The Matching Status Between Donor and Recipient Hepatitis B Seroepidemiology Makes a Difference in Liver Transplantation for Hepatocellular Carcinoma

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INTRODUCTION: **Antibody to hepatitis B core antigen (HBcAb) is known to be related with the prognosis for patients with hepatocellular carcinoma (HCC). This study aims to evaluate the prognostic capacity of HbcAb and other donor/recipient hepatitis B seroepidemiological indexes in transplantation for HCC.**

METHODS: **Based on the national liver transplant registry, we analyzed the prognostic capacity of HBcAb in liver transplantation for patients with HCC of different etiological backgrounds. The hepatitis B virus (HBV)-related HCC cohort was further studied regarding donor/recipient hepatitis B seroepidemiology, and then divided into a training cohort (n = 1,222) and a validation cohort (n = 611) to develop a pretransplant recurrence-risk predicting nomogram.**

RESULTS: **Positive HbcAb in recipients was related to an increased risk of post-transplant tumor recurrence in HBV-related (n = 1,833, $P = 0.007$), HCV-related (n = 79, $P = 0.037$), and non-B non-C HCC (n = 313, $P = 0.017$). In HBV-related HCC (n = 1,833), donor hepatitis B surface antigen (HbsAg) was also associated with post-transplant tumor recurrence ($P = 0.020$). Multivariate analysis showed that the matching status of recipient HbcAb and donor HbsAg (MSHB) was an independent prognostic factor ($P = 0.017$). HbcAb-positive recipients matched with HbsAg-positive donors displayed the worst post-transplant outcomes ($P < 0.001$). In the training cohort (n = 1,222), a risk-predicting nomogram was established based on α-fetoprotein, Milan criteria, and MSHB. The model showed excellent prognostic capacity and safely expanded Milan criteria in both training and validation cohorts ($P < 0.001$).**

DISCUSSION: **Positive HbcAb in recipients increases the risk of post-transplant tumor recurrence in HCC with different etiological backgrounds. The nomogram based on MSHB is effective in predicting tumor recurrence after transplantation for HBV-related HCC.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A259, http://links.lww.com/CTG/A260, http://links.lww.com/CTG/A261, http://links.lww.com/CTG/A262

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INTRODUCTION

Liver cancers are the fifth most prevalent malignancy worldwide, and the related mortality ranks the third (1). Among them, hepatocellular carcinoma (HCC) is the largest entity. China has the heaviest HCC burden, owing to the high prevalence of hepatitis B virus (HBV) infection. It is estimated that China accounts for around 55% of all newly diagnosed HCC cases and 45% of HCC-related mortality (2). Although the development of treatment techniques and anticancer drugs has improved its long-term survival, the overall prognosis remains poor (3). Liver transplantation is currently considered the most radical treatment option for selected patients with HCC, and Milan criteria are the golden candidate selection criteria to ensure excellent prognosis for patients with HCC (4). However, growing experience raised concerns that Milan criteria are rather restrictive and not precise enough for candidate selection (5).

HBV infection and replication are known to promote the carcinogenesis and progression of HCC. As a reflection of HBV

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infection status, hepatitis B seroepidemiology has been frequently studied for its role in the prediction of postoperative outcomes (6,7). Among them, antibody to hepatitis B core antigen (HbcAb) has always been attracting attention because it affects tumor recurrence in both HBV-related and non-HBV HCC (7,8). Still, the importance of hepatitis B seroepidemiology was often neglected when other predictors, such as tumor size or number, were introduced. The related information is very limited for HCC patients undergoing liver transplantation.

Meanwhile, with the expansion of marginal donor livers, hepatitis B surface antigen (HbsAg)-positive donor livers are generally considered safe for recipients with HBV-related end-stage diseases (9). The use of HbsAg-positive donor livers in transplantation increased the heterogeneity among the recipients regarding hepatitis B seroepidemiology. In this study, we first studied the role of recipient HbcAb in post-transplant recurrence of HCC of different etiological backgrounds. Specifically for the 1,833 HBV-related patients with HCC undergoing transplantation, we analyzed the prognostic capacity of donor-recipient matching status in hepatitis B seroepidemiology and established a novel risk-predicting nomogram with excellent prognostic capacity.

METHODS

Patient selection and data collection

We gratefully acknowledge the China Liver Transplant Registry (CLTR) and the contributing transplant centers from Mainland China. All the study cohorts were extracted from the CLTR database (from January 1, 2015, to July 31, 2018). After excluding the following cases: (i) patients with preoperative sign of extrahepatic or macrovascular invasion, (ii) patients who died within 1 month after transplantation, (iii) patients with the lack of essential data, (iv) patients with the follow-up length less than 6 months and without recurrence, and (v) child liver transplantation (<18 years old) or retransplantation, a total of 1,833 HBV-related patients with HCC were enrolled for the analysis. All of the recipients were HbsAg positive. Among them, 1,646 (89.8%) were men. The average age was 51.5 years (ranging from 19 to 77 years old). The endpoint of the follow-up was January 31, 2019. The average follow-up length was 19.4 months. The prophylaxis of HBV reinfection was routinely performed using hepatitis B immunoglobulin and antivirals (entecavir/tenofovir) unless matched with HbsAg-positive donors.

A cohort of 79 HCV-related HCC recipients was also enrolled from January 1, 2015, to July 31, 2018. The exclusion criteria were the same as above. One case with HBV coinfection was also excluded. Sixty-five (82.3%) among them are men. The average age was 54.4 years, ranging from 40 to 67 years old. The endpoint of the follow-up was January 31, 2019. The average follow-up length was 19.7 months.

Anti-HBV therapy with nucleotide analog or anti-HCV therapy was routinely performed for the patients on the diagnosis of HBV/HCV before transplantation. However, some patients are not aware of HBV/HCV infection until the very late stage of liver diseases, so they will not have sufficient antivirus treatments before transplantation.

