Original Article

Liver transplantation for hepatocellular carcinoma: a proposal for including preoperative serological indicators improves the Milan criteria expanded

Ning Jiao[1,2,3,4#], Cheng Yan[1,2#], Li He[1,2#], Hai-Long Jin[1], Shuang Oiu[1], Chao Li[1], Zhi-Sheng Zheng[5], Bin Lu[1], Feng-Dong Wu[1], Yang Yang[1], Xin-Guo Chen[1], Qing Zhang[1]

[1]Department of Organ Transplantation, Third Medical Center of Chinese PLA General Hospital, Beijing, China; [2]School of Clinical Medicine, Shandong Second Medical University, Weifang, China; [3]Department of Health Statistics, Fourth Military Medical University, Xi'an, China; [4]Department of Gastroenterology, People's Hospital of Jiyang County, Jinan, China; [5]Department of Orthopaedics, People's Hospital of Jiyang County, Jinan, China

Contributions: (I) Conception and design: N Jiao; (II) Administrative support: Q Zhang; (III) Provision of study materials or patients: Q Zhang; (IV) Collection and assembly of data: HL Jin, S Oiu, C Li, ZS Zheng, B Lu, FD Wu, Y Yang, XG Chen; (V) Data analysis and interpretation: C Yan, L He; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Qing Zhang, PhD. Department of Organ Transplantation, Third Medical Center of Chinese PLA General Hospital, No. 69 Yongding Road, Haidian District, Beijing 100039, China. Email: zqy6920@sina.com.

Background: Liver transplantation (LT) is the most effective and radical treatment for hepatocellular carcinoma (HCC). Most LT criteria are based on the morphological characteristics of tumors, which are not enough to predict the risk of tumor recurrence. It is found that some serological biomarkers can predict tumor recurrence and may be a good indicator for selecting suitable HCC patients for LT. This article aims to evaluate the predictive effect of preoperative serological indicators on long-term overall survival (OS) and tumor recurrence-free survival (TFS) of patients with HCC after LT, and to explore its significance for expanding the Milan criteria.

Methods: Clinical data of 253 patients after LT in HCC were collected retrospectively. The receiver operating characteristic curve was used to calculate the best cut-off value. χ^2 test was used to analyze the correlation between preoperative serological indicators and tumor pathological features. Univariate and multivariate analyses were used to analyze the risk factors affecting the OS and TFS rates and the predictive values of different LT criteria were compared. Nomogram model was used to predict the OS and TFS rates of patients exceeding Milan criteria.

Results: Independent risk factors for poor OS and TFS rates were alpha-fetoprotein (AFP) >200 ng/mL, gamma-glutamyl transpeptidase (GGT) >80 IU/L, total tumor diameter (TTD) >8 cm and microsatellite lesions. Nomogram model showed patients beyond Milan criteria had better survival when AFP ≤200 ng/mL and GGT ≤80 IU/L or AFP >200 ng/mL, GGT ≤80 IU/L and TTD ≤8 cm. According to Milan criteria, AFP, GGT and TTD, Milan-AFP-GGT-TTD (M-AGT) criteria was established. There was no significant difference in OS and TFS rates among patients in M-AGT, Milan, Hangzhou, Malaya and the University of California at San Francisco (UCSF) criteria.

Conclusions: Preoperative serological indicators AFP and GGT can effectively predict long-term OS and TFS in HCC patients after LT. Establishing M-AGT criteria based on serological indicators is helpful to supplement the Milan criteria.

Keywords: Hepatocellular carcinoma (HCC); liver transplantation (LT); preoperative serological indicators; prognosis

Received: 01 April 2024; Accepted: 22 July 2024; Published online: 14 October 2024.

doi: 10.21037/tgh-24-40

View this article at: https://dx.doi.org/10.21037/tgh-24-40

Introduction

Hepatocellular carcinoma (HCC) is among the most common malignant tumors and the third leading cause of cancer-associated death worldwide (1). A predicted 1.3 million people could die from liver cancer in 2040 (2). Liver transplantation (LT) is one of the most effective treatments for the radical cure of HCC (3), but it is limited by organ shortage and the higher postoperative tumor recurrence. Thus, it is particularly important to select appropriate HCC recipients for LT.

Milan criteria are recognized as the gold standard for LT with HCC, which is based on tumor morphology features, can effectively reduce tumor recurrence rate and improve patient prognosis post-LT (4,5). However, Milan criteria are too strict, resulting in some HCC patients who may benefit from LT being excluded (6,7). Thus, several expanded criteria beyond the Milan criteria have been proposed, such as the University of California at San Francisco (UCSF) (8), Asan (9), "Up to seven" (10), Kyoto (11) and Shanghai criteria (12), etc. These expanded criteria were also mainly based on the tumor morphological characteristics, such as tumor number or tumor nodule size. However, there are deficiencies in detecting tumor morphological features using imaging techniques, which can lead to the inaccuracy of preoperative evaluation for LT (13). Several reports have indicated that as many as 20–25% of HCC patients who undergo LT have inaccurately staged morphological characteristics by imaging techniques (14). Additionally, in some expanded LT criteria, such as Hangzhou (15) and Malatya criteria (16), tumor histological differentiation needs to be assayed by liver biopsy, which is a kind of invasive examination with potential risks and is not necessary for every patient with HCC before LT. These limitations have promoted people's interest in identifying the effective prognostic serologic biomarker for HCC patients with LT (17).

Alpha-fetoprotein (AFP) as a serum marker of HCC, has been identified as an independent predictor of tumor recurrence and survival after LT in HCC patients (15,18-21), and has been included as a vital index in several expanded criteria, such as Hangzhou criteria and Metroticket2.0 criteria (21). However, the efficacy of the AFP is limited, because the level of AFP was negative and not significantly elevated in 30–40% of patients with HCC (22,23), while in some individuals without HCC, the AFP level has increased (24). Therefore, it is necessary to find more feasible serum indicators to make up for the limitations of AFP and the Milan criteria, so as to select suitable HCC recipients for LT.

Previous studies had reported that serum liver enzyme indicators for detecting and evaluating hepatic injury were related to HCC and its prognosis (25-27). However, few studies have investigated their role as risk factors in predicting the outcomes of HCC patients after LT. The study evaluated the prognostic role of preoperative serum liver enzyme indicators on tumor recurrence and survival of HCC patients who received LT, and established appropriate LT criteria based on serological indicators to improve Milan criteria expanded. We present this article in accordance with the TRIPOD reporting checklist (available at https://tgh.amegroups.com/article/view/10.21037/tgh-24-40/rc).

