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Original Article

Significant association between serum Wisteria floribunda agglutinin-positive Mac-2-binding protein and prognosis of hepatocellular carcinoma after surgical treatment

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Abstract: Serum Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA$^+$-M2BP) is a novel marker for evaluating fibrosis and predicting the development of hepatocellular carcinoma (HCC). However, the role of WFA$^+$-M2BP in the prognosis of HCC patients after curative surgery remains unknown. In this study, we aimed to evaluate the prognostic role of serum WFA$^+$-M2BP in HCC patients after curative resection and liver transplantation. We enrolled 460 HCC patients (357 resection and 103 transplantation) to analyze the risk factors for HCC recurrence and patient's survival. We employed time-to-event models using univariate and multivariable Cox proportional hazards regression analyses and calculated the hazard ratios (HRs) and adjusted HRs with their corresponding 95% confidence intervals (CIs). The levels of WFA$^+$-M2BP were 0.19-14.51 COI (median 1.08) in patients of hepatectomy and 0.47-19.90 COI (median 6.0) in transplant patients. The levels of WFA$^+$-M2BP in liver transplant patients is much higher than that of hepatectomy patients. Overall, liver fibrotic stage was positively correlated to WFA$^+$-M2BP levels ($P<0.0001$). This study demonstrated that elevated WFA$^+$-M2BP level (COI ≥0.75) was associated with a higher HCC recurrence rate in the resection group ($P<0.001$). Survival analysis showed that an elevated WFA$^+$-M2BP level (COI ≥1.43) is associated with a higher mortality risk after surgical resection ($P=0.0088$) in the univariate analysis only. In liver transplant patients, WFA$^+$-M2BP level (COI ≥3.81) did not predict HCC recurrence at all, but was associated poor survival after transplantation, with a borderline significance ($P=0.0943$). Serum WFA$^+$-M2BP is a reliable marker for liver fibrosis in the present study. It is also reliable marker to predict prognosis of HCC after surgical resection. However, the prognostic role of WFA$^+$-M2BP in HCC related transplants is equivocal, which is different from that of surgical resection.

Keywords: Hepatocellular carcinoma, liver fibrosis, WFA$^+$-M2BP, hepatitis B, hepatitis C

Introduction

Liver fibrosis is important for the prognosis of chronic hepatitis B, chronic hepatitis C, and hepatocellular carcinoma [1]. It is very important to identify patients with significant liver fibrosis to prevent liver fibrosis progression and subsequent decompensation [2]. In the past, liver biopsy has been the gold standard for diagnosing liver fibrosis, but it has life-threatening complications such as internal bleeding, infection, and sampling error [3, 4]. With the advancement of antiviral treatment for patients with chronic hepatitis B or C virus infection, it is extremely important to find a reliable and noninvasive tool for regular evaluation and follow-up of patients with liver fibrosis after treatment. As a result, the use of noninvasive techniques for

diagnosing liver fibrosis and HCC is extremely important, especially in high-risk patients who need to undergo liver biopsy. Liver sonography is most commonly used to evaluate liver fibrosis and HCC as it is noninvasive and cost-effective; however, this method has a lower sensitivity for diagnosing cirrhosis [5]. A previous study demonstrated that FibroScan, FIB-4, and aspartate aminotransferase-to-platelet ratio index (APRI) could be used in evaluating liver fibrosis and possess high accuracy for diagnosing cirrhosis [6]. However, in clinical practice, the results of FibroScan, FIB-4, and APRI may be affected by obesity and acute hepatitis due to various etiologies. On the contrary, multiple-detector computed tomography, liver computed tomography, and dynamic magnetic resonance imaging are most commonly used for HCC detection, but the risk of exposure to contrast and radiation is high; hence, these approaches cannot be frequently used for clinical examination [7, 8]. Mac 2-Binding Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA+-M2BP), also known as Mac-2 binding protein glycosylation isomer (M2BPGi), is a reliable marker used for assessing liver fibrosis and HCC induced by various liver diseases including hepatitis B and C [9-12]. Yoon et al. demonstrated that the Mac-2 binding protein is a useful marker for evaluating liver fibrosis and HCC development [13]. Basically, WFA+-M2BP secreted from hepatic stellate cells (HSCs) can induce Mac-2 expression in Kupffer cells (KCs), which transforms HSCs into fibrogenic cells [14]. HSCs and Kupffer cells play important roles in the progression of liver fibrosis [15]. Therefore, serum WFA+-M2BP is a highly specific marker of liver fibrosis. Mak et al. also demonstrated a high correlation between WFA+-M2BP with liver fibrosis and that this marker could be used to predict the regression of liver fibrosis [12]. It is important to monitor the status of patients with liver fibrosis after an efficient antiviral treatment. Although it is an important marker related to liver fibrosis and HCC development, only a few studies have explored its role in HCC prognosis after curative treatment [16-18]. There was no comprehensive study of WFA+-M2BP on liver transplant patients in the past. Therefore, in this study, we aimed to comprehensively explore the clinical application of WFA+-M2BP in the prognosis of HCC patients after curative surgery and liver transplant.