Meanwhile, another cohort of 313 non-B non-C patients with HCC was enrolled from January 1, 2015, to July 31, 2018. The exclusion criteria were the same as above. Non-B non-C HCC is defined as those negative for both markers of HBV and hepatitis C virus infection. The average age was 55.4 years old, ranging from 24 to 78 years. Among them, 264 (84.3%) were men. Thirteen patients had autoimmune hepatitis, and 113 had primary biliary cholangitis. The endpoint of the follow-up was January 31, 2019. The average follow-up length was 19.7 months.

The HBV-related cohort (n = 1,833) was then specifically analyzed using univariate and multivariate methods. The matching status between the donor and recipient hepatitis B seroepidemiology was also analyzed for its prognostic capacity. The 1,833 patients were further randomly divided into a training cohort (n = 1,222) and a validation cohort (n = 611) according to a 2:1 ratio to develop and validate a prognostic nomogram.

The procedures of this study were in accordance with the Declaration of Helsinki. All livers were acquired from the deceased donors strictly according to the guidelines of the China donation after citizen's death. No donor organs were obtained from executed prisoners or other institutionalized persons. The study was approved by CLTR, the only national registry in Mainland China, according to the Regulations on Human Organ Transplant and national legal requirements. Patient consents were obtained. The study protocol was also approved by the Human Ethics Committee of First Affiliated Hospital, Zhejiang Univeristy School of Medicine.

Pretransplant factors that may potentially be related to recurrence were selected in this study, including age, gender, cirrhosis, tumor size (cm), number of tumor nodules, pretransplant hepatectomy, transcatheter arterial chemoembolization (TACE), or radiofrequency ablation. Preoperative laboratory values of serum albumin (ALB, g/mL), α-fetoprotein (AFP, ng/mL), donor and recipient hepatitis B seroepidemiology, and total bilirubin (TB, umol/L) were also included in the analysis. Hepatitis B seroepidemiology includes HbsAg, antibody to hepatitis B surface antigen (HbsAb), hepatitis B e-antigen (HbeAg), antibody to hepatitis B e-antigen (HbeAb), and HbcAb. The follow-up survey includes patient death and tumor recurrence.

Statistical analysis

The χ^2 test was used for categorical variables, and the Student *t* test was used for continuous variables. The Kaplan–Meier method was used for survival, and recurrence-risk analysis was performed by using the the log-rank test. Overall survival (OS) was calculated from the date of transplant to the date of death. The recurrence-free survival was calculated from the date of transplant to the date when tumor recurrence was diagnosed. If recurrence was not diagnosed, the cases were censored at the date of death or the last date of follow-up. The Cox proportional hazards regression (backward stepwise) was used to determine the independent factors on survival and recurrence. The RMS package by *R* was used to establish a risk-predicting nomogram. We used the Area Under the Receiver Operating Characteristic curve (AUROC) and Harrell concordance index (c-index) to evaluate the efficiency of the nomogram. The other statistical analyses were performed by SPSS 19.0 (SPSS, Chicago, IL). A *P* value below 0.05 was considered statistically significant.

RESULTS

Recipient HbcAb predicted post-transplant tumor recurrence in HCC of different etiological backgrounds

In the 1,833 HBV-related HCC recipients, HbcAb was positive in 94.0% cases and was significantly associated with an increased risk of post-transplant tumor recurrence (Figure 1a, $P = 0.007$), but not OS (see Figure 1a, $P = 0.230$, Supplementary Digital Content 1, http://links.lww.com/CTG/A259).