Highlight box

Key findings

- Preoperative serological indicators alpha-fetoprotein (AFP) and gamma-glutamyl transpeptidase (GGT) can effectively predict long-term overall survival (OS) and tumor recurrence-free survival (TFS) in hepatocellular carcinoma (HCC) patients after liver transplantation (LT). Establishing Milan-AFP-GGT-TTD (M-AGT) criteria based on serological indicators is helpful to supplement the Milan criteria.

What is known and what is new?

- LT is the most effective and radical treatment for HCC.
- This study found that preoperative serum GGT combined with AFP could effectively predict the long-term prognosis of HCC after LT. The proposed M-AGT criteria can help to screen suitable HCC patients before LT. The nomogram model improves the credibility of M-AGT criteria, has not been used in the previous LT criteria.

What is the implication, and what should change now?

- M-AGT criteria are helpful to supplement Milan criteria and may benefit more HCC patients from LT.

Methods

Patients and data collection

From January 2015 and January 2019, 255 patients with HCC underwent LT with donation after cardiac death (DCD) or donation after brain death plus cardiac death (DBCD) were included in this study at the Third Medical Center of Chinese PLA General Hospital, Beijing, China. All of them had histologically proven HCC in pre-LT or intraoperative examination. Inclusion criteria: (I) patients with imaging and pathological diagnosis of HCC; (II)

patients treated with DCD or DBCD LT; (III) age ≥18 years, and (IV) all patients had completely preoperative baseline data postoperative and follow-up data. Exclusion criteria: (I) macrovascular invasion and lymph node or distant metastasis, (II) multi-visceral and combined organ retransplantation, (III) death due to non-tumor diseases within 1 month after LT. According to the inclusion and exclusion criteria, 253 HCC patients were enrolled in this study. *Table 1* summarizes the baseline characteristics of 253 HCC patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Third Medical Center of Chinese PLA General Hospital (No. 2011-042) and informed consent was obtained from all individual participants.

In the current study, there were 220 (87%) men and 33 (13%) women, with a median age of 54 (range, 20–77) years. AFP level and liver enzyme indicators were detected within 1 month before LT. The median serum AFP level was 34.3 mg/L (range, 1.15–60,500 mg/L). The median serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) levels were 38.50 IU/L (range, 11–6,034 IU/L), 32.00 IU/L (range, 4–4,398 IU/L), and 75.50 IU/L (range, 8–1,384 IU/L), respectively. Among the 253 patients, 198 were serum HBsAg positive, 32 were HBeAg positive and 48 patients were hepatitis B virus (HBV) DNA positive (>1 IU/mL). Before LT, 47 (18.6%) patients received anti-tumor treatments. There were 96 (37.9%) patients who met the Milan criteria.

Diagnosis and assessment

HCC was diagnosed before LT by measuring serum AFP and combining two imaging techniques [ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), or histologically by liver biopsy. HCC was diagnosed by histopathological examination during the operation. The resected liver of HCC recipients was examined by two experienced pathologists. Record the number of tumors, the size of tumors (the largest diameter of tumor nodules), the degree of differentiation (well, moderately, or poorly differentiated) and whether there is invasion of blood vessels or lymph nodes around the liver.

Immunosuppressive therapy and follow-up

Postoperative immunosuppression was based on the calcineurin-inhibitor (tacrolimus or ciclosporin) combined with mycophenolate mofetil (MMT) and prednisone. Prednisone was gradually stopped within 3 months after LT, and the recipients who stopped tacrolimus (FK506) or cyclosporine 3 months after transplantation began to use sirolimus. The HCC patients after LT were followed monthly for the first year and every 3–6 months after that. All patients underwent routine laboratory tests such as blood routine, biochemical tests, drug concentration, tumor markers, and imaging examinations, etc.

During the follow-up period, tumor recurrence or metastasis was assessed routinely by AFP level and abdominal ultrasonography once a month, and the whole-body CT or MRI examinations every 6–12 months. If necessary, the recurrence of tumor was confirmed histologically by liver biopsy examination.

The overall survival (OS) time was calculated from the date of operation to the date of death or the most recent follow-up visit. The tumor recurrence-free survival (TFS) time was calculated from the date of operation to the first day of diagnosis of tumor recurrence.

Statistical analysis

The primary endpoint was OS, and the secondary endpoints were TFS rates. Frequencies, means, and medians were calculated for the clinical data. The optimal cut-off values were calculated by the receiver operating characteristic (ROC) curves. OS and TFS rates were assessed by the log-rank test, and Kaplan-Meier survival analysis was performed. Multivariate stepwise Cox regression was used to find the independent factors influencing survival. Nomogram model was used to predict OS and TFS rates in HCC patients who do not meet the Milan criteria. By comparing the area under ROC curve (AUC) and prognosis, the predictive power of different LT criteria was evaluated. Statistical significance was defined as a two-tailed $P<0.05$. All statistics was carried out by SPSS Statistics software (ver. 23. SPSS Inc., Chicago, IL, USA). This article follows the SAMPL guidelines for statistics.

Results

Survival and recurrence

During the follow-up period, 1 case was lost to follow-up, 1 case died of non-tumor diseases within 1 month after LT, and the remaining 253 patients met the conditions of this study. The patient selection process is shown in *Figure 1*.

Table 1 Main clinical and pathological characteristics of patients included in this study (N=253)

Variables	N (%)
Gender	
Male	220 (87.0)
Female	33 (13.0)
Age (years)	
≤60	202 (79.8)
>60	51 (20.2)
GGT (IU/L)	
≤80	133 (52.6)
>80	120 (47.4)
AST (IU/L)	
≤47	155 (61.3)
>47	98 (38.7)
AFP (ng/mL)	
≤200	169 (66.8)
>200	84 (33.2)
ALT (IU/L)	
≤30	115 (45.5)
>30	138 (54.5)
HBV DNA (copies/mL)	
≤1	205 (81.0)
>1	48 (19.0)
Microsatellite lesions	
Absent	116 (45.8)
Present	137 (54.2)
Venous invasion	
Absent	210 (83.0)
Present	43 (17.0)
TTD (cm)	
≤8	141 (55.7)
>8	112 (44.3)
Membrane invasion	
Absent	166 (65.6)
Present	87 (34.4)
Tumor-node score	
1	112 (44.3)
>1	141 (55.7)
MELD score	
≤15	199 (78.7)
>15	54 (21.3)
Child-Pugh score	
≤6	100 (39.5)
7–9	100 (39.5)
>9	53 (20.9)
TNM staging	
1	67 (26.5)
2	33 (13.0)
3	98 (38.7)
4-6	55 (21.7)
Histopathologic grading	
Grade I	4 (1.6)
Grade II	237 (93.7)
Grade III	12 (4.7)
Fit Milan criteria	
No	157 (62.1)
Yes	96 (37.9)
Fit Hangzhou criteria	
No	47 (18.6)
Yes	206 (81.4)
Fit Malatya criteria	
No	114 (45.1)
Yes	139 (54.9)
Fit UCSF criteria	
No	138 (54.5)
Yes	115 (45.5)
Survival	
No	112 (44.3)
Yes	141 (55.7)
Recurrence	
No	166 (65.6)
Yes	87 (34.4)

GGT, gamma-glutamyl transpeptidase; AST, aspartate aminotransferase; AFP, serum alpha fetoprotein; ALT, alanine aminotransferase; HBV, hepatitis B virus; TTD, total tumor diameter; MELD, Model for End-Stage Liver Disease; UCSF, University of California at San Francisco.