Materials and methods

A total of 460 patients who underwent surgery for HCC (segmentectomy, lobectomy, or liver transplantation) between April 30, 2010, and December 19, 2018 in Kaohsiung Chang Gung Memorial Hospital, Taiwan were included in this retrospective study (Supplementary Material). More than 1,000 HCC patients who underwent curative surgery (resection or transplantation) were initially screened; patients with no complete data or stored serum samples for further analysis were excluded. This study was conducted in accordance with the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL) guidelines [19, 20]; all patients with early-stage HCC and good liver reserve function were selected for hepatectomy. However, if patients had cirrhosis (compensated or decompensated) and developed cirrhosis complications (esophageal or gastric varices, ascites, hepatic encephalopathy, etc.) but had early-stage HCC and met the UCSF criteria (solitary tumor ≤6.5 cm, or ≤3 nodules with a largest lesion size of ≤4.5 cm and a total tumor diameter of ≤8 cm) [21, 22], the patients may be referred for liver transplantation. The blood samples were all taken prior to operation on the operative day. We analyzed the data on patients' age, sex, Child-Pugh classification, liver fibrosis status (METAVIR score), HCC stage (AJCC system, The American Joint Committee on Cancer, 7th edition), hepatitis B or C status, serum WFA+-M2BP level (COI), serum alpha-fetoprotein (AFP) level, serum alanine aminotransferase (ALT) and albumin level, and albumin-bilirubin (ALBI) grade.

Among these factors, serum WFA+-M2BP levels were measured by conducting through a lectin-Ab sandwich immunoassay method using the HISCL WFA+-M2BP reagent kit (Sysmex, Japan) on an automatic immunoanalyzer HISCL-800. The serum WFA+-M2BP level was expressed as a cut-off index (COI) and was calculated based on the following equation: $COI = ([M2BPGi]_{sample} - [M2BPGi]_{NC})/([M2BPGi]_{PC} - [M2BPGi]_{NC})$. The $[M2BPGi]_{sample}$ represented the WFA+-M2BP concentration in the patient serum sample. $[M2BPGi]_{PC}$ and $[M2BPGi]_{NC}$ were used as positive and negative controls, respectively.

The surgical specimens were analyzed and evaluated by pathologists who were experts in

the field of hepatology. The Metavir staging system was used to grade the degree of liver fibrosis, and the specimens were graded from F0 to F4 [23]. According to the Metavir staging system, in the F0 stage, the liver tissue showed absence of fibrosis. In the F1 stage, the liver tissue showed a fibrous portal expansion. In the F2 stage, the liver tissue showed few bridges or septa. In the F3 stage, the liver tissue showed numerous bridges or septa. The F4 stage was defined as cirrhosis. The Child-Pugh classification is used to assess clinical ascites, hepatic encephalopathy, serum prothrombin time, bilirubin total, and albumin level, and identifies the patients as class A, B, and C according to the severity of the disease [24]. The albumin-bilirubin (ALBI) grade was calculated according to the following formula: ($\log_{10}$ bilirubin total ×0.66) + (albumin ×-0.085), with bilirubin measured in micromol/L and albumin in g/L. According to the formula, the ALBI grade was divided as grade 1 (score ≤-2.60), grade 2 (score >-2.60 to ≤-1.39), and grade 3 (>-1.39) [25]. The cut-off value of WFA+-M2BP levels were obtained by AUROC curve according to the events of HCC recurrence or death after hepatectomy and liver transplantation. The cut-off values were used for further Kaplan-Meier and Cox regression hazards Model analyses.