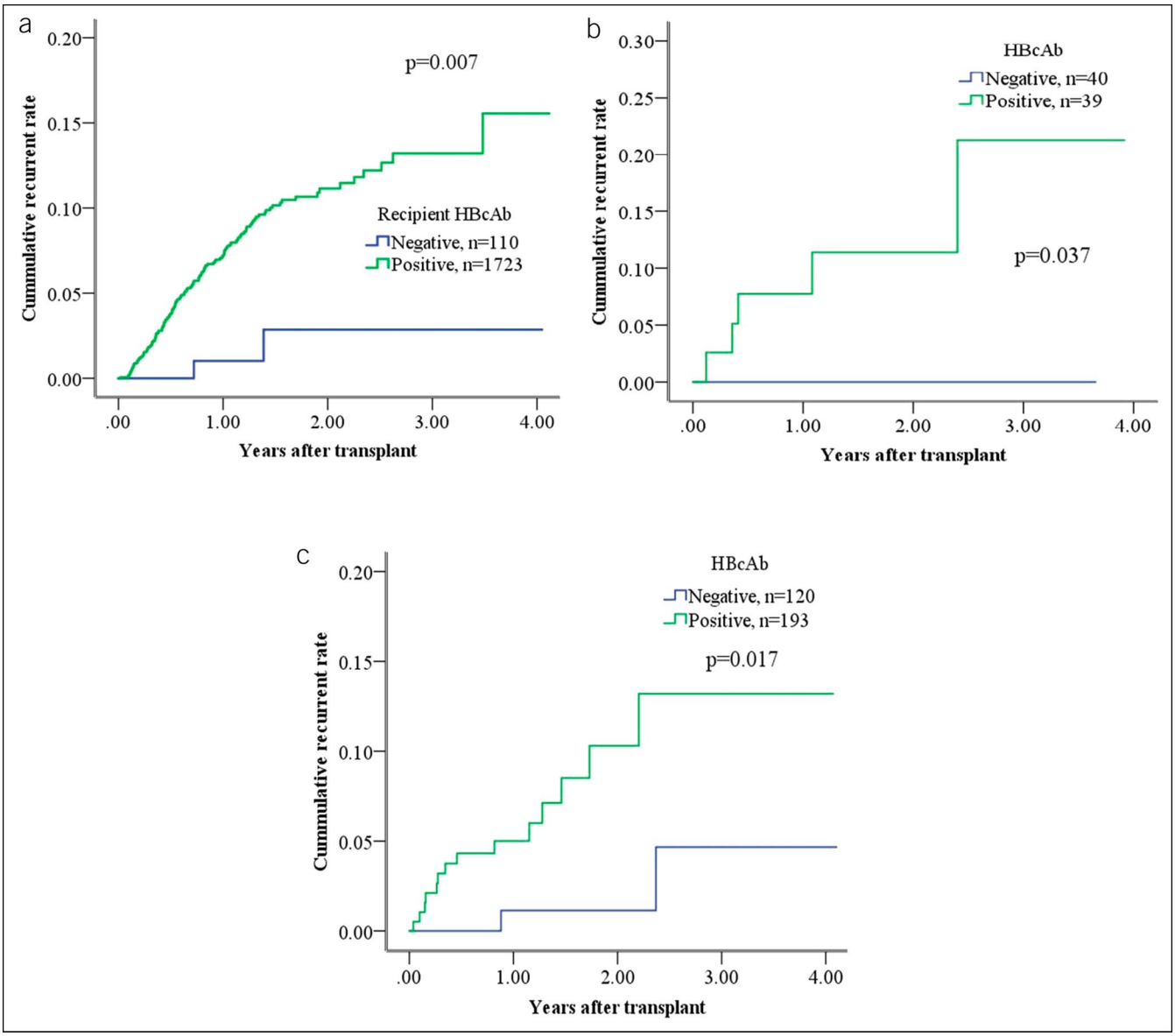

Figure 1. The recurrence-predicting capacity of HbcAb in liver transplantation for HCC of different etiological backgrounds. (**a**) In liver transplantation for HBV-related HCC (n = 1,833), positive HbcAb in recipients was significantly associated with an increased risk of post-transplant tumor recurrence (P = 0.007). (**b** and **c**) Positive HbcAb in recipients increased the recurrence risk in both HCV-related HCC (n = 79, P = 0.037) and non-B non-C HCC (n = 313, P = 0.017). HbcAb, antibody to hepatitis B core antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

To study the prognostic capacity of HbcAb in HCC of other etiological backgrounds, we also enrolled 79 HCV-related recipients and 313 non-B non-C patients with HCC. Survival analysis also showed that positive HbcAb increased the recurrence risk in both HCV-related HCC (P = 0.037, Figure 1b) and non-B non-C HCC (P = 0.017, Figure 1c). However, recipient HbcAb was not related to OS in both HCV-related HCC and non-B non-C HCC (see Figure 1b and 1c, Supplementary Digital Content 1, http://links.lww.com/CTG/A259).

The matching state of recipient HbcAb and donor HbsAg-predicted tumor recurrence after liver transplantation for HBV-related HCC

HBV-related HCC cohort (n = 1,833) was further analyzed. Recipient HbcAb significantly correlated with AFP, ALB, TB, MELD, HbsAb, and HbeAb (Table 1). Interestingly, we found that recipients with positive HBcAb had lower TB level and MELD score, but higher ALB and AFP levels ($P < 0.01$, see Figure 1d, Supplementary Digital Content 1, http://links.lww.com/CTG/A259). Univariate analysis further found pretransplant TACE, AFP, ALB, tumor size, and tumor number as significant predictors of tumor recurrence (Table 1). Meanwhile, although part of the cases had missing information in HbsAb, HbeAg, and HbeAb state, survival analysis based on the existing data showed that they were not related to tumor recurrence (Table 1).

On the other hand, HbsAb was positive in 118 of the 1,833 donors (6.4%). We also found that donor HbsAg was related to tumor recurrence (P = 0.020, Figure 2a), but not OS (see

Table 1. Univariate survival analysis of recipient demographic, clinical features and their association with HbcAb

	n	HbcAb positive	χ^2	3-year RFS	Kaplan–Meier
Gender					0.466
Male	1,646	93.7%	0.090	87.1%	
Female	187	96.8%		90.9%	
Age, yrs					0.466
≤50	767	94.0%	0.995	88.0%	
>50	1,066	94.0%		87.2%	
Pretransplant hepatectomy					0.080
None	1,524	94.2%	0.519	88.0%	
Yes	309	93.2%		85.0%	
Pretransplant TACE					<0.001
None	1,271	93.8%	0.561	88.7%	
Yes	562	94.5%		84.6%	
Pretransplant RFA					0.958
None	1,515	93.9%	0.778	87.1%	
Yes	318	94.3%		88.8%	
Alpha-fetoprotein, ng/mL					<0.001
≤100	1,172	93.1%	0.029	91.4%	
>100	661	95.6%		80.2%	
Albumin, g/L					0.021
≥35	974	95.7%	0.001	90.6%	
<35	895	92.1%		85.1%	
Total bilirubin, μmol/L					0.074
≤50	1,027	95.4%	<0.001	85.7%	
>50	806	92.2%		90.1%	
MELD					0.057
≤26	1,327	95.5%	<0.001	85.9%	
>26	506	90.1%		92.1%	
Tumor number					<0.001
Single	1,055	93.4%	0.183	89.6%	
Multifocal	778	94.9%		84.6%	
Tumor size, cm					<0.001
≤5	1,404	93.9%	0.863	90.8%	
>5	429	94.2%		75.5%	
Liver cirrhosis					0.534
No	230	95.7%	0.259	87.8%	
Yes	1,603	93.8%		87.4%	
Body mass index, kg/m^2					0.189
≤25	1,290	94.2%	0.603	85.8%	
>25	543	93.6%		91.3%	
Milan criteria					<0.001
Inside	1,053	93.4%	0.248	93.9%	
Outside	780	94.7%		78.4%	
HbsAb[a]					0.236
Negative	1,705	94.3%	0.025	87.7%	

