
4. Guan YS, Ahmad Al-Shatouri M, He Q, Liu WM. Hepatocellular carcinoma: carcinogenesis, establishment, progression, and therapies. Biomed Res Int. 2014;2014:706142. doi:10.1155/2014/706142.
5. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127:S35–50.
6. Anzola M. Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat. 2004;11:383–93. doi:10.1111/j.1365-2893.2004.00521.x.
7. Ishikawa T, Ichida T, Yamagiwa S, Sugahara S, Uehara K, Okoshi S, et al. High viral loads, serum alanine aminotransferase and gender are predictive factors for the development of hepatocellular carcinoma from viral compensated liver cirrhosis. J Gastroenterol Hepatol. 2001;16:1274–81.
8. Wei L. Natural history of chronic hepatitis B virus infection: what determines prognosis after cirrhotic decompensation? J Gastroenterol Hepatol. 2008;23:1631–2. doi:10.1111/j.1440-1746.2008.05576.x.
9. Fattovich G. Natural history and prognosis of hepatitis B. Semin Liver Dis. 2003;23:47–58. doi:10.1055/s-2003-37590.
10. Jonas S, Bechstein WO, Steinmuller T, Herrmann M, Radke C, Berg T, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. Hepatology. 2001;33:1080–6. doi:10.1053/jhep.2001.23561.
11. Senkerikova R, Frankova S, Sperl J, Oliverius M, Kieslichova E, Filipova H, et al. Incidental hepatocellular carcinoma: risk factors and long-term outcome after liver transplantation. Transplant Proc. 2014;46:1426–9. doi:10.1016/j.transproceed.2014.03.010.
12. Hsieh CH, Wei CK, Yin WY, Chang CM, Tsai SJ, Wang LY, et al. Vascular invasion affects survival in early hepatocellular carcinoma. Mol Clin Oncol. 2015;3:252–6. doi:10.3892/mco.2014.420.
13. Sumie S, Nakashima O, Okuda K, Kuromatsu R, Kawaguchi A, Nakano M, et al. The significance of classifying microvascular invasion in patients with hepatocellular carcinoma. Ann Surg Oncol. 2014;21:1002–9. doi:10.1245/s10434-013-3376-9.
14. Figueras J, Ibanez L, Ramos E, Jaurrieta E, Ortiz-de-Urbina J, Pardo F, et al. Selection criteria for liver transplantation in early-stage hepatocellular carcinoma with cirrhosis: results of a multicenter study. Liver Transpl. 2001;7:877–83. doi:10.1053/jlts.2001.27856.
15. Pawlik TM, Delman KA, Vauthey JN, Nagorney DM, Ng IO, Ikai I, et al. Tumor size predicts vascular invasion and histologic grade: implications for selection of surgical treatment for hepatocellular carcinoma. Liver Transpl. 2005;11:1086–92. doi:10.1002/lt.20472.
16. Shirabe K, Toshima T, Kimura K, Yamashita Y, Ikeda T, Ikegami T, et al. New scoring system for prediction of microvascular invasion in patients with hepatocellular carcinoma. Liver Int. 2014;34:937–41. doi:10.1111/liv.12459.
17. Suh SW, Lee KW, Lee JM, You T, Choi Y, Kim H, et al. Prediction of aggressiveness in early-stage hepatocellular carcinoma for selection of surgical resection. J Hepatol. 2014;60:1219–24. doi:10.1016/j.jhep.2014.01.027.
18. Kim BK, Han KH, Park YN, Park MS, Kim KS, Choi JS, et al. Prediction of microvascular invasion before curative resection of hepatocellular carcinoma. J Surg Oncol. 2008;97:246–52. doi:10.1002/jso.20953.
19. Esnaola NF, Lauwers GY, Mirza NQ, Nagorney DM, Doherty D, Ikai I, et al. Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation. J Gastrointest Surg. 2002;6:224–32. discussion 232.
20. Hsu C-Y, Lee Y-H, Huang Y-H, Hsia C-Y, Su C-W, Lin H-C, et al. Ascites in patients with hepatocellular carcinoma: prevalence, associated factors, prognostic impact, and staging strategy. Hepatol Int. 2013;7:188–98. doi:10.1007/s12072-011-9338-z.
21. Chen LP, Li C, Wang C, Wen TF, Yan LN, Li B. Risk factors of ascites after hepatectomy for patients with hepatocellular carcinoma

and hepatitis B virus-associated cirrhosis. Hepatogastroenterology. 2012;59:292–5. doi:10.5754/hge11399.