Statistical analyses

We examined whether the continuous variables were normally distributed. The median and interquartile range (IQR) were used to express the differences between quartile 1 (Q1) and quartile 3 (Q3), while the Mann-Whitney U test was used to determine whether the variables have non-normal distribution. For dichotomous variables, the percentage of proportion was used to illustrate the distribution, and a chi-square test was performed to examine the statistical significance. To investigate the effect of WFA+-M2BP on the HCC recurrence or mortality rates, we employed the time-to-event models using univariate and multivariable Cox proportional hazards regression and calculated the hazard ratios (HRs) and adjusted HRs with their corresponding 95% confidence intervals (CIs), respectively. Two outcomes, HCC recurrence and mortality, were treated in different time-to-event models. For the HCC recurrence model, the follow-up time was calculated from the operative date to the date of recurrence and treated as an event; otherwise, those who were followed up until the end of December 2019 or until the date of death were treated as censored cases. For the mortality model, those who died from HCC were classified as events and followed up until the date of death; otherwise, they were classified as censored cases and followed up until the end of 2019. We used the Youden index defined as (sensitivity + specificity -1) as a criterion for selecting the optimal cut-off point for continuous variables. In addition to baseline age, sex, and WFA+-M2BP as compulsive variables, we applied stepwise selection with a *P*-value of <0.05 to identify the potential confounding factors for multivariable adjustment. All analyses were performed using the SAS software (version 9.4). A two-tailed *P*-value of <0.05 was considered significant.

Results

Baseline characteristics and comparison

The baseline characteristics of the 460 enrolled patients are presented in **Table 1**. Among the 460 studied patients, 357 underwent partial hepatectomy and 103 underwent liver transplantation; the median follow-up time from surgery until the date of death was 186.5 weeks, while the median follow-up from surgery until the date of HCC recurrence was 150 weeks. The other baseline characteristics of included patients were as follows: male sex (352, 76.5%), chronic hepatitis B carriers (242 patients, 52.6%), cirrhotic (216, 47%), AJCC stage 2 (234, 50.9%), mean age of 60 years, median AFP of 10.3 ng/mL, median WFA+-M2BP level (COI) of 1.39, and median ALT of 34 U/L. In liver fibrosis analysis, ratio of advanced liver fibrosis was predominant in the transplant patients (**Table 1**). The levels of WFA+-M2BP were 0.19-14.51 COI (median 1.08) in patients of hepatectomy and 0.47-19.90 COI (median 6.0) in transplant patients. The levels of WFA+-M2BP in liver transplant patients is much higher than that of hepatectomy patients (**Table 1**). The effect might be attributed to the much higher percentage of cirrhotic patients in transplant patients. Overall, liver fibrotic stage was positively correlated with WFA+-M2BP levels (P<0.0001, **Figure 1**).

For recurrence status after surgery (including hepatectomy and liver transplantation), the data showed that old age, male sex, higher ALT

Table 1. Baseline characteristics of 460 hepatocellular carcinoma patients post-surgery

Variable	Classification (unit)	Numbers of cases or range	Mean ± SD	Median (IQR)
Patient number		460		
Operation Date		2010/04/30-2018/12/19		
Surgical type	hepatectomy/transplantation	357/103		
Gender	Female/male	108/352		
Hepatitis type	B(-)C(-)/B(+)/C(+)/B(+)C(+)	65/242/137/16		
Liver fibrosis (Metavir) (H)*	0/1/2/3/4	24/77/85/52/119		
Liver fibrosis (Metavir) (T)*	0/1/2/3/4	0/2/4/0/97		
Child-Pugh classification	A/B/C	397/43/20		
AJCC 7	1/2/3/4	152/234/71/3		
Age		28-85	60.45±10.85	62 (14)
WFA+-M2BP (H)*	(COI)	0.19-14.51	1.64±1.84	1.08 (1.08)
WFA+-M2BP (T)*	(COI)	0.47-19.90	6.98±4.81	6.00 (8.30)
AFP	(ng/mL)	0-289172	3154.82±22057.92	10.3 (64.9)
ALT	(U/L)	5-1494	57.37±98.52	34 (32)
Albumin	(g/dL)	1.20-5.05	3.89±0.65	4.04 (0.87)
Total bilirubin	(mg/dL)	0.2-30.9	1.26±2.18	0.8 (0.5)
PT	(INR)	0.87-3.34	1.11±0.20	1.05 (0.11)
ALBI grade	1/2/3	264/163/33		
Recurrence	No/Yes	286/174		
HCC death	No/Yes	400/60		

SD: standard deviation; IQR: interquartile range; AFP: alpha-fetoprotein; ALT: alanine aminotransferase; PT: prothrombin time; ALBI: albumin-bilirubin; HCC: hepatocellular carcinoma; *H: hepatectomy; *T: transplantation.