Table 1. (*continued*)

	n	HbcAb positive	χ^2	3-year RFS	Kaplan–Meier
Positive	87	88.5%		81.3%	
HbeAg[b]					0.504
Negative	1,297	95.1%	0.072	87.1%	
Positive	508	92.9%		88.7%	
HbeAb[c]					0.758
Negative	635	94.8%	0.005	85.6%	
Positive	852	97.5%		86.1%	

HbcAb, antibody to hepatitis B core antigen; HbeAb, antibody to hepatitis B e-antigen; HbeAg, hepatitis B e-antigen; HbsAb, antibody to hepatitis B surface antigen; HbsAg, hepatitis B surface antigen; MELD, model for end-stage liver disease; RFA, radiofrequency ablation; RFS, recurrence-free survival; TACE, transcatheter arterial chemoembolization.
[a]Excluding the 1 case without HbsAb information.
[b]Excluding the 28 cases without HbeAg information.
[c]Excluding the 346 cases without HbeAb information.

Figure 2a, $P = 0.150$, Supplementary Digital Content 2, http://links.lww.com/CTG/A260), whereas donor HbcAb was not ($P = 0.873$, 8 case were excluded because of missing data).

By entering recipient HbcAb and donor HbsAg into cox regression, we found that both of them were the risk factors for tumor recurrence ($P = 0.018$ and $P = 0.026$, Table 2). We thereby combined them into a new index, the matching status between recipient HbcAb and donor HbsAg (MSHB). According to MSHB, we further classified the patients into 3 groups, that is, subtype A (recipient HbcAb negative), subtype B (recipient HbcAb positive and donor HbsAg negative), and subtype C (recipient HbcAb positive and donor HbsAg positive). Survival analysis showed significant differences in between the 3 subtypes, with a 3-year recurrence-free survival rate of 97.1%, 87.2%, and 81.3%, respectively (Figure 2b, $P < 0.001$). OS was not different among the 3 subtypes (see Figure 2b, $P = 0.185$, Supplementary Digital Content 2, http://links.lww.com/CTG/A260).

According to the results of univariate analysis shown above, pretransplant TACE, AFP, ALB, tumor size, tumor number, and MSHB were further entered into multivariate Cox regression. Pretransplant TACE, AFP, tumor size, tumor number, and MSHB were found to be independent risk factors for post-transplant tumor recurrence (Figure 2c).

A novel recurrence-risk predicting nomogram in liver transplantation for HBV-related HCC

In the HBV-related HCC cohort of 1,833 cases, the Milan criteria showed excellent predicting capacity regarding tumor recurrence and OS (see Figure 3a and 3b, $P < 0.001$, Supplementary Digital Content 3, http://links.lww.com/CTG/A261). In those 1,053 patients fulfilling the Milan criteria, only 40 had tumor recurrence and the 3-year tumor-free survival rate was 93.9%. However, in the 780 patients exceeding the Milan criteria, only 122 had recurrence, whereas 658 did not, and the 3-year recurrence-free survival rate was 78.4%.

We then randomly divided the patients into a training cohort (n = 1,222) and a validation cohort (n = 611) according to a 2:1 ratio. In the training cohort (n = 1,222), pretransplant AFP, Milan criteria, and MSHB were the independent risk factors for tumor recurrence. A risk-predicting nomogram was then established (Figure 3a). The AUROC value was 0.748, and the calibration curve showed a good correlation between the predicted and actual 3-year recurrence-free survival rates, and the c-index was 0.754 (Figure 3b). The patients in the training cohort were classified into the low-risk group (n = 852) and the high-risk group (n = 370) according to the optimal cutoff value of 2.24 by Youden index. The 2 groups had significantly different post-transplant outcomes, with the 3-year recurrence-free survival rate of 93.9% and 71.5%, respectively ($P < 0.001$, Figure 3c). The OS was also significantly different ($P < 0.001$, see Figure 4a, Supplementary Digital Content 4, http://links.lww.com/CTG/A262). The 159 patients exceeding the Milan criteria but in the low-risk group had acceptable outcomes comparable with those 705 patients inside the Milan criteria ($P = 0.118$, Figure 3d and see Figure 4b, Supplementary Digital Content 4, http://links.lww.com/CTG/A262). The 3-year recurrence-free survival rate was 92.5% and 94.0%, respectively.

In the validation cohort, the nomogram also showed excellent prognostic capacity, with an AUROC value of 0.706 (Figure 4a). The calibration curve showed a good correlation between the predicted and actual 3-year recurrence-free survival rates, and the c-index was 0.706 (Figure 4b). The low-risk group (n = 423) showed significantly decreased recurrence risk compared with the high-risk group (n = 188), with a 3-year recurrence-free survival rate of 93.3% and 74.4%, respectively ($P < 0.001$, Figure 4c). The OS was also significantly different ($P < 0.001$, see Figure 4c, Supplementary Digital Content 4, http://links.lww.com/CTG/A262). The 80 patients exceeding the Milan criteria but in the low-risk group also had acceptable outcomes, comparable with those 348 patients inside the Milan criteria ($P = 0.363$, Figure 4d and see Figure 4d, Supplementary Digital Content 4, http://links.lww.com/CTG/A262). The 3-year recurrence-free survival rate was 90.8% and 93.8%, respectively.

DISCUSSION

HbcAb is a classical serological marker for HBV infection and is routinely detected in patients with end-stage liver diseases (10). Positive HbcAb is considered to be an indicator of either past or existing HBV infection, typically with lifelong persistence (11). Occult HBV infection, that is, HbsAg negative but HbcAb positive, has been reported to increase the risk of HCC in patients with HCV-related liver cirrhosis and in patients with non-B non-C cirrhosis (12,13). Early in 1984, Sjogren et al. (14) found that the presence of HbcAb indicates high risks for the development of HBV-related HCC.