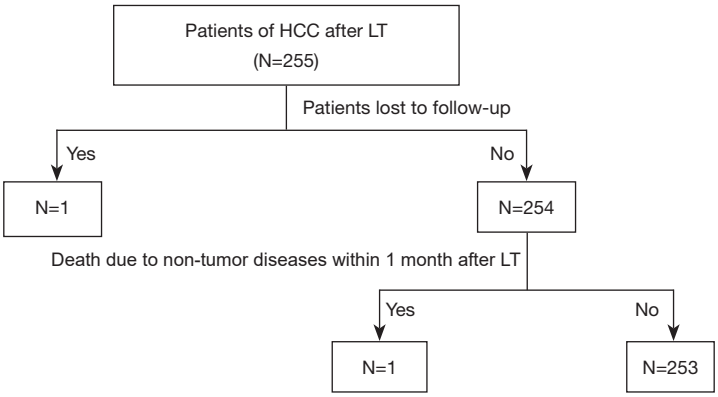

Figure 1 The patient selection process. HCC, hepatocellular carcinoma; LT, liver transplantation.

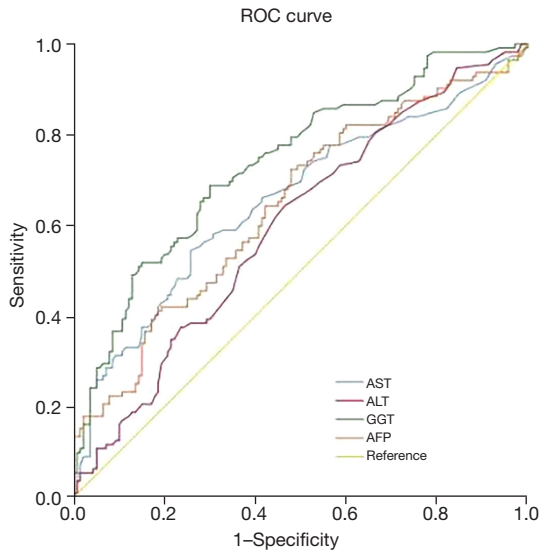

Figure 2 An ROC analysis was performed on preoperative serum GGT, AST, ALT and AFP. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; AFP, serum alpha fetoprotein; ROC, receiver operating characteristic.

Among them, 112 (44.3%) patients died, and 87 (34.4%) patients experienced tumor recurrence. The median survival time was 49.00 months. After LT, these patients had 1-, 3-, 5- and 7-year OS rates of 82%, 60%, 56%, and 54%, respectively. Their corresponding TFS rates were 70%, 57%, 54%, and 53% respectively.

Cutoff values of pre-LT serum GGT, AST, ALT and AFP levels

The analysis of the ROC curve revealed that the optimal cutoff values of serum GGT, AST, ALT and AFP levels were 80 IU/L (AUC =0.739, P<0.001), 47 IU/L (AUC =0.658, P<0.001), 30 IU/L (AUC =0.599, P=0.007), and 200 ng/mL (AUC =0.644, P<0.001), respectively (*Figure 2*). According to the best intercept, the above indicators were converted into dichotomous variables, further stratified analysis.

Relationship of pre-LT serum GGT, AST and ALT with clinicopathologic features

The level of serum GGT was significantly associated with total tumor diameter (TTD) (≤8 *vs.* >8 cm) (P<0.001), tumor number (≤1 *vs.* >1) (P<0.001), venous invasion (P<0.001), microsatellite lesions (P<0.001), membrane invasion (P<0.001), TNM staging (P<0.001), Model for End-Stage Liver Disease (MELD) score (P=0.007) and HBV DNA (P=0.006). The level of serum AST was significantly associated with TTD (≤8 *vs.* >8 cm) (P<0.001), tumor number (≤1 *vs.* >1) (P=0.001), venous invasion (P<0.001), microsatellite lesions (P=0.01), membrane invasion (P<0.001), TNM staging (P<0.001), MELD score (P<0.001) and HBV DNA (P=0.001). The level of serum ALT was significantly associated with TTD (≤8 *vs.* >8 cm) (P<0.001), tumor number (≤1 *vs.* >1) (P=0.01), venous invasion (P<0.001), microsatellite lesions (P=0.03), membrane invasion (P<0.001), TNM staging (P<0.001), MELD score (P<0.001), HBV DNA (P=0.002).

Univariate analysis of the risk factors for OS and TFS survival

The pre-LT factors that significantly affect post-LT poor OS were preoperative serum AFP >200 ng/mL (P<0.001),

GGT >80 IU/L (P<0.001), AST >47 IU/L (P<0.001), ALT >30 IU/L (P=0.002), tumor number >1 (P<0.001), TTD >8 cm (P<0.001), venous invasion (P<0.001), microsatellite lesions (P<0.001), and TNM staging (P<0.001). The significant factors affecting poor post-LT TFS were serum AFP >200 ng/mL (P<0.001), GGT >80 IU/L (P<0.001), AST >47 IU/L (P<0.001), ALT >30 IU/L (P=0.01), tumor number >1 (P<0.001), TTD >8 cm (P<0.001), venous invasion (P<0.001), microsatellite lesions (P<0.001) and TNM staging (P<0.001). The 1-, 3-, 5-, and 7-year OS and TFS rates of these indices are shown in *Table 2*.

