Original Article

A scoring model based on plasma fibrinogen concentration for predicting recurrence of hepatocellular carcinoma after liver transplantation

Kai-Ning Zeng [a], Ying-Cai Zhang [b], Gen-Shu Wang [a], Jian Zhang [a], Yi-Nan Deng [a], Shi-Hui Li [a], Qi Zhang [a], Hua Li [a], Guo-Ying Wang [a, *], Yang Yang [b, **], Gui-Hua Chen [a]

[a] *Department of Hepatic Surgery and Liver Transplant Program of the Third Affiliated Hospital, Organ Transplantation Institute, Sun Yat-sen University; Organ Transplantation Research Center of Guangdong Province, Guangzhou, China*
[b] *Guangdong Key Laboratory of Liver Disease Research, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China*

ABSTRACT

Background: We investigated the prognostic value of preoperative fibrinogen levels in hepatocellular carcinoma patients receiving liver transplantation by building a scoring model for predicting tumor recurrence.
Methods: Cox regression analysis was used to identify factors that predicted tumor recurrence, and a scoring model was generated by assigning a value of 0 or 1 to each independent risk factor. The cut-off value for fibrinogen was determined by receiver operating characteristic curve analysis.
Results: Preoperative fibrinogen concentration was significantly higher in patients with *vs.* without tumor recurrence (3.27 g/L *vs.* 2.34 g/L, $P < 0.001$), with *vs.* without macrovascular invasion (3.54 g/L *vs.* 2.82 g/L, $P = 0.007$), and with >400 *vs.* ≤400 ng/mL plasma alpha-fetoprotein concentration (3.43 g/L *vs.* 2.76 g/L, $P = 0.007$). The 5-year disease-free survival rate was significantly lower for patients with elevated (≥2.68 g/L) *vs.* normal (<2.68 g/L) fibrinogen concentration (37.2% *vs.* 78.4%, $P = 0.001$). Macrovascular invasion, >3 tumor nodules, and elevated fibrinogen concentration were independent risk factors for tumor recurrence. A scoring model based on these risk factors predicted recurrence with a sensitivity of 68.3% and a specificity of 87.5%.
Conclusions: Elevated preoperative plasma fibrinogen concentration is associated with tumor recurrence in HCC patients after liver transplantation. A new scoring model predicted recurrence with good sensitivity and specificity.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor and the third leading cause of cancer-related deaths worldwide. An estimated 0.5 to 1 million new cases of HCC are diagnosed annually.[1] In Eastern Asia, hepatitis B virus (HBV) infection is the leading cause of liver cirrhosis and HCC; in China, nearly 80% of HCC cases are caused by HBV.[2,3] Since the first operation was conducted in the 1960s, orthotopic liver transplantation (LT) has been regarded as one of the best therapies for small, unresectable HCC with cirrhosis. Up to 40% of LT in China are performed for HCC.[4] However, prior to 1966, when the Milan criteria for LT were introduced, the long-term outcomes of LT for HCC were disappointing, with a 5-year survival rate of only 18—40%.[5] The Milan criteria, which restrict LT to patients with a single tumor nodule ≤5 cm or two to three nodules ≤3 cm, improved the outcome of LT for HCC significantly, and it has since been adopted by the United Network for Organ Sharing for allocation of organs for LT in the United States.

As experience of LT for HCC has increased, it has been suggested that HCC patients who would not qualify based on the Milan criteria may also benefit from LT.[6] Thus, several expanded criteria, such as the UCSF and Hangzhou criteria, have been proposed.[7,8] However, the implementation of expanded criteria for selection of HCC patients remains controversial, due partly to the increased approval of patients with high risk for vascular invasion and tumor recurrence and partly to the additional need for donor organs, thereby further extending waiting times for LT.[9] Despite improved surgical techniques and selection criteria, however, approximately 20% of patients undergoing LT for HCC develop recurrence within 5 years.[4] These observations highlight the urgent need to identify risk factors for HCC recurrence after LT and to establish a new model or scoring system for predicting prognosis. The results could be used to select HCC patients who would most benefit from LT.