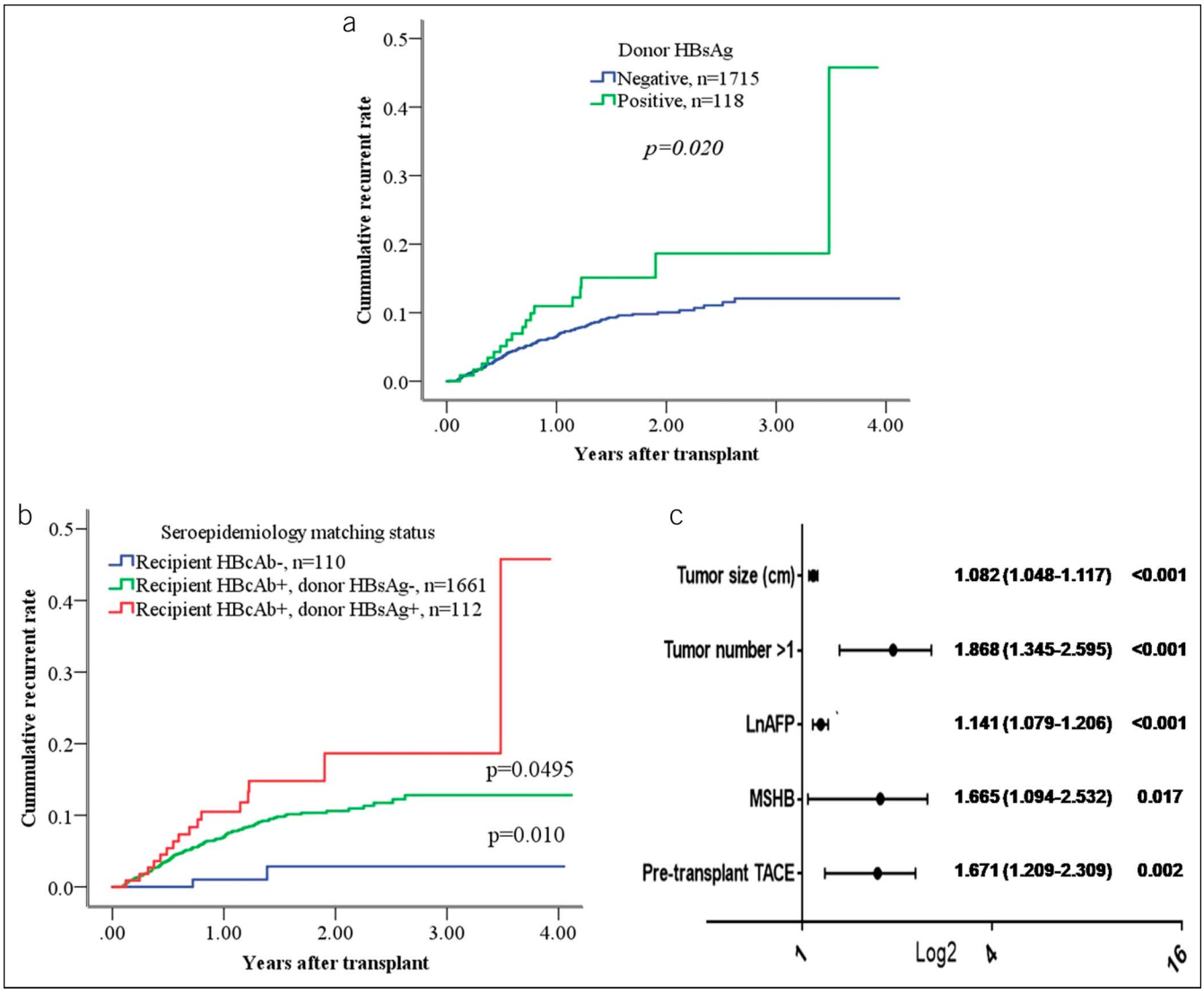

Figure 2. The recurrence-predicting capacity of donor/recipient hepatitis B seroepidemiology in liver transplantation for HBV-related HCC. (**a**) In liver transplantation for HBV-related HCC (n = 1,833), positive HbsAg in donors was related to an increased risk of post-transplant tumor recurrence (P = 0.020). (**b** and **c**) In liver transplantation for HBV-related HCC (n = 1,833), the MSHB was associated with post-transplant tumor recurrence ($P < 0.001$), and was an independent prognostic factor by cox regression. HbcAb, antibody to hepatitis B core antigen; HbsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MSHB, matching status of recipient HbcAb and donor HbsAg.

Moreover, it has frequently been reported to affect the treatment outcomes of HCC. Li et al. (7) found that positive HBcAb was associated with a higher risk of early recurrence and poorer survival after curative resection in HBV-related HCC. For other etiological backgrounds, that is, HCV-related or non-B non-C HCC, the role of HbcAb is in controversy. Okamura et al. (8) reported that positive HBcAb is rather a favorable predictor for outcomes after curative resection in non-B non-C HCC, whereas Itoh et al. (15) found no correlation between HbcAb and tumor recurrence in non-B non-C HCC and HCV-related HCC. On the other hand, there are also studies reporting positive HbcAb as a risk factor for postoperative tumor recurrence in non-B non-C HCC (16–18). In this present study, we found that recipient HbcAb was a potent predictor for tumor recurrence after liver transplantation in HBV-related, HCV-related, and non-B non-C HCC. Given the high tumor recurrence risk, those HbcAb-positive recipients with HCC should have a close follow-up and may need enhanced antitumor recurrence therapy after transplantation.