Multivariate analysis of the independent risk factors for OS and TFS survival

Multivariate analysis included venous invasion, microsatellite lesions, TNM staging, tumor number, TTD, AFP level, GGT level, AST level, and ALT level were included. Independent predictors of poor survival after LT were AFP >200 ng/mL [hazarad ratio (HR) =1.912, P=0.005], GGT >80 IU/L (HR =1.544, P=0.02), TTD >8 cm (HR =2.116, P=0.001) and microsatellite lesions (HR =2.432, P<0.001) (*Table 3*). Independent predictors of TFS after LT were AFP >200 ng/mL (HR =1.768, P=0.01), GGT >80 IU/L (HR =1.742, P=0.004), TTD >8 cm (HR =1.924, P=0.003) and microsatellite lesions (HR =2.684, P<0.001) (*Table 4*).

The predictive value of serum AFP combined with GGT

Analysis of preoperative serum AFP in combination with GGT showed that patients with AFP ≤200 ng/mL and GGT ≤80 IU/L (103 patients) had higher OS and TFS rates at 5 years postoperatively than those with only AFP ≤200 ng/mL (169 patients) or AFP ≤200 ng/mL and GGT >80 IU/L (66 patients) (*Figure 3A*,*3B*, both P<0.05); OS and TFS rates at 5 years after surgery were higher in patients with AFP >200 ng/mL and GGT ≤80 IU/L (30 patients) than in patients with AFP >200 ng/mL and GGT >80 IU/L (54 patients) (*Figure 3C*,*3D*, both P<0.05).

Table 2 Univariate analysis of overall survival and tumor recurrence-free survival risk factors

Variables	Overall survival (%)					Tumor recurrence-free survival (%)				
	1-year	3-year	5-year	7-year	P value	1-year	3-year	5-year	7-year	P value
Gender					0.80					0.47
Male	83	60	56	54		69	56	53	50	
Female	79	60	60	60		73	61	60	60	
Age (years)					0.58					0.38
≤60	82	59	55	53		71	57	54	50	
>60	82	67	61	61		73	65	61	55	
AFP (ng/mL)					<0.001*					<0.001*
≤200	90	68	63	61		80	66	61	60	
>200	67	44	41	41		50	39	39	34	
AST (IU/L)					<0.001*					<0.001*
≤47	88	72	67	65		81	69	64	60	
>47	72	41	38	38		53	37	37	37	
ALT (IU/L)					0.002*					0.01*
≤30	87	70	67	63		81	66	62	55	
>30	78	53	48	48		59	49	47	47	

Table 2 (*continued*)

Table 2 (*continued*)

Variables	Overall survival (%)					Tumor recurrence-free survival (%)				
	1-year	3-year	5-year	7-year	P value	1-year	3-year	5-year	7-year	P value
GGT (IU/L)					<0.001*					<0.001*
≤80	89	77	74	71		84	75	71	65	
>80	74	40	35	35		54	37	35	35	
Tumor number					<0.001*					<0.001*
1	94	80	78	76		91	80	77	71	
>1	73	45	39	38		55	41	37	35	
TTD (cm)					<0.001*					<0.001*
≤8	89	77	76	74		83	74	72	67	
>8	74	39	32	31		53	35	31	30	
Venous invasion					<0.001*					<0.001*
Absent	85	65	62	60		75	65	61	56	
Present	70	40	30	30		53	28	26	26	
Microsatellite lesions					<0.001*					<0.001*
No	92	78	78	76		87	79	76	74	
Yes	74	45	39	37		58	41	37	32	
TNM staging					<0.001*					<0.001*
1	93	84	82	78		88	83	78	78	
2	85	73	73	73		78	72	72	62	
3	85	56	52	50		69	52	47	47	
4–6	64	33	24	24		41	24	24	18	
Child-Pugh score					0.52					0.48
≤6	86	62	58	58		79	60	58	51	
7–9	83	61	58	54		69	59	54	52	
>9	74	57	51	51		60	55	51	51	
MELD score					0.40					0.65
≤15	84	61	57	56		71	57	54	51	
>15	74	57	53	50		64	54	51	50	
Histopathologic grading					0.22					0.25
Grade I	100	100	100	100		100	100	100	100	
Grade II	81	59	56	54		70	56	52	50	
Grade III	67	58	58	58		66	58	58	58	

*, statistically significant. AFP, serum alpha fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; TTD, total tumor diameter; MELD, Model for End-Stage Liver Disease.

Table 3 Multivariate proportional hazard model for overall survival after liver transplantation in 253 patients with hepatocellular carcinoma

Variables	B	SE	Wald	P	HR (95% CI)
Satellite, no *vs.* yes	0.889	0.231	14.834	<0.001*	2.432 (1.547–3.822)
GGT level, ≤80 *vs.* >80 IU/L	0.434	0.195	4.973	0.02*	1.544 (1.054–2.262)
AFP level, ≤200 *vs.* >200 ng/mL	0.648	0.231	7.873	0.005*	1.912 (1.216–3.007)
TTD, ≤8 *vs.* >8 cm	0.750	0.235	10.219	0.001*	2.116 (1.336–3.351)

*, statistically significant. SE, standard error; HR, hazard ratio; CI, confidence interval; GGT, gamma-glutamyl transpeptidase; AFP, serum alpha fetoprotein; TTD, total tumor diameter.

Table 4 Multivariate proportional hazard model for tumor recurrence-free survival after liver transplantation in 253 patients with hepatocellular carcinoma

Variables	B	SE	Wald	P	HR (95% CI)
Satellite, no *vs.* yes	0.987	0.226	19.127	<0.001*	2.684 (1.724–4.178)
GGT level, ≤80 *vs.* >80 IU/L	0.555	0.190	8.520	0.004*	1.742 (1.200–2.529)
AFP level, ≤200 *vs.* >200 ng/mL	0.570	0.221	6.659	0.01*	1.768 (1.147–2.725)
TTD, ≤8 *vs.* >8 cm	0.655	0.224	8.565	0.003*	1.924 (1.241–2.983)

*, statistically significant. SE, standard error; HR, hazard ratio; CI, confidence interval; GGT, gamma-glutamyl transpeptidase; AFP, serum alpha fetoprotein; TTD, total tumor diameter.