Several studies have shown that plasma fibrinogen concentration correlates with tumor progression in malignancies, including HCC.[10,11] In the present study, we sought to further investigate the relationship between preoperative plasma fibrinogen level and HCC recurrence after LT. We examined the associations between preoperative plasma fibrinogen concentration and a number of clinicopathological factors, including survival rates, and incorporated the independent risk factors into a scoring model for predicting HCC recurrence.

2. Methods

2.1. Patient selection

We enrolled 99 patients with HCC who were treated with LT at the Third Affiliated Hospital of Sun Yat-sen University (China) from 2003 to 2008. Demographic, clinical, and laboratory data, including age, gender, blood neutrophil and lymphocyte counts, serum alpha-fetoprotein (AFP) concentration, preoperative plasma fibrinogen concentration, preoperative imaging data (tumor size, number, and macrovascular invasion), and history of pre-transplant tumor therapy were recorded. The diagnosis of HCC was confirmed by pathological examination of explanted liver. The inclusion criteria included: (i) HBV infection, (ii) > 18 years of age, and (iii) complete clinical and follow-up data available. The exclusion criteria included: (i) missing preoperative blood test or imaging data, and (ii) HCC not confirmed pathologically.[12] This study was approved by the Institutional Review Board of the Key Lab of Liver Disease Research in Guangdong Province. Written informed consent was obtained according to the Declaration of Helsinki.

2.2. Surgery and postoperative management

LT was performed without venovenous bypass unless portal vein was detected to be involved by the tumor during operation. The postoperative immunosuppression regimen included tacrolimus and/or rapamycin, glucocorticoids (discontinued within 3 months), and basiliximab (administered on the day of operation and postoperative day 4).[12]

2.3. Follow-up

Patients were followed up at the outpatient clinic. Abdominal and chest computed tomographys were conducted every 3 months for the first 2 postoperative years, followed by every 6 months thereafter. Liver function and plasma AFP concentration were assessed every month in the first postoperative year and then every 3 months thereafter.[12]

2.4. Statistical analysis

All statistical analyses were performed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL). Data were presented as the mean ± standard deviation (SD) unless noted. Predictive factors for recurrence were examined using Cox regression analysis. Factors with a *P*-value <0.05 in univariate analysis were entered into the multivariate model. Significant independent risk factors for recurrence were used to construct a scoring system using the logistic regression model. Overall survival (OS) and disease-free survival (DFS) were compared using the Kaplan−Meier method. A receiver operating characteristic (ROC) curve analysis was performed to compare the predictive value of the proposed model with currently existing systems. Independent samples *t*-test was used to analyze group differences in clinicopathological features.[12] Statistical significance was set at *P* < 0.05.

3. Results

3.1. Patient demographics and outcomes

The 99-patient cohort consisted of 90 male (90.1%) and 9 female (9.9%). The mean age at LT was 48.4 ± 9.4 years, and the mean follow-up time was 33.6 ± 21.5 months. Thirty-four patients (34.3%) received preoperative anti-tumor therapy. Forty patients (40.4%) died during follow-up, and 41 patients (41.4%) developed tumor recurrence. Of 41 recurred patients, 26 (63.4%) experienced recurrence within 1 year and 37 (90.2%) within 2 years after LT. The 1-, 3-, and 5-year OS and DFS rates for all patients were 62.8% and 49.6%, 41.8% and 59.8%, and 55.8% and 53.3%, respectively.

3.2. Selection of cut-off value for preoperative plasma fibrinogen concentration

A ROC curve was constructed to determine the optimal cut-off value for assigning normal and high preoperative plasma fibrinogen concentrations. The area under the ROC curve (AUC) was 0.787 (95% confidence interval 0.663−0.910). A cut-off value of 2.68 g/L was selected with a sensitivity of 80.5% and a specificity of 75%.