22. Lee SS, Shin HS, Kim HJ, Lee SJ, Lee HS, Hyun KH, et al. Analysis of prognostic factors and 5-year survival rate in patients with hepatocellular carcinoma: a single-center experience. Korean J Hepatol. 2012;18:48–55. doi:10.3350/kjhep.2012.18.1.48.
23. Lencioni R, Bartolozzi C, Caramella D, Paolicchi A, Carrai M, Maltinti G, et al. Treatment of small hepatocellular carcinoma with percutaneous ethanol injection. Analysis of prognostic factors in 105 Western patients. Cancer. 1995;76:1737–46.
24. Savastano S, Miotto D, Casarrubea G, Teso S, Chiesura-Corona M, Feltrin GP. Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with Child's grade A or B cirrhosis: a multivariate analysis of prognostic factors. J Clin Gastroenterol. 1999;28:334–40.
25. Dvorchik I, Carr BI. A simple prognostic scoring system for patients with unresectable hepatocellular carcinoma treated by chemoembolization. Cancer Detect Prev. 2007;31:154–60. doi:10.1016/j.cdp.2007.02.003.
26. Ikai I, Hatano E, Hasegawa S, Fujii H, Taura K, Uyama N, et al. Prognostic index for patients with hepatocellular carcinoma combined with tumor thrombosis in the major portal vein. J Am Coll Surg. 2006;202:431–8. doi:10.1016/j.jamcollsurg.2005.11.012.
27. Sun HC, Zhang W, Qin LX, Zhang BH, Ye QH, Wang L, et al. Positive serum hepatitis B e antigen is associated with higher risk of early recurrence and poorer survival in patients after curative resection of hepatitis B-related hepatocellular carcinoma. J Hepatol. 2007;47:684–90. doi:10.1016/j.jhep.2007.06.019.
28. Kim WR, Gores GJ. Recurrent hepatocellular carcinoma: it's the virus. J Clin Oncol. 2013;31:3621–2. doi:10.1200/JCO.2013.51.8381.
29. Jang JW, Choi JY, Bae SH, Yoon SK, Woo HY, Chang UI, et al. The impact of hepatitis B viral load on recurrence after complete necrosis in patients with hepatocellular carcinoma who receive transarterial chemolipiodolization: implications for viral suppression to reduce the risk of cancer recurrence. Cancer. 2007;110:1760–7. doi:10.1002/cncr.22984.
30. Urata Y, Kubo S, Takemura S, Uenishi T, Kodai S, Shinkawa H, et al. Effects of antiviral therapy on long-term outcome after liver resection for hepatitis B virus-related hepatocellular carcinoma. J Hepatobiliary Pancreat Sci. 2012;19:685–96. doi:10.1007/s00534-011-0489-z.
31. DiMaio CJ, Krishnan S, Roayaie S. EUS-guided ethanol ablation for management of metastatic hepatocellular carcinoma. J Interv Gastroenterol. 2014;4(1):13–14. doi:10.7178/jig.138.
32. Chong CC, Wong GL, Lai PB. Impact of antiviral therapy on post-hepatectomy outcome for hepatitis B-related hepatocellular carcinoma. World J Gastroenterol. 2014;20:6006–12. doi:10.3748/wjg.v20.i20.6006.
33. Verslype C, Rosmorduc O, Rougier P, Group EGW. Hepatocellular carcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23 Suppl 7:vii41–8. doi:10.1093/annonc/mds225.
34. Shyamala K, Girish HC, Murgod S. Risk of tumor cell seeding through biopsy and aspiration cytology. J Int Soc Prev Community Dent. 2014;4:5–11. doi:10.4103/2231-0762.129446.
35. Supriya M, Denholm S, Palmer T. Seeding of tumor cells after fine needle aspiration cytology in benign parotid tumor: a case report and literature review. Laryngoscope. 2008;118:263–5. doi:10.1097/MLG.0b013e318158f718.
36. Huang C, Sheng Y, Jia J, Chen L. Identification of melanoma biomarkers based on network modules by integrating the human signaling network with microarrays. J Cancer Res Ther. 2014;10 Suppl:C114–124. doi:10.4103/0973-1482.145816.
37. Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. Ann Surg. 2007;245:435–42. doi:10.1097/01.sla.0000250420.73854.ad.
38. Stigliano R, Burroughs AK. Should we biopsy each liver mass suspicious for HCC before liver transplantation?–no, please don't. J Hepatol. 2005;43:563–8. doi:10.1016/j.jhep.2005.07.015.
39. Malek NP, Schmidt S, Huber P, Manns MP, Greten TF. The diagnosis and treatment of hepatocellular carcinoma. Dtsch Arztebl Int. 2014;111:101–6. doi:10.3238/arztebl.2014.0101.