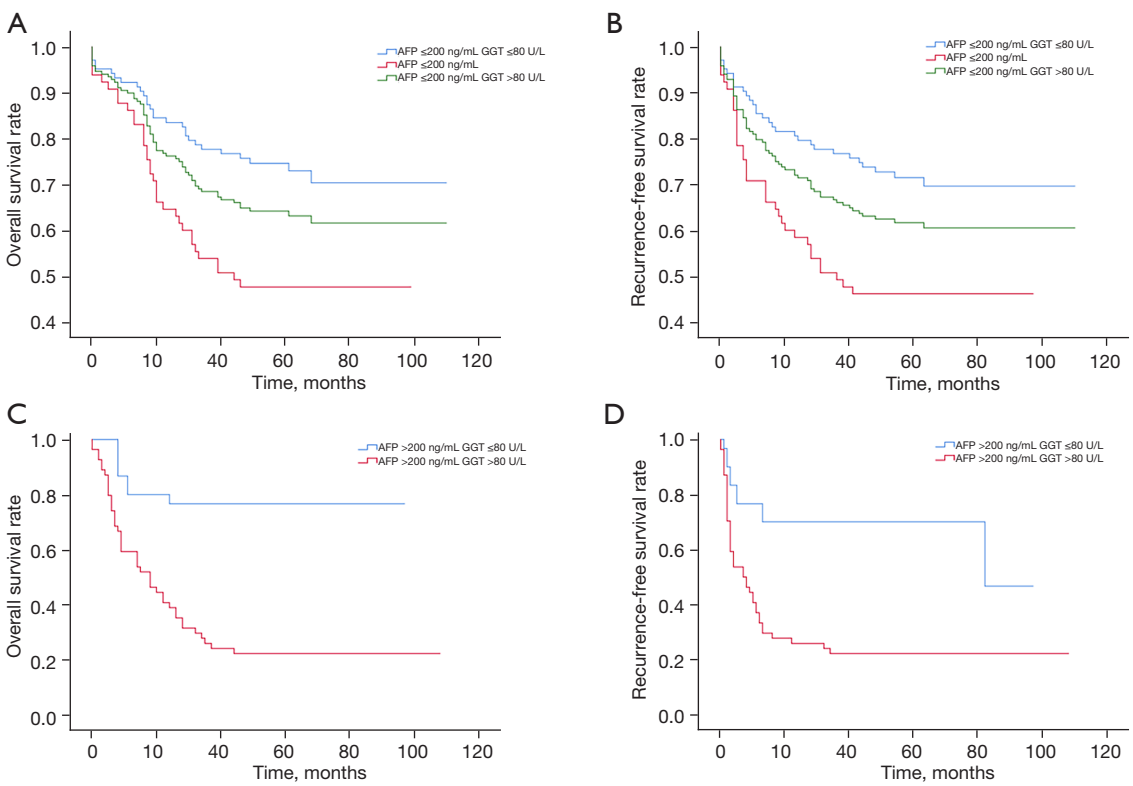

Figure 3 Cumulative overall survival curves (A,C) and tumor recurrence-free survival curves (B,D) of patients of AFP combined with GGT. AFP, serum alpha fetoprotein; GGT, gamma-glutamyl transpeptidase.

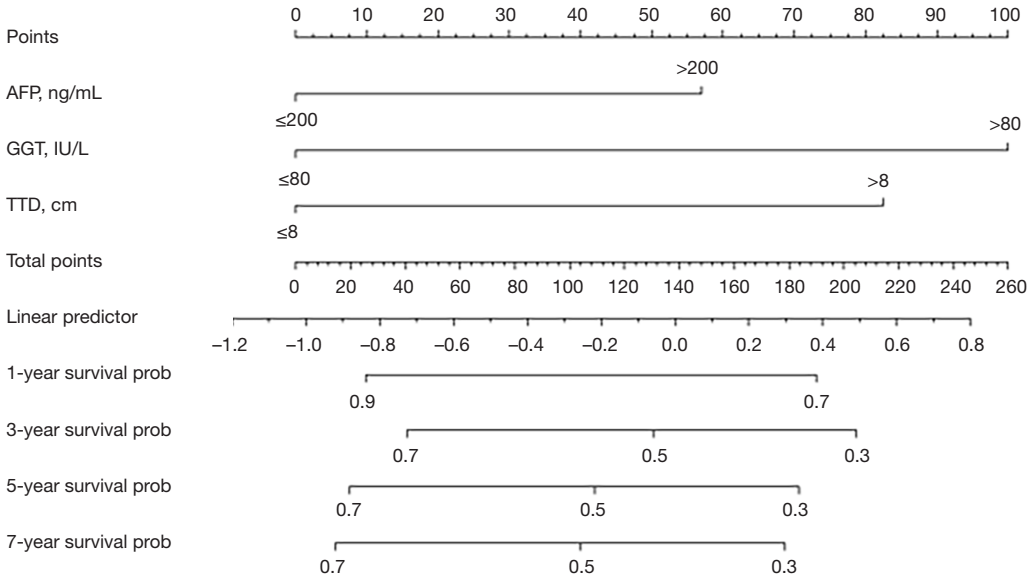

Figure 4 Nomogram model of patients after LT for HCC beyond the Milan criteria. LT, liver transplantation; HCC, hepatocellular carcinoma; AFP, serum alpha fetoprotein; GGT, gamma-glutamyl transpeptidase; TTD, total tumor diameter.

Nomogram model of patients after LT for HCC beyond the Milan criteria

For the candidates who exceed Milan criteria, we construct a nomogram for low-grade endometrial stromal sarcoma (LG-ESS) according to AFP, GGT, and TTD. Through the nomogram model, we can obtain when AFP ≤200 ng/mL and GGT ≤80 IU/L or AFP >200 ng/mL, GGT ≤80 IU/L and TTD ≤8 cm, patients of HCC after LT beyond the Milan criteria had better OS and TFS rates (*Figure 4*). The 1-year OS rates of HCC patients after LT are between 70% and 90%, and the 1-year TFS rates are more excellent than 70%. The OS and TFS rates of 3-, 5- and 7-year in HCC patients after LT are between 50% and 70%.

Proposal of the Milan-AFP-GGT-TTD (M-AGT) criteria

Then, we based on Milan criteria and these independent risk factors beyond Milan criteria, TTD and serological indexes (AFP and GGT), proposed the M-AGT criteria: (I) patients met Milan criteria; (II) patients beyond the Milan criteria met (i) AFP ≤200 ng/mL and GGT ≤80 IU/L, or (ii) AFP >200 ng/mL, GGT ≤80 IU/L and TTD ≤8 cm.

The analysis showed that for patients who fit the M-AGT criteria (n=151), the 1-, 3-, 5- and 7-year OS and TFS rates were 92%, 77%, 74%, 71% and 85%, 74%, 71%, 66%, respectively, which were significantly higher than those who were unfit for the current criteria (all P<0.001) (*Figure 5*). For patients who fit the Milan criteria (n=96), the 1-, 3-, 5- and 7-year OS and TFS rates were 93%, 83%, 82%, 80% and 90%, 83%, 79%, 72%, respectively (*Table 5*). The OS and TFS rates of patients who fit the M-AGT criteria were not significantly different from those who fit the Milan criteria (all P>0.05, *Table 5*).