3.3. Risk factors for HCC recurrence after LT

Table 1 shows the results of univariate and multivariate analysis of preoperative factors affecting DFS. More than 3 tumor nodules, largest tumor size >5 cm, macrovascular invasion, preoperative fibrinogen concentration >2.68 g/L, and age >50 years were all significant risk factors for postoperative recurrence in univariate analysis. In multivariate analysis, >3 tumor nodules, macrovascular invasion, and preoperative plasma fibrinogen concentration >2.68 g/L remained independent risk factors for HCC recurrence after LT.

Of the 99 patients in the study, 34 (34.3%) had >3 tumor nodules in preoperative examinations. This group had a significantly lower 5-year DFS rate than the remaining patients (24.3% *vs.* 69.7%, *P* < 0.001, Fig. 1A).

Thirty patients (30.3%) had macrovascular invasion; of these, 21 patients developed recurrence, all within 18 months of LT. The 5-year DFS rates of the patients with and without macrovascular invasion were 18.6% and 66.8%, respectively (*P* < 0.001, Fig. 1B).

The 62 patients (61.6%) who had elevated preoperative plasma fibrinogen concentrations (>2.68 g/L) had a significantly lower 5-year DFS rate compared with those with normal preoperative plasma fibrinogen concentrations (37.2% *vs.* 78.4%, *P* = 0.001; Fig. 1C).

Table 1
Preoperative factors affecting DFS in HCC patients after LT.

Category	Subcategory (N)	HR	95%CI	Univariate analysis	Multivariate analysis
Gender	Male (90)	0.461	0.111−1.910	0.274	NS
	Female (9)				
Age	<50 years (55)	0.505	0.261−0.905	0.038	NS
	≥50 years (44)				
Preoperative treatment	Yes (34)	0.588	0.294−1.175	0.128	NS
	No (65)				
Maximum tumor diameter	>5 cm (40)	3.348	1.791−6.257	<0.001	NS
	≤5 cm (59)				
Number of tumor nodules	>3 (34)	4.004	2.153−7.448	<0.001	<0.001
	≤3 (65)				
Macrovascular invasion	Yes (30)	6.360	3.360−12.040	<0.001	<0.001
	No (69)				
AFP	>400 ng/mL (41)	1.443	0.777−2.678	0.243	NS
	≤400 ng/mL (58)				
Fibrinogen concentration	>2.68 g/L (61)	3.391	1.563−7.358	0.001	0.005
	≤2.68 g/L (38)				

Abbreviations: HR, hazard ratio; CI, confidence interval; NS, not significant; AFP, alpha-fetoprotein.
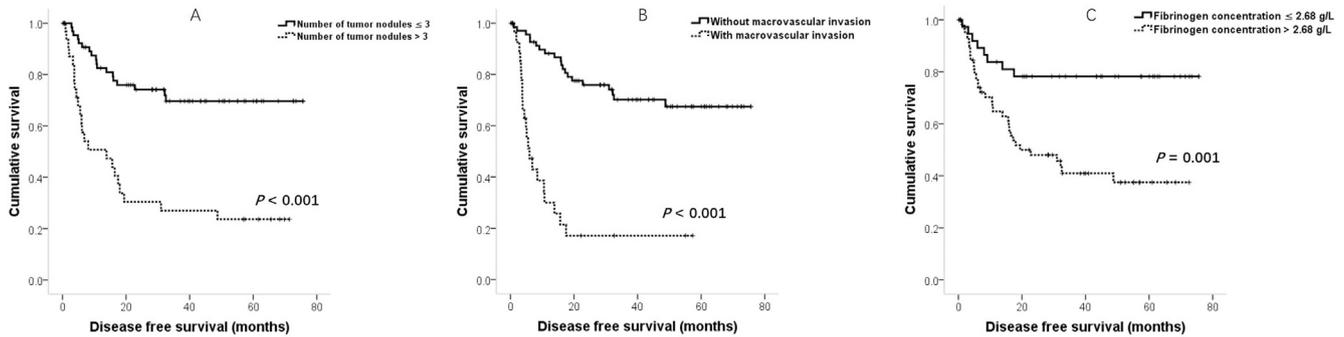
Fig. 1. DFS of HCC patients stratified by tumor nodule number, macrovascular invasion, and fibrinogen concentration. Kaplan−Meier survival curves for patients with (A) > 3 vs. ≤3 tumor nodules (B) with vs. without macrovascular invasion, and (C) elevated (>2.68 g/L) vs. normal (≤2.68 g/L) plasma fibrinogen concentration. Abbreviations: DFS, disease-free survival; HCC, hepatocellular carcinoma.