40. Fitzmorris P, Shoreibah M, Anand BS, Singal AK. Management of hepatocellular carcinoma. J Cancer Res Clin Oncol. 2014. doi:10.1007/s00432-014-1806-0.
41. Sotiropoulos GC, Tagkalos E, Fouzas I, Vernadakis S, Mathe Z, Treckmann J, et al. Liver transplantation for hepatocellular carcinoma using extended criteria donor grafts. Transplant Proc. 2012;44:2730–3. doi:10.1016/j.transproceed.2012.09.021.
42. Yao FY, Ferrell L, Bass NM, Bacchetti P, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: comparison of the proposed UCSF criteria with the Milan criteria and the Pittsburgh modified TNM criteria. Liver Transpl. 2002;8:765–74. doi:10.1053/jlts.2002.34892.
43. You Z, Chen LP, Ye H. Predictors of microvascular invasion in patients with solitary small hepatitis B related hepatocellular carcinoma. Pak J Med Sci. 2014;30:331–4.
44. Lim KC, Chow PK, Allen JC, Chia GS, Lim M, Cheow PC, et al. Microvascular invasion is a better predictor of tumor recurrence and overall survival following surgical resection for hepatocellular carcinoma compared to the Milan criteria. Ann Surg. 2011;254:108–13. doi:10.1097/SLA.0b013e31821ad884.
45. Thuluvath PJ. Vascular invasion is the most important predictor of survival in HCC, but how do we find it? J Clin Gastroenterol. 2009;43:101–2. doi:10.1097/MCG.0b013e318191e64f.
46. McHugh PP, Gilbert J, Vera S, Koch A, Ranjan D, Gedaly R. Alpha-fetoprotein and tumour size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. HPB (Oxford). 2010;12:56–61. doi:10.1111/j.1477-2574.2009.00128.x.
47. Kuiper JJ, de Man RA, van Buuren HR. Review article: management of ascites and associated complications in patients with cirrhosis. Aliment Pharmacol Ther. 2007;26 Suppl 2:183–93. doi:10.1111/j.1365-2036.2007.03482.x.
48. Nguyen VX, Nguyen CC, De Petris G, Sharma VK, Das A. Confocal endomicroscopy (CEM) improves efficiency of Barrett surveillance. J Interv Gastroenterol. 2012;2(2):61-65. doi:10.4161/jig.22175.
49. Lee YH, Hsu CY, Huang YH, Hsia CY, Chiou YY, Su CW, et al. Vascular invasion in hepatocellular carcinoma: prevalence, determinants and prognostic impact. J Clin Gastroenterol. 2014;48:734–41. doi:10.1097/MCG.0b013e3182a8a254.
50. Xu J, Shi J, Wang YP, Lin Y, Chen YX, Lu J, et al. Milder liver cirrhosis and loss of serum HBeAg do not imply lower risk for hepatocellular carcinoma development in HBV-related cirrhosis. Med Sci Monit. 2009;15:Cr274–9.
51. Kim HY, Park JW, Joo J, Kim H, Woo SM, Lee WJ, et al. Worse outcome of sorafenib therapy associated with ascites and Child-Pugh score in advanced hepatocellular carcinoma. J Gastroenterol Hepatol. 2013;28:1756–61. doi:10.1111/jgh.12310.
52. Chan KM, Lee CF, Wu TJ, Chou HS, Yu MC, Lee WC, et al. Adverse outcomes in patients with postoperative ascites after liver resection for hepatocellular carcinoma. World J Surg. 2012;36:392–400. doi:10.1007/s00268-011-1367-1.
53. Hsu SJ, Huang HC. Management of ascites in patients with liver cirrhosis: recent evidence and controversies. J Chin Med Assoc. 2013;76:123–30. doi:10.1016/j.jcma.2012.11.005.
54. Gines P, Cardenas A. The management of ascites and hyponatremia in cirrhosis. Semin Liver Dis. 2008;28:43–58. doi:10.1055/s-2008-1040320.

55. Wang L, Liu X, Wang H, Wang S. Correlation of the expression of vascular endothelial growth factor and its receptors with microvessel density in ovarian cancer. Oncol Lett. 2013;6:175–80. doi:10.3892/ol.2013.1349.

56. Yabushita H, Shimazu M, Noguchi M, Kishida T, Narumiya H, Sawaguchi K, et al. Vascular endothelial growth factor activating matrix metalloproteinase in ascitic fluid during peritoneal dissemination of ovarian cancer. Oncol Rep. 2003;10:89–95.