Table 2. Multivariate analysis including recipient HBcAb and donor HBsAg for tumor recurrence after liver transplantation

Variables	B	Exp (B)	95% CI, *df*	*P* value
Recipient HBcAb	1.68	5.4	1.31–21.8	0.018
Donor HBsAg	0.57	1.8	1.07–2.93	0.026

CI, confidence interval; HBcAb, antibody to hepatitis B core antigen; HBsAg, hepatitis B surface antigen.

However, the above-mentioned studies were performed based on patients with HCC undergoing liver resection, in which the remnant liver with HBV-DNA integration may provide favorable "soil" for recurrence. Interestingly, we found in this study that recipient HbcAb,

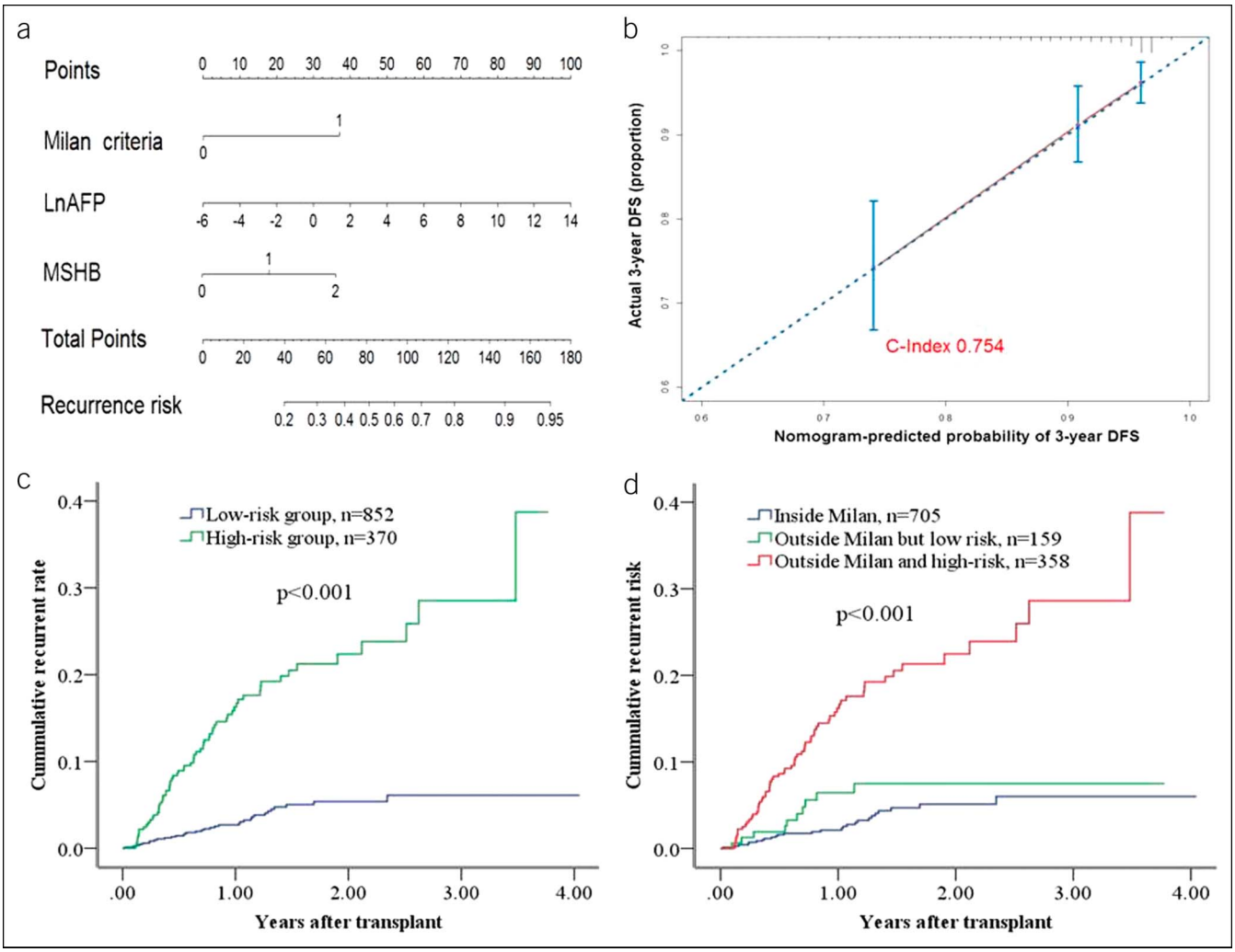

Figure 3. The nomogram can effectively predict post-transplant tumor recurrence in the training cohort. (**a**) The nomogram predicting tumor recurrence after liver transplantation for HBV-related HCC. (**b**) The calibration curve showed a good correlation between predicted and actual 3-year recurrence-free survival rate for the established nomogram, and the c-index was 0.754. (**c**) The patients in the training cohort were then divided into the low-risk group (n = 852) and the high-risk group (n = 370) according to the nomogram, and the 2 groups had significantly different post-transplant recurrence risk ($P < 0.001$). (**d**) Those patients exceeding Milan criteria but in the low-risk group had acceptable outcomes comparable to those inside Milan criteria (3-year recurrence-free survival rates: 92.5% vs 94.0%, $P = 0.118$). HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