3.4. Clinicopathological characteristics of patients with normal and high preoperative plasma fibrinogen concentrations

We analyzed the correlation between preoperative plasma fibrinogen concentration and various clinicopathological parameters. Significantly higher preoperative plasma fibrinogen concentrations were detected in patients with vs. without macrovascular invasion (3.54 g/L vs. 2.82 g/L, $P = 0.007$; Fig. 2A), in patients with AFP >400 ng/mL vs. ≤400 ng/mL (3.43 g/L vs. 2.76 g/L, $P = 0.007$; Fig. 2B), and in patients who did vs. did not develop recurrence after LT (3.27 g/L vs. 2.34 g/L, $P = 0.002$; Fig. 2C).

3.5. A preoperative prognostic scoring model based on macrovascular invasion, tumor nodule frequency, and plasma fibrinogen concentration

We established a scoring model based on the three significant preoperative prognostic factors on multivariate analysis. Scores of 0 were assigned to no microvascular invasion, ≤3 tumor nodules, and no tumor recurrence and a score of 1 was assigned to the presence of invasion, >3 nodules, and recurrence, resulting in four scoring categories of 0, 1, 2 and 3. DFS rates for patients with each preoperative prognostic score are shown in Fig. 3A. The 5-year DFS
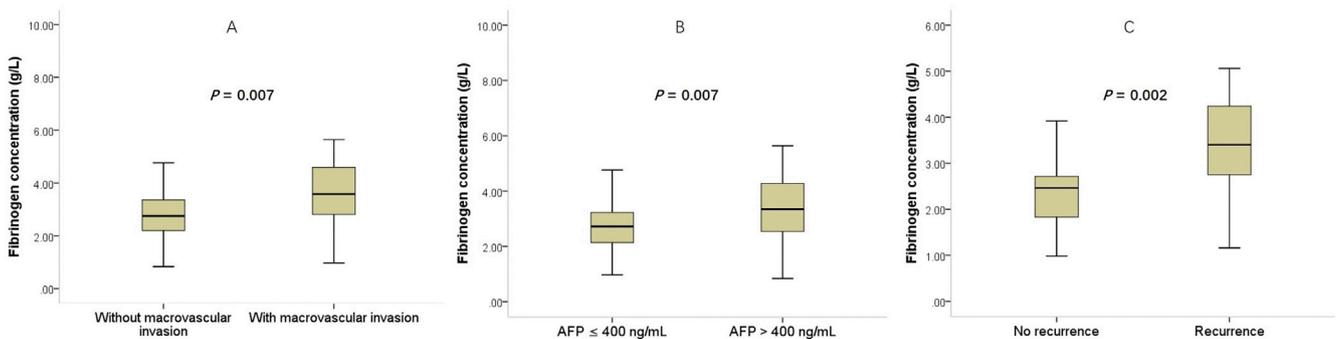
Fig. 2. Preoperative plasma fibrinogen concentrations in patients stratified by macrovascular invasion, AFP level, and recurrence. Preoperative plasma fibrinogen levels in patients (A) with vs. without macrovascular invasion, (B) with AFP >400 ng/mL vs. ≤400 ng/mL, and (C) with vs. without recurrence. The horizontal line, box edges (lower and upper edges), and bars represent the median, first and third quartiles, and minimum/maximum values, respectively. Abbreviation: AFP, alpha-fetoprotein.
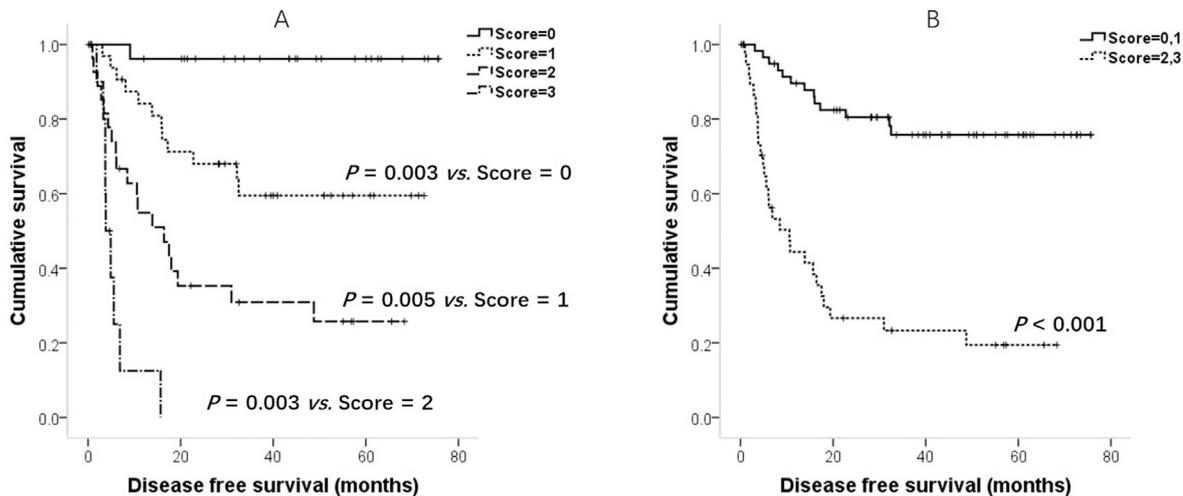
Fig. 3. DFS of patients stratified using the new scoring model. Kaplan–Meier DFS curves of patients with (A) scores of 0, 1, 2, and 3, and (B) scores of 0/1 or 2/3 ($P < 0.001$). Abbreviation: DFS, disease-free survival.