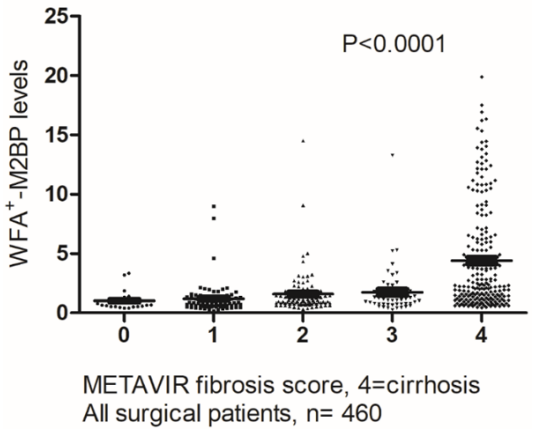

Figure 1. WFA+-M2BP showing a significant correlation with different METAVIR liver fibrosis grade (P<0.0001). The figure shows that the values of WFA+-M2BP (COI) increased with liver fibrosis progression in 460 patients.

level, higher AFP level, higher bilirubin level, advanced liver fibrosis status, advanced HCC stage, Child-Pugh classification grade A, ALBI grade 1, and hepatectomy (versus liver transplantation) were associated with higher HCC recurrence (**Table 2**). On the contrary, old age, male sex, higher ALT level, lower albumin level, advanced HCC stage, and ALBI grades 2 and 3 were related to postoperative mortality. Kaplan-Meier analysis revealed that different prognosis between the two surgical groups. Hepatectomy patients had higher hepatocellular carcinoma (HCC) recurrent rate than liver transplantation patients (P<0.001, **Figure 2A**). However, hepatectomy patients had a similar disease specific survival rate to liver transplant patients (P>0.05, **Figure 2B**).

HCC recurrence rate according to the type of surgical approach

After AUROC analysis, we used the optimal WFA+-M2BP cut-off value (WFA+-M2BP COI =0.75) to evaluate the HCC recurrence rate and demonstrated that a higher WFA+-M2BP level was associated with a higher tumor recurrence rate in the hepatectomy group (P<0.001, **Figure 3A**). However, no statistical significance was observed in the liver transplantation group by cut-off value of (WFA+-M2BP COI =3.81) (P>0.05, **Figure 3B**). Univariate and multivariable Cox regression analyses were performed to further evaluate the HCC recurrence rate according to the type of surgical approach (partial hepatectomy and liver transplantation)

Table 2. Demographic data and factor distribution by recurrence and mortality rate