The predictive value of M-AGT criteria as the Milan criteria expanded

Among 253 HCC patients, the OS and TFS rates of patients who fit M-AGT criteria are not different from those of Milan, Hangzhou, Malatya and UCSF criteria (*Table 5*). The results of the ROC curve analysis showed that the AUC values of the OS and TFS rates of patients who fit the M-AGT were 0.705 and 0.691, which were significantly different from Hangzhou criteria (0.624 and 0.612, all P=0.01) and not substantially different from Milan criteria (0.703 and 0.699, all P>0.05), Malatya criteria (0.691 and 0.686, all P>0.05) and UCSF criteria (0.735 and 0.735, all P>0.05) (*Figure 6*).

Furthermore, the patients who did not meet the Milan criteria (n=157), 35% patients fit the M-AGT criteria, and

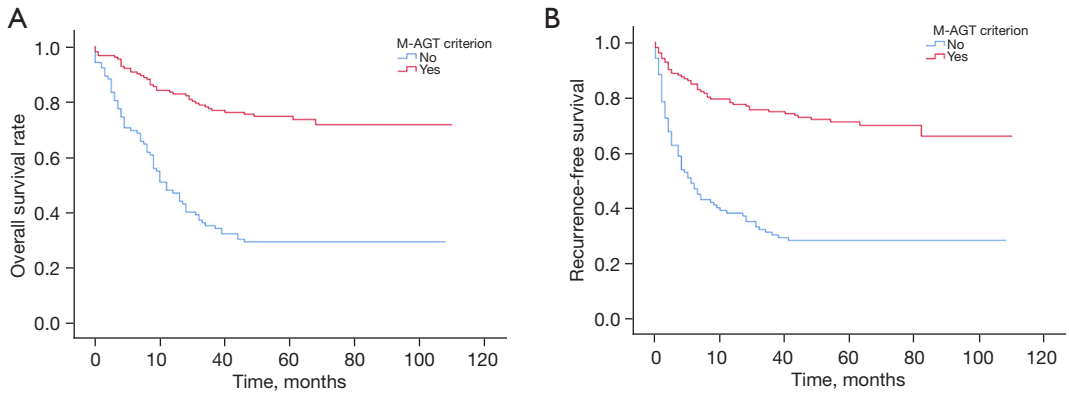

Figure 5 Cumulative overall survival curves (A) and tumor recurrence-free survival curves (B) of patients classified in M-AGT criteria. M-AGT, Milan-AFP-GGT-TTD.

Table 5 Comparison of overall survival and recurrence-free survival rates of different liver transplant criteria in HCC patients

LT criteria	N	Overall survival (%)					Tumor recurrence-free survival (%)				
		1-year	3-year	5-year	7-year	P value	1-year	3-year	5-year	7-year	P value
M-AGT	151	92	77	74	71	–	85	74	71	66	–
Milan	96	93	83	82	80	0.16	90	83	79	72	0.15
Hangzhou	206	88	68	65	62	0.06	79	66	61	58	0.07
UCSF	115	92	82	80	79	0.22	89	81	78	73	0.13
Malatya	139	91	76	73	70	0.77	84	74	71	65	0.93

LT, liver transplant; M-AGT, Milan-AFP-GGT-TTD; UCSF, University of California at San Francisco.

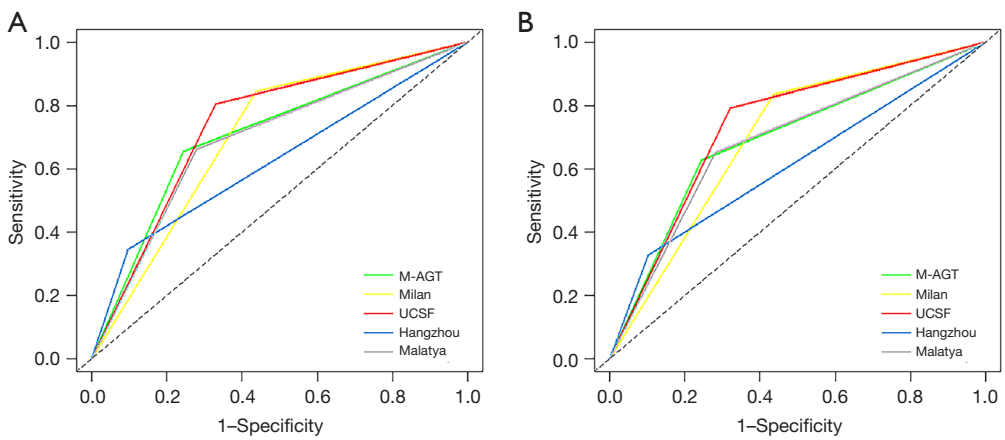

Figure 6 ROC curves for the overall survival and recurrence-free survival rates of different criteria after liver transplantation. ROC, receiver operating characteristic; M-AGT, Milan-AFP-GGT-TTD; UCSF, University of California at San Francisco.

the 1-, 3-, 5- and 7-year OS and TFS rates were 87%, 65%, 61%, 56% and 74%, 60%, 56%, 56%, respectively. There was no significant difference in OS and TFS rates between patients who did not meet Milan criteria but met M-AGT criteria and those who met the Hangzhou, Malatya and UCSF criteria (all P>0.05).

Discussion

Morphological characteristics of tumor, such as tumor size and number of nodules, are insufficient to indicate the biologic features of HCC (28). In recent years, many extended LT criteria include tumor biomarkers (for example, AFP) in the selection criteria and are not limited to tumor size and number (15,29). The practitioner found that AFP still had some false positive or false negative rates in HCC patients (24). Therefore, we need to find other serological markers to compensate for the deficiency of AFP. This study found that preoperative serum GGT combined with AFP could effectively predict the long-term prognosis of HCC after LT. The proposed M-AGT criteria can help to screen suitable HCC patients before LT. The nomogram model improves the credibility of M-AGT criteria, but has not been used in the previous LT criteria.

GGT is a key enzyme that catalyzes the transpeptidation and hydrolysis of the glutamyl group of glutathione, and its expression level in HCC is abnormally high (30). GGT may play an important role in HCC progression and poor prognosis through various signaling pathways and mechanisms (31), which gives GGT increasing attention. Our study found that GGT was closely related to the TTD, tumor number, venous invasion, microsatellite lesions, membrane invasion, and TNM stage in HCC patients (both P<0.05), which was consistent with the research results of Zhang *et al.* (32) and Fu *et al.* (33). We believe that GGT may be a molecular marker that can reflect the biological characteristics of HCC. Further studies showed that GGT was an independent risk factor for long-term OS and TFS rates after LT for HCC, consistent with the results of studies by Fu *et al.* (33) and Ince *et al.* (16). Our results further confirmed the predictive value of preoperative serum GGT level in HCC patients who underwent LT. In addition, our study found the 5-year OS rate of HCC patients after LT with serum GGT ≤80 IU/L reached 74%, and the 5-year TFS rate reached 71%. Therefore, we speculate that GGT has the potential as a serological marker to predict the long-term prognosis of LT in HCC.