for patients with scores of 0, 1, 2, and 3 were 96.2% ($N = 27$), 59.8% ($N = 32$), 25.3% ($N = 27$), and 0 ($N = 13$), respectively. Of the 13 patients with a score of 3, 9 developed tumor recurrence within 16 months after LT, and none survived beyond 28 months. The median DFS for patients with scores of 0/1, 2, and 3 were 60, 14.6, and 4.7 months, respectively.

To compare the predictive value of our preoperative prognostic scoring method with that of the Milan, UCSF, and Hangzhou criteria, we performed ROC curve analysis and obtained AUC of 0.871, 0.776, 0.802, and 0.755, respectively. The new scoring method had a sensitivity of 68.3% and a specificity of 87.5% when the cut-off value was set between 1 and 2. The 5-year DFS rate of patients with scores of 0 or 1 was significantly higher than that of patients with scores of 2 or 3 ($P < 0.001$; Fig. 3B).

3.6. A preoperative prognostic scoring model for HCC patients without macrovascular invasion

Patients with tumor invasion of main vessels, such as the portal veins, hepatic veins, or vena cava, were previously deemed ineligible for LT due to the high rate of tumor recurrence. Therefore, we

analyzed the prognostic value of our scoring model for the 69 patients in this study without preoperative macrovascular invasion by assigning scores of 0, 1, or 2 based on plasma fibrinogen level and tumor nodule number. The 5-year DFS rates for patients with scores of 0, 1, and 2 were 96.2%, 63.8%, and 25.0%, respectively (Fig. 4A). The DFS rate of patients with scores of 0 or 1 was significantly higher than that of patients with a score of 2 ($P < 0.001$; Fig. 4B). Collectively, these results reveal that elevated preoperative plasma fibrinogen concentration and >3 tumor nodules were indicators of HCC recurrence after LT in patients without macrovascular invasion.