Variable	Classification	Recurrence		*P*-value	Death		*P*-value
		Non-recurrence (n=286)	Recurrence (n=174)		Alive (n=400)	HCC death (n=60)	
		N (%)/median (IQR)	N (%)/median (IQR)		n(%)/median (IQR)	N (%)/median (IQR)	
Age	(continuous)	60.5 (12)	64.0 (15)	0.0052	61.0 (13.0)	65.5 (13.5)	0.0277
Gender	Female	79 (73.1%)	29 (26.9%)	0.0072	100 (92.6%)	8 (7.4%)	0.0468
	Male	207 (58.8%)	145 (41.2%)		300 (85.2%)	52 (14.8%)	
ALT	(IU/L)	48.9 (94.7)	71.3 (103.2)	<0.0001	33.0 (30.5)	46 (34.5)	0.0023
AFP	(ng/mL)	8.1 (43.1)	14.1 (172.0)	0.0018	9.8 (4.3)	12.0 (6.1)	0.2808
Albumin	g/dL	4.0 (1.0)	4.1 (0.6)	0.0830	4.1 (0.8)	3.8 (1.0)	0.0339
Total bilirubin	mg/dL	0.8 (0.6)	0.8 (0.4)	0.0142	0.8 (0.5)	0.9 (0.6)	0.4454
Hepatitis	B(-) and C(-)	45 (69.2%)	20 (30.8%)	0.3387	57 (87.7%)	8 (12.3%)	0.3289
	B(+) and C(-)	149 (61.6%)	93 (38.4%)		215 (88.8%)	27 (11.2%)	
	B(-) and C(+)	80 (58.4%)	57 (41.6%)		116 (84.7%)	21 (15.3%)	
	B(+) and C(+)	12 (75.0%)	4 (25.0%)		12 (75.0%)	4 (25.0%)	
PT	(INR)	1.07 (0.15)	1.04 (0.08)	0.0083	1.05 (0.11)	1.07 (0.14)	0.3591
WFA+-M2BP	(COI)	1.4 (3.3)	1.2 (1.5)	0.1141	1.3 (1.9)	1.8 (3.3)	0.1372
Fibrosis score	F0	17 (70.8%)	7 (29.2%)	0.0181	23 (95.8%)	1 (4.2%)	0.3026
	F1	51 (64.6%)	28 (35.4%)		69 (87.3%)	10 (12.7%)	
	F2	48 (53.9%)	41 (46.1%)		72 (80.9%)	17 (19.1%)	
	F3	24 (46.2%)	28 (53.8%)		46 (88.5%)	6 (11.5%)	
	F4	146 (67.6%)	70 (32.4%)		190 (88.0%)	26 (12.0%)	
AJCC stage	1	114 (75.0%)	38 (25.0%)	<0.0001	142 (93.4%)	10 (6.6%)	<0.0001
	2	144 (61.5%)	90 (38.5%)		205 (87.6%)	29 (12.4%)	
	3	28 (39.4%)	43 (60.6%)		53 (74.7%)	18 (25.3%)	
	4	0 (0.0%)	3 (100.0%)		0 (0.0%)	3 (100.0%)	
Child-Pugh	A	229 (57.7%)	168 (42.3%)	<0.0001	347 (87.4%)	50 (12.6%)	0.1678
classification	B	37 (86.1%)	6 (13.9%)		34 (79.1%)	9 (20.9%)	
	C	20 (100.0%)	0 (0.0%)		19 (95.0%)	1 (5.0%)	
ALBI grade	1	156 (59.1%)	108 (40.9%)	0.0005	241 (91.3%)	23 (8.7%)	0.0059
	2	99 (60.7%)	64 (39.3%)		132 (81.0%)	31 (19.0%)	
	3	31 (93.9%)	2 (6.1%)		27 (81.8%)	6 (18.2%)	
Surgical type	Hepatectomy	193 (54.1%)	164 (45.9%)	<0.0001	312 (87.4%)	45 (12.6%)	0.6032
	Transplant	93 (90.3%)	10 (9.7%)		88 (85.4%)	15 (14.6%)	

ALBI: Albumin-Bilirubin, IQR: interquartile range (Q3-Q1).

(**Table 3**). In hepatectomy patients, the ALT level, chronic hepatitis C status, prothrombin time, liver fibrosis status, AJCC tumor stage, ALBI grade, and WFA+-M2BP level were all important factors for HCC recurrence in the univariate analysis. In the multivariable analysis, only the higher ALT level (HR 1.002, 1.001-1.003), chronic viral hepatitis C status (HR 1.76, 1.03-3.02), advanced AJCC tumor stage (HR 1.84, 1.47-2.30), and higher WFA+-M2BP level (HR 1.87, 1.23-2.83) were important factors for HCC recurrence. In liver transplantation patients, only chronic dual viral hepatitis B and C status (HR 8.35, 0.92-76.21), lower fibrotic stages (HR 0.34, 012-0.91) and advanced AJCC tumor stage (HR 7.13, 1.35-37.83), were important and independent factors for HCC recurrence in the multivariable analysis (**Table 3**).