but not donor HbcAb, affects post-transplant tumor recurrence. The mechanism involved is still not clear. In liver transplantation, the solid tumor lesions are removed together with the whole HBV-infected liver. Therefore, the mechanism responsible for post-transplant tumor recurrence shall be more related to the invasiveness of the tumor cells with HBV-DNA integration. Actually, it is found that HBV covalently closed circular DNA level in tumor tissues, but not that in adjacent liver tissues, correlates with post-transplant tumor recurrence risk (19), also proving the key roles of infected tumor cells. Meanwhile, HbcAg is closely related to the hepatic covalently closed circular DNA level (20) and has the potential to regulate tumor cell biology (21–23). In our study, we can also see that positive HBcAb in the recipients is related to higher AFP levels, (see Figure 1d, Supplementary Digital Content 1, http://links.lww.com/CTG/A259), indicating higher malignancy. Therefore, HbcAb, produced as an antibody to HbcAg in immunological reaction, reflects a more invasive phenotype of HCC. In addition, we observed lower total bilirubin level and MELD score, and higher albumin level in those HBcAb-positive recipients (see Figure 1d, Supplementary Digital Content 1, http://links.lww.com/CTG/A259). Imazeki et al. (24) also found more conserved liver function in HBcAb-positive patients with HCC . It indicates that patients with positive HBcAb may develop HCC at an earlier stage of liver disease, also implicating the oncogenic potential. For donor livers with positive HBcAb, where HBV-DNA may have been integrated into the liver genomes during occult infection, we found that it is not related to post-transplant recurrence. A study from Hongkong also found that donor HBcAb status did not impact on the risk of HCC recurrence after liver transplantation (25). Therefore, we speculate that highly malignant phenotype of HCC in HBcAb-positive recipients and proinflammation effect of HBcAb may be the reasons for post-transplant tumor recurrence.

Although previous studies reported safety in the use of HbsAg-positive donor liver (26,27), long-term outcomes for HCC recipients have not been ensured. In this study, we found that positive donor HbsAg, but not HbcAb, is associated with an increased risk of tumor recurrence after liver transplantation for HBV-related HCC,

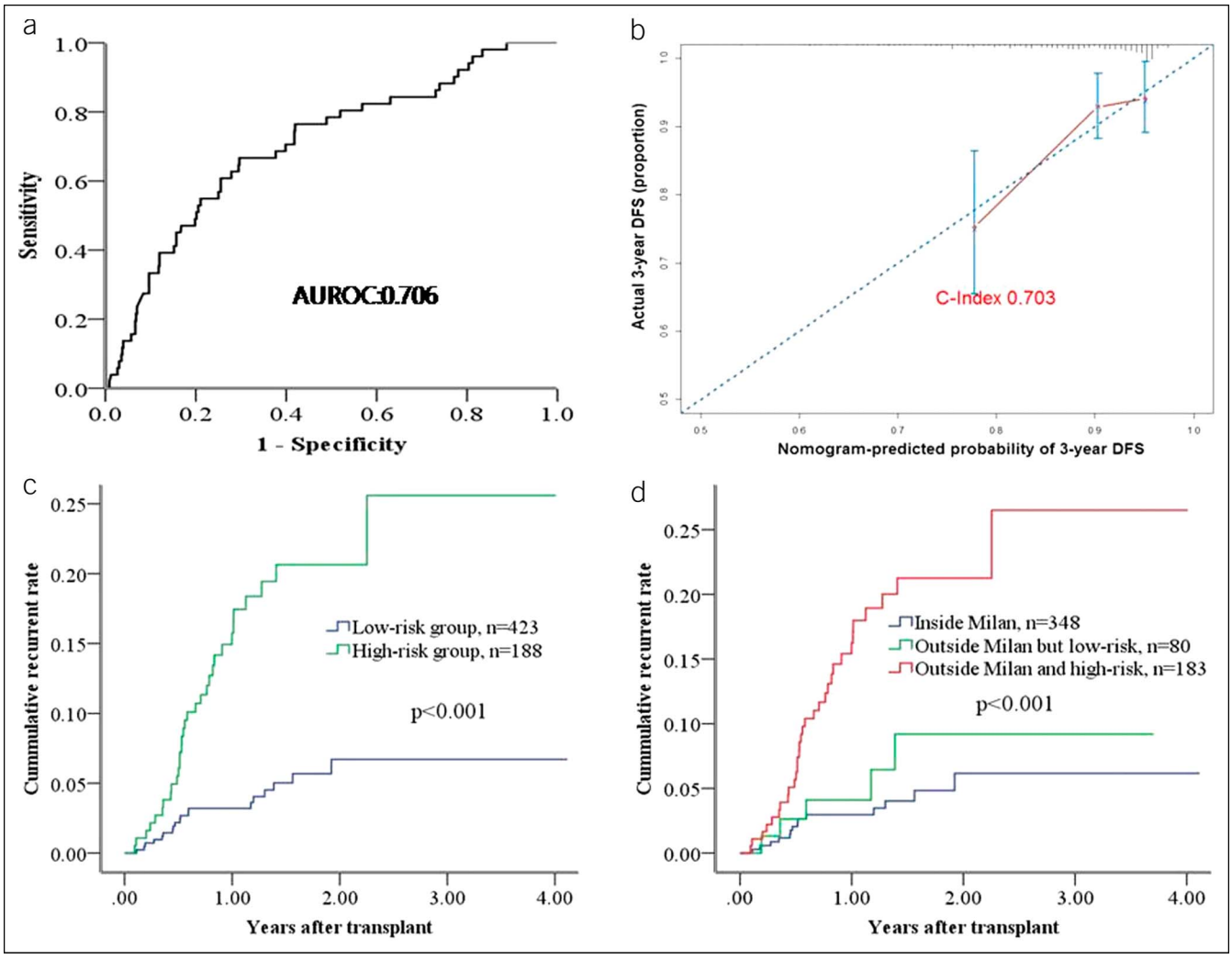

Figure 4. The nomogram showed excellent recurrence-predicting capacity in the validation cohort. (**a**) The ROC curve of the nomogram score. (**b**) The calibration curve showed a good correlation between predicted and actual 3-year recurrence-free survival rate, and the c-index was 0.706. (**c**) According to the nomogram, the low-risk group (n = 423) showed significantly decreased recurrence risk compared to the high-risk group (n = 188), with a 3-year tumor-free survival rate of 94.9% and 76.0% ($P < 0.001$). (**d**) Those patients exceeding Milan criteria but in the low-risk group had acceptable outcomes comparable to those inside Milan criteria (3-year recurrence-free survival rates: 90.8% vs 93.8%, $P = 0.363$). ROC, receiver operating characteristic curve.