4. Discussion

4.1. Plasma fibrinogen concentration is an independent risk factor for HCC recurrence after LT

In the present study of a cohort of 99 Chinese patients, we found that preoperative plasma fibrinogen concentration >2.68 g/L, >3 tumor nodules, and macrovascular invasion were independent risk factors for HCC recurrence after LT. Fibrinogen is converted by
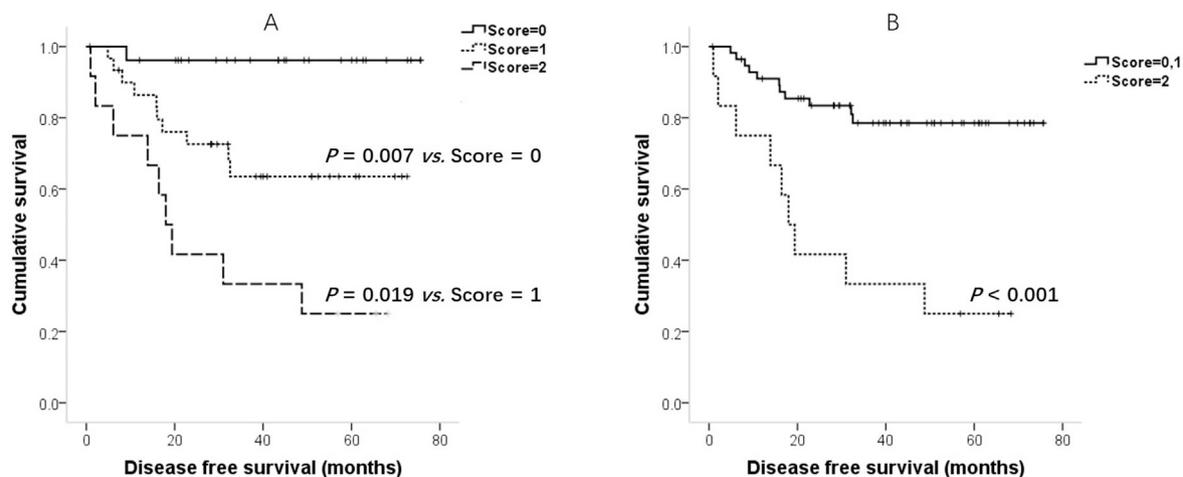
Fig. 4. DFS of patients without macrovascular invasion stratified using the new scoring model. Kaplan–Meier DFS curves of patients with (A) scores of 0, 1, and 2, and (B) scores of 0/1 or 2 ($P < 0.001$). Abbreviation: DFS, disease-free survival.

thrombin to fibrin when the hemostatic system is activated and is a crucial factor in the maintenance of hemostasis. Fibrinogen also contributes to many inflammatory processes, such as angiogenesis, cell adhesion, and transendothelial cell migration.[13,14] Plasma fibrinogen concentrations are often elevated in patients with malignant tumors, probably due to the inflammatory reaction to tumor growth and the hypercoagulable state of these patients,[15,16] and elevated levels have been associated with tumor progression or recurrence.[15,17–23] Expression of *FGG* mRNA, which encodes the fibrinogen γ chain, has also been detected at significantly higher levels in HCC tumors than in normal liver tissue.[24]

We identified preoperative plasma fibrinogen concentration >2.68 g/L to be an independent risk factor for HCC after LT with a sensitivity of 80.5% and a specificity of 75%. Elevated preoperative plasma fibrinogen concentration correlated with the presence of macrovascular invasion, high AFP levels, and HCC recurrence. Another independent risk factor for recurrence in the present study was >3 tumor nodules on preoperative examination. This finding is consistent with other studies showing that tumor nodule number can predict recurrence after LT for HCC patients;[25] however, it is not yet clear whether it is the number of nodules or the total tumor mass that determines the outcome of LT.[26–28] In our study, univariate analysis identified a size of >5 cm for the largest tumor as a significant risk factor for recurrence after LT. Further analysis showed that this variable correlated significantly with macrovascular invasion, which might have contributed to it exclusion in multivariate analysis.