Disease specific survival rate according to the type of surgical approach

In the survival analysis, a higher WFA+-M2BP cut-off value (WFA+-M2BP COI ≥1.43) was associated with a higher mortality risk (P=0.0078) in the hepatectomy patients (**Figure 4A**). However, there is no association of WFA+-M2BP value (WFA+-M2BP COI ≥3.81) with mortality risk in transplant patients (**Figure 4B**). In the univariate analysis, WFA+-M2BP level was a significant factor affecting the survival of patients who underwent partial hepatectomy (**Table 4**). However, it no longer serves as an important factor in the multivariable analysis. Male sex (HR 3.62, 1.10-11.94), advanced HCC stage (HR 2.10, 1.39-3.19), and ALBI grade (HR 4.24, 0.91-19.73) were important factors affecting patient's survival in the multivariable analysis.

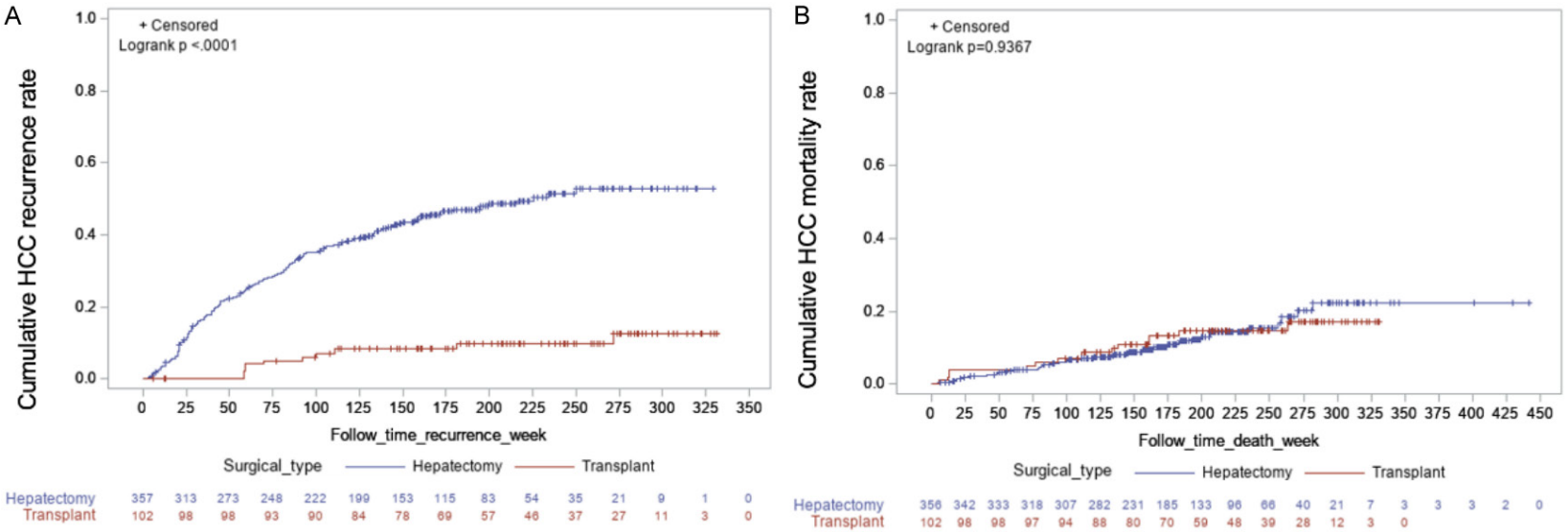

Figure 2. Differences in the prognosis between the two surgical groups. A. Hepatectomy patients had higher hepatocellular carcinoma (HCC) recurrent rate than liver transplantation patients (P<0.001). B. Hepatectomy had a similar survival rate with liver transplantation for HCC treatment (P>0.05).