especially in the HbcAb-positive recipients. Although there are no studies yet reporting the impact of HbsAg-positive donor on tumor recurrence, post-transplant HBV reinfection has already been known as a risk factor for tumor recurrence (28,29). HBsAg-positive donor livers means a persist status of post-transplant HBV infection. This will increase the incidence and severity of hepatic inflammation by HBV activation and replication, especially under the post-transplant immune suppression status, and eventually induce tumor recurrence. However, the allocation of HBsAg-positive donor livers is not a simple procedure. The patients will usually be more anxious and more severe in disease status so as agreeing to receive HBsAg-positive donor livers, which may also interfere with our results. Therefore, whether HBsAg-positive donor liver will cause tumor recurrence needs further randomized clinical trials. After all, we proposed a new concept concerning the matching status of recipient HbcAb and donor HbsAg (MSHB) and stratified the patients into 3 subsets as described above. Among them, HbcAb-positive recipients matched with HbsAg-positive donors had the highest risk of recurrence.

For hepatitis B seroepidemiology in liver transplantation, elevated HBV-DNA level is also known as a risk factor for post-transplant HCC recurrence (30) and should not be neglected. HBV-DNA replication may promote the process of post-transplant recurrence owing to its pro-oncogenic effects, as we mentioned above. Moreover, HBV-DNA level has a good correlation with the expression of HbcAg (31–33), which, in turn, corresponds to HbcAb. However, our data are from a national registry which includes over 80 centers with different HBV monitoring strategies for liver transplantation. Therefore, the information of HBV-DNA level is incomplete for our cohort. Further studies are needed to clarify whether HBV-DNA levels can substitute or surpass HbcAb in predicting post-transplant recurrence.

As the most frequently used candidate selection criteria for HCC recipients, Milan criteria have been challenged in numerous studies that aim to safely expand the candidate pool (34–36). However, most of them are limited to morphological features and have limited effects because the survival will decrease more or less compared with the Milan criteria. China has the largest HCC

population. Hepatectomy is usually the first surgical treatment option for small HCC patients (inside Milan criteria) with preserved liver function (37). Transplantations will thereby be performed on relatively advanced HCC in China. Therefore, much more transplant recipients will exceed the Milan criteria compared with the western countries, and different centers might use different criteria according to local policies. In our study, 780 patients exceeded Milan criteria, but 84.4% of them did not have recurrence. To improve the prognostic capacity of Milan criteria, we established a novel nomogram combing Milan criteria, pretransplant AFP, and MSHB in the training cohort. The nomogram had excellent prognostic capacity and safely expanded the candidate pool by a round 1/4 compared with Milan criteria. The validation cohort also proved the efficiency of our nomogram.

However, there are limitations in this study. Besides the HBV-DNA level that we mentioned above, it is a pity that some critical information for post-transplant tumor recurrence is also missing, for instance, des-gamma carboxy prothrombin (DCP). In 2007, Ito et al. proposed the Kyoto criteria, which defines the transplantable HCC patients as with tumor number ≤10, maximal diameter ≤5 cm, and serum DCP ≤400 mAU/mL (38). This set of criteria has been validated to be highly efficient in the selection of eligible HCC recipients (39), indicating the essentialness to include DCP in the pretransplant prediction of tumor. Actually, DCP has been proved to be a more efficient marker than AFP for early HCC and can be also be used as a potent biomarker of microvascular invasion (40), which is the key pathological event responsible for post-transplant tumor recurrence. Increasing evidences showed the usefulness of integrating AFP with DCP to overcome the weakness when used alone (41,42). However, not all liver transplant centers routinely detects DCP in China, we are currently not able to analyze the impact of DCP based on the present cohort. Further optimization of the Chinese liver transplant database is needed especially for those critical parameters such as DCP, and studies are needed to validate its impact nationwide in the future.

In conclusion, positive HbcAb in recipients increases the risk of post-transplant tumor recurrence in HBV-related, HCV-related and non-B non-C HCC. The MSHB can be an effective predictor for post-transplant tumor recurrence. The nomogram based on MSHB is effective predicting tumor recurrence after liver transplantation for HBV-related HCC.

CONFLICTS OF INTEREST

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Specific author contributions: Di Lu, PhD, Fan Yang, MD, and Jianyong Zhuo, MD, contributed equally to this work. Conceptualization: X.X. and S.Z.; Methodology: X.X. and D.L.; Formal Analysis: D.L., F.Y. and Z.L.: Investigation: F.Y., J.Z., M.Y. and B.C.; Data Curation: P.J., X.C.; Draft Preparation: J.W. and X.W.; Writing, Review & Editing: all authors. All the authors approved the final draft submitted.

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Study Highlights

WHAT IS KNOWN

- ✓ HBcAb is related with the outcomes of HCC.

WHAT IS NEW HERE

- ✓ Positive HbcAb in recipients increases post-transplant tumor recurrence risk in HCC of different etiological backgrounds.
- ✓ The matching status of recipient HbcAb and donor HbsAg predicts tumor recurrence after transplantation for HBV-related HCC.
- ✓ We developed and validated an effective pretransplant prognostic nomogram for HBV-related HCC.

TRANSLATIONAL IMPACT

- ✓ The matching Status between donor and recipient hepatitis B seroepidemiology can be used as an effective indicator for post-transplant tumor recurrence in patients with HBV-related HCC.

LIVER

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