Macrovascular invasion was another independent risk factor for recurrence of HCC after LT in the present study. This factor has been regarded as a contra-indicator for LT in most selection criteria, including the Milan, UCSF, and Hangzhou criteria. In China, most patients with HCC are diagnosed when macrovascular invasion is already present, and this is the case for about a third of patients who undergo LT at our center. Without treatment, most patients with macrovascular invasion live no longer than 3 months, but LT or liver resection can prolong their survival and improve their quality of life.[29] Studies in Japan and Korea found that patients whose donors were living relatives had a better prognosis after LT.[30,31] Our results showed that macrovascular invasion was associated with significantly worse outcomes, indicating that this parameter should remain as a contra-indicator for LT.

AFP concentration is the most commonly used biomarker of HCC and has also been reported to be an independent prognostic factor for HCC.[32] In our study, the AFP level was not an independent risk factor for predicting recurrence of HCC after LT, although this does not mean that AFP is not associated with recurrence. Indeed, we found a significant correlation between elevated preoperative plasma fibrinogen concentration and the AFP level, suggesting that AFP was still associated with HCC recurrence after LT despite being excluded in the multivariate analysis.

4.2. Comparison of the new scoring model with currently used selection criteria for LT

Our preoperative prognostic scoring model had an AUC of 0.871 compared with 0.785, 0.807, and 0.789 for the Milan, UCSF, and Hangzhou criteria, respectively. The sensitivity and specificity of our model for predicting HCC recurrence after LT were 68.3% and 87.5%, respectively, when the cut-off score was set between 1 and 2. Our cohort of patients with a score of 0 or 1 ($N = 59$) had a 5-year DFS of 76.1%, compared with 70.7% of patients using the Hangzhou criteria ($N = 60$).

In conclusion, our study identified elevated preoperative plasma fibrinogen concentration as a risk factor for HCC recurrence after LT, suggesting that this parameter may aid in the selection of patients

who would most benefit from LT. Our preoperative scoring model, which was based on three risk factors, was sensitive and specific in predicting recurrence of HCC after LT. The limitations of the study include its single-center and retrospective nature, and further multi-center and prospective studies will be needed to validate and improve the accuracy of the model. The underlying mechanism by which fibrinogen might affect the recurrence and metastasis of HCC is also unclear and merits further research.

Authors' contributions

Conception and design: K.-N. Zeng, Y.-C. Zhang, G.-Y. Wang. Administrative support: Y. Yang, G.-H. Chen. Provision of study materials or patients: G.-S. Wang, J. Zhang, H. Li, Q. Zhang. Collection and assembly of data: K.-N. Zeng, Y.-C. Zhang, Y.-N. Deng, S.-H. Li. Data analysis and interpretation: K.-N. Zeng, Y.-C. Zhang. Manuscript writing and final approval of manuscript: all authors. K.-N. Zeng and Y.-C. Zhang contributed equally to this work.

Conflict of interest

The authors declare that they have no conflict of interest.

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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.
2. Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology*. 2004;127:S5–S16.
3. Yuen MF, Hou JL, Chutaputti A. Hepatocellular carcinoma in the Asia pacific region. *J Gastroenterol Hepatol*. 2009;24:346–353.
4. Chen GH. Liver transplantation in China: retrospect and prospect. *Chin Med J (Engl)*. 2009;122:2229–2230.
5. Mazzaferro V, Chun YS, Poon RT, et al. Liver transplantation for hepatocellular carcinoma. *Ann Surg Oncol*. 2008;15:1001–1007.
6. Broelsch CE, Frilling A, Malago M. Should we expand the criteria for liver transplantation for hepatocellular carcinoma–yes, of course!. *J Hepatol*. 2005;43:569–573.
7. Yao FY, Ferrell L, Bass NM, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology*. 2001;33:1394–1403.
8. Zheng SS, Xu X, Wu J, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation*. 2008;85:1726–1732.
9. Hiatt JR, Carmody IC, Busuttil RW. Should we expand the criteria for hepatocellular carcinoma with living-donor liver transplantation?–no, never. *J Hepatol*. 2005;43:573–577.
10. Kinoshita A, Onoda H, Imai N, et al. Elevated plasma fibrinogen levels are associated with a poor prognosis in patients with hepatocellular carcinoma. *Oncology*. 2013;85:269–277.
11. Wang GY, Jiang N, Yi HM, et al. Pretransplant elevated plasma fibrinogen level is a novel prognostic predictor for hepatocellular carcinoma recurrence and patient survival following liver transplantation. *Ann Transplant*. 2016;21:125–130.
12. Wang GY, Yang Y, Li H, et al. A scoring model based on neutrophil to lymphocyte ratio predicts recurrence of HBV-associated hepatocellular carcinoma after liver transplantation. *PLoS One*. 2011;6, e25295.
13. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357:539–545.
14. Palumbo JS, Potter JM, Kaplan LS, Talmage K, Jackson DG, Degen JL. Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice. *Cancer Res*. 2002;62:6966–6972.
15. Seebacher V, Polterauer S, Grimm C, et al. The prognostic value of plasma fibrinogen levels in patients with endometrial cancer: a multi-centre trial. *Br J Cancer*. 2010;102:952–956.
16. Gouin-Thibault I, Achkar A, Samama MM. The thrombophilic state in cancer patients. *Acta Haematol*. 2001;106:33–42.