AFP is the most commonly used prognostic marker in the treatment decision-making of HCC patients, and has been included in many LT criteria (15,21,34). But the cutoff value of AFP was different in different LT criteria, such as in the Hangzhou criteria, AFP is 400 ng/mL (15), and two levels of 100 ng/mL and 1,000 ng/mL are set in the AFP model (34). In this study, AFP ≤200 ng/mL was an excellent independent predictor for HCC patient survival after LT, consistent with the results of the Malatya criteria (21). However, it is reported that the AFP level will increase when patients experience some gastrointestinal tumors, even during pregnancy (24). Therefore, we further considered AFP combined with the serological marker GGT to complement the limitations of false positive rate AFP. Our study found that the OS and TFS rates of patients after LT in HCC with AFP ≤200 ng/mL and GGT ≤80 IU/L were significantly better than those with only AFP ≤200 ng/mL or AFP ≤200 ng/mL and GGT >80 IU/L (P<0.05). The overall OS and TFS rates of patients after LT in HCC with AFP >200 ng/mL and GGT ≤80 IU/L were significantly better than those with AFP >200 ng/mL and GGT >80 IU/L. Therefore, we believe that the level of serum AFP combined with GGT can better predict the long-term prognosis of HCC after LT.

Of course, the preoperative tumor size cannot be ignored in HCC after LT patients. In this study, TTD >8 cm was an independent risk factor affecting the OS and TFS rates of HCC after LT. It has been confirmed in previous studies and used in the expanded criteria for LT (8). As we all know, the HCC patients who meet Milan criteria have a good 5-year survival rate (35). However, only 37.9% of patients met the Milan criteria in this study, and almost 62.1% of patients exceeded the Milan criteria. Therefore, we developed a nomogram model with AFP, GGT and TTD to predict the prognosis of HCC patients who exceeded Milan criteria, which was not used in previous LT criteria. The nomogram model showed that when AFP ≤200 ng/mL and GGT ≤80 IU/L or AFP >200 ng/mL, GGT ≤80 IU/L and TTD ≤8 cm, the survival rate of patients with HCC exceeding Milan criteria after LT was better, and the 7-year OS and TFS rates are over 50%. Based on Milan criteria and predictive factors TTD, AFP and GGT, we proposed the M-AGT criteria.

Among patients who exceeded the Milan criteria, more than 35% (55 of 157) met the M-AGT criteria were allowed to receive LT and achieved favorable long-term survival. Our research shows that the post-LT survival of HCC patients who fit M-AGT criteria was not significantly different from that of patients who fit the Milan criteria.

It indicates the population of HCC patients who fit the M-AGT criteria would benefit from LT was larger than that of the Milan criteria. Therefore, the author believes that M-AGT criteria are helpful to supplement Milan criteria and may benefit more HCC patients from LT.

The Hangzhou and Malatya criteria are expanded criteria for HCC after LT, including molecular marker. Due to Hangzhou criteria has no restrictions on TTD, it includes more candidates than M-AGT criteria. However, the AUC of both OS and TFS rates who met the M-AGT criteria was significantly better than the patients who met the Hangzhou criteria. M-AGT criteria does not require liver biopsy to obtain tumor histological grade of tumors, which reduces the potential risk of needle tract seeding and hematogenous dissemination and simpler and easier. The Malatya criteria also include indicators such as AFP, GGT and TTD, which further proves the reliability of M-AGT criteria. The difference between Malatya and M-AGT criteria is that the cutoff values for GGT and TTD are different, and the Malatya criteria also includes the degree of tumor differentiation. Our study found no difference in OS and TFS between the Malatya and M-AGT criteria, but the M-AGT criteria have more beneficiaries and avoid defects in pathological biopsy. UCSF criteria is a commonly used expanded criteria for HCC after LT, and it also takes TTD =8 cm as the critical value (8). In our study, there was no statistical difference in OS and TFS rates between patients who met M-AGT criteria and UCSF criteria. However, the M-AGT criteria included more candidates than the UCSF criteria, and the AUC of both OS and TFS rates was higher. The above shows that the M-AGT criteria have good predictive power.

This study has several limitations. This is a retrospective study design and single-center experience with a small sample size. There may be unrecognized selection bias could have influenced the outcomes analysis. Our selection criteria in the study need to be independently verified, which should be further confirmed by multicenter and prospective study. Therefore, we used various methods, such as Nomogram model and Multivariate analysis, to clarify the important prognosis value of the predictive factors in the M-AGT criteria for LT among HCC patients.

Conclusions

In summary, the results of this study show that GGT has the potential as a serological marker to predict the prognosis of LT in HCC. M-AGT criteria could effectively predict the long-term survival rate after LT in HCC, and can be used as a supplementary criteria of Milan criteria.

Acknowledgments

Funding: This work was supported by the National Natural Science Foundation of China (No. 81372595).

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tgh.amegroups.com/article/view/10.21037/tgh-24-40/rc

Data Sharing Statement: Available at https://tgh.amegroups.com/article/view/10.21037/tgh-24-40/dss

Peer Review File: Available at https://tgh.amegroups.com/article/view/10.21037/tgh-24-40/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tgh.amegroups.com/article/view/10.21037/tgh-24-40/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Third Medical Center of Chinese PLA General Hospital (No. 2011-042) and informed consent was obtained from all individual participants.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics

2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Rumgay H, Arnold M, Ferlay J, et al. Global burden of primary liver cancer in 2020 and predictions to 2040. J Hepatol 2022;77:1598-606.
3. Vibert E, Schwartz M, Olthoff KM. Advances in resection and transplantation for hepatocellular carcinoma. J Hepatol 2020;72:262-76.
4. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693-9.
5. Shimamura T, Goto R, Watanabe M, et al. Liver Transplantation for Hepatocellular Carcinoma: How Should We Improve the Thresholds? Cancers (Basel) 2022;14:419.
6. Akbulut S, Koc C. Do We Need to Be Limited by Matching Milan Criteria for Survival in Living Donor Liver Transplantation? J Gastrointest Cancer 2020;51:1107-13.
7. Costentin CE, Bababekov YJ, Zhu AX, et al. Is It Time to Reconsider the Milan Criteria for Selecting Patients With Hepatocellular Carcinoma for Deceased-Donor Liver Transplantation? Hepatology 2019;69:1324-36.
8. 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;33:1394-403.
9. 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;14:935-45.
10. Takada Y, Ito T, Ueda M, et al. Living donor liver transplantation for patients with HCC exceeding the Milan criteria: a proposal of expanded criteria. Dig Dis 2007;25:299-302.
11. Takada Y, Uemoto S. Liver transplantation for hepatocellular carcinoma: the Kyoto experience. J Hepatobiliary Pancreat Sci 2010;17:527-32.
12. Fan J, Zhou J, Xu Y, et al. Indication of liver transplantation for hepatocellular carcinoma: Shanghai Fudan Criteria. Zhonghua Yi Xue Za Zhi 2006;86:1227-31.
13. Singh S, Hoque S, Zekry A, et al. Radiological Diagnosis of Chronic Liver Disease and Hepatocellular Carcinoma: A Review. J Med Syst 2023;47:73.
14. Sotiropoulos GC, Malagó M, Molmenti E, et al. Liver transplantation for hepatocellular carcinoma in cirrhosis: is clinical tumor classification before transplantation realistic? Transplantation 2005;79:483-7.
15. Zheng SS, Xu X, Wu J, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. Transplantation 2008;85:1726-32.
16. Ince V, Akbulut S, Otan E, et al. Liver Transplantation for Hepatocellular Carcinoma: Malatya Experience and Proposals for Expanded Criteria. J Gastrointest Cancer 2020;51:998-1005.
17. Lingiah VA, Niazi M, Olivo R, et al. Liver Transplantation Beyond Milan Criteria. J Clin Transl Hepatol 2020;8:69-75.
18. Mahmud N, John B, Taddei TH, et al. Pre-transplant alpha-fetoprotein is associated with post-transplant hepatocellular carcinoma recurrence mortality. Clin Transplant 2019;33:e13634.
19. Costentin C, Piñero F, Degroote H, et al. R3-AFP score is a new composite tool to refine prediction of hepatocellular carcinoma recurrence after liver transplantation. JHEP Rep 2022;4:100445.
20. Tran BV, Moris D, Markovic D, et al. Development and validation of a REcurrent Liver cAncer Prediction ScorE (RELAPSE) following liver transplantation in patients with hepatocellular carcinoma: Analysis of the US Multicenter HCC Transplant Consortium. Liver Transpl 2023;29:683-97.
21. Mazzaferro V, Llovet JM, Miceli R, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol 2009;10:35-43.
22. Jing W, Peng R, Zhu M, et al. Differential Expression and Diagnostic Significance of Pre-Albumin, Fibrinogen Combined with D-Dimer in AFP-Negative Hepatocellular Carcinoma. Pathol Oncol Res 2020;26:1669-76.
23. Luo P, Wu S, Yu Y, et al. Current Status and Perspective Biomarkers in AFP Negative HCC: Towards Screening for and Diagnosing Hepatocellular Carcinoma at an Earlier Stage. Pathol Oncol Res 2020;26:599-603.
24. Fu J, Li Y, Li Z, et al. Clinical utility of decarboxylation prothrombin combined with α-fetoprotein for diagnosing primary hepatocellular carcinoma. Biosci Rep 2018;38:BSR20180044.
25. Guo J, Liu S, Gao S, et al. γ-Glutamyltranspeptidase as a Prognostic Biomarker in Advanced Hepatocellular Carcinoma Treated with Transarterial Chemoembolization. J Vasc Interv Radiol 2021;32:419-428.e2.

26. Guo C, Liang H, Yuan W, et al. Analysis on the value of soluble intercellular adhesion molecule-1 (sICAM-1), alpha fetoprotein (AFP), and aspartate aminotransferase/platelet ratio index (APRI) in predicting the prognostic survival of patients with primary liver cancer after radiofrequency ablation. Ann Palliat Med 2021;10:4760-7.
27. Li ZC, Li Z, Li C, et al. Letter: elevated aspartate aminotransferase and aspartate aminotransferase to alanine aminotransferase ratio predicts poor survival in hepatocellular carcinoma after resection. Aliment Pharmacol Ther 2020;52:1762-3.
28. Gunsar F. Liver Transplantation for Hepatocellular Carcinoma Beyond the Milan Criteria. Exp Clin Transplant 2017;15:59-64.
29. Mehta N, Bhangui P, Yao FY, et al. Liver Transplantation for Hepatocellular Carcinoma. Working Group Report from the ILTS Transplant Oncology Consensus Conference. Transplantation 2020;104:1136-42.
30. Tsutsumi M, Sakamuro D, Takada A, et al. Detection of a unique gamma-glutamyl transpeptidase messenger RNA species closely related to the development of hepatocellular carcinoma in humans: a new candidate for early diagnosis of hepatocellular carcinoma. Hepatology 1996;23:1093-7.
31. Xia J, Song P, Sun Z, et al. Advances of diagnostic and mechanistic studies of γ-glutamyl transpeptidase in hepatocellular carcinoma. Drug Discov Ther 2016;10:181-7.
32. Zhang LX, Lv Y, Xu AM, et al. The prognostic significance of serum gamma-glutamyltransferase levels and AST/ALT in primary hepatic carcinoma. BMC Cancer 2019;19:841.
33. Fu SJ, Zhao Q, Ji F, et al. Elevated Preoperative Serum Gamma-glutamyltranspeptidase Predicts Poor Prognosis for Hepatocellular Carcinoma after Liver Transplantation. Sci Rep 2016;6:28835.
34. Notarpaolo A, Layese R, Magistri P, et al. Validation of the AFP model as a predictor of HCC recurrence in patients with viral hepatitis-related cirrhosis who had received a liver transplant for HCC. J Hepatol 2017;66:552-9.
35. Song P, Cai Y, Tang H, et al. The clinical management of hepatocellular carcinoma worldwide: A concise review and comparison of current guidelines from 2001 to 2017. Biosci Trends 2017;11:389-98.

doi: 10.21037/tgh-24-40
Cite this article as: Jiao N, Yan C, He L, Jin HL, Oiu S, Li C, Zheng ZS, Lu B, Wu FD, Yang Y, Chen XG, Zhang Q. Liver transplantation for hepatocellular carcinoma: a proposal for including preoperative serological indicators improves the Milan criteria expanded. Transl Gastroenterol Hepatol 2024;9:63.