17. Takeuchi H, Ikeuchi S, Kitagawa Y, et al. Pretreatment plasma fibrinogen level correlates with tumor progression and metastasis in patients with squamous cell carcinoma of the esophagus. *J Gastroenterol Hepatol*. 2007;22:2222–2227.

18. Yamashita H, Kitayama J, Kanno N, Yatomi Y, Nagawa H. Hyperfibrinogenemia is associated with lymphatic as well as hematogenous metastasis and worse clinical outcome in T2 gastric cancer. *BMC Cancer*. 2006;6:147.

19. Guo Q, Zhang B, Dong X, et al. Elevated levels of plasma fibrinogen in patients with pancreatic cancer: possible role of a distant metastasis predictor. *Pancreas*. 2009;38:e75–e79.

20. Tang L, Liu K, Wang J, Wang C, Zhao P, Liu J. High preoperative plasma fibrinogen levels are associated with distant metastases and impaired prognosis after curative resection in patients with colorectal cancer. *J Surg Oncol*. 2010;102:428–432.

21. Jones JM, McGonigle NC, McAnespie M, Cran GW, Graham AN. Plasma fibrinogen and serum C-reactive protein are associated with non-small cell lung cancer. *Lung Cancer*. 2006;53:97–101.

22. Polterauer S, Grimm C, Seebacher V, et al. Plasma fibrinogen levels and prognosis in patients with ovarian cancer: a multicenter study. *Oncologist*. 2009;14:979–985.

23. Polterauer S, Seebacher V, Hefler-Frischmuth K, et al. Fibrinogen plasma levels are an independent prognostic parameter in patients with cervical cancer. *Am J Obstet Gynecol*. 2009;200(e1-7):647.

24. Zhu WL, Fan BL, Liu DL, Zhu WX. Abnormal expression of fibrinogen gamma (FGG) and plasma level of fibrinogen in patients with hepatocellular carcinoma. *Anticancer Res*. 2009;29:2531–2534.

25. Duffy JP, Vardanian A, Benjamin E, et al. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg*. 2007;246:502–509.

26. Toso C, Trotter J, Wei A, et al. Total tumor volume predicts risk of recurrence following liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl*. 2008;14:1107–1115.

27. Toso C, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology*. 2009;49:832–838.

28. Germani G, Gurusamy K, Garcovich M, et al. Which matters most: number of tumors, size of the largest tumor, or total tumor volume? *Liver Transpl*. 2011;17:S58–S66.

29. Pesi B, Ferrero A, Grazi GL, et al. Liver resection with thrombectomy as a treatment of hepatocellular carcinoma with major vascular invasion: results from a retrospective multicentric study. *Am J Surg*. 2015;210:35–44.

30. Ito T, Takada Y, Ueda M, et al. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl*. 2007;13:1637–1644.

31. Lee SG, Hwang S, Moon DB, et al. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl*. 2008;14:935–945.

32. Johnson PJ. Role of alpha-fetoprotein in the diagnosis and management of hepatocellular carcinoma. *J Gastroenterol Hepatol*. 1999;14:S32–S36.