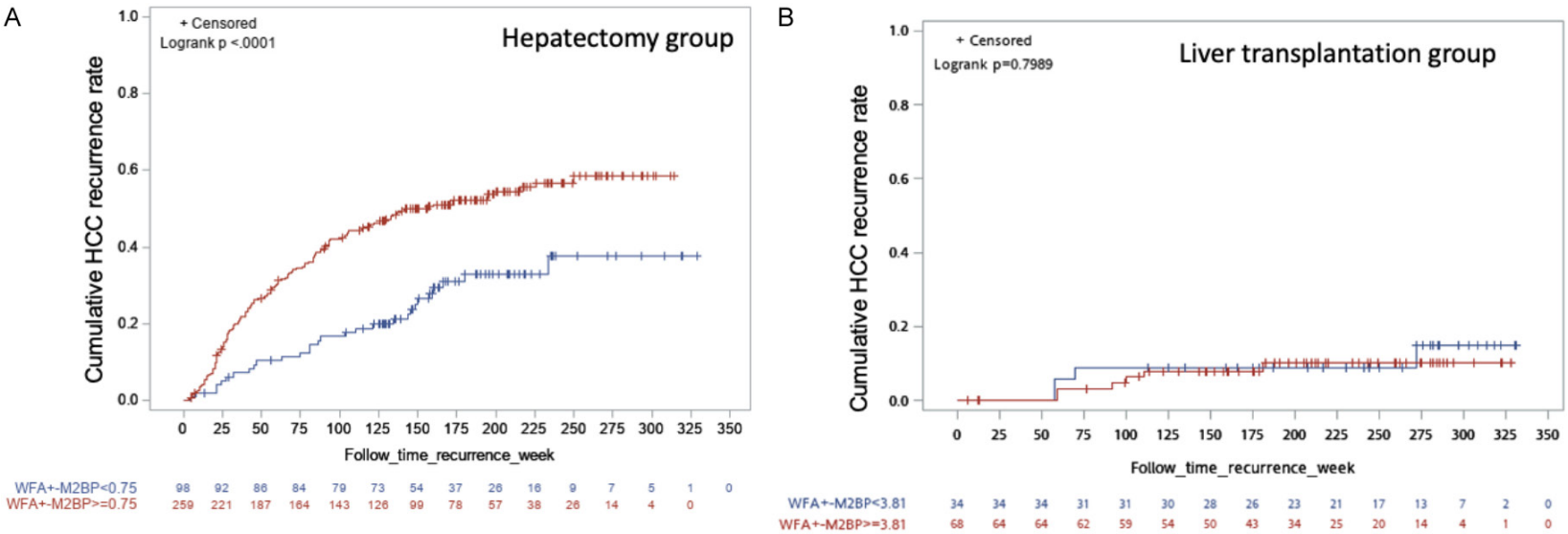

Figure 3. Clinical significance of serum WFA+-M2BP in HCC recurrence in different surgical types after calculating the optimal cut-off level. Higher WFA+-M2BP levels (COI ≥0.75) had higher tumor recurrence rate in the hepatectomy group (P<0.001, A), but no statistical significance was observed in liver transplantation group by cut of levels WFA+-M2BP levels (COI =3.81) (P>0.05, B).

Table 3. Univariate and multivariable Cox regression analyses of HCC recurrence by surgical type

Variable	Type	Hepatectomy				Transplant			
		Univariate		Multivariable		Univariate		Multivariable	
		HR (95% CI)	*P*-value	adj. HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	adj. HR (95% CI)	*P*-value
Age		1.01 (1.00, 1.03)	0.0779	1.01 (0.99, 1.02)	0.3352	0.95 (0.88, 1.03)	0.1863	0.94 (0.85, 1.06)	0.3128
Gender	M vs F	1.40 (0.92, 2.13)	0.1134	1.52 (0.98, 2.37)	0.0630	1.48 (0.38, 5.73)	0.5701	0.64 (0.13, 3.32)	0.5995
ALT		1.00 (1.001, 1.003)	0.0008	1.002 (1.001, 1.003)	0.0006	100 (1.001, 1.004)	0.5988	---	
AFP		1.00 (1.00, 1.00)	0.2610	---		1.01 (0.99, 1.02)	0.2327	---	
Total bilirubin		1.13 (0.84, 1.52)	0.4068	---		0.81 (0.51, 1.30)	0.3823	---	
Hepatitis	B(+)C(-)	1.21 (0.73, 2.01)	0.0158	1.14 (0.68, 1.90)	0.0139	0.88 (0.16, 4.81)	0.0780	1.06 (0.15, 7.67)	0.0489
[vs. B(-)C(-)]	B(-)C(+)	1.84 (1.08, 3.14)		1.76 (1.03, 3.02)		0.38 (0.05, 2.69)		0.30 (0.04, 2.67)	
	B(+)C(+)	0.45 (0.11, 1.94)		0.31 (0.07, 1.36)		4.86 (0.68, 34.75)		8.35 (0.92, 76.21)	
PTINR		12.09 (1.98, 74.02)	0.0070	---		0.32 (0.02, 5.18)	0.4197	---	
Fibrosis score		1.19 (1.06, 1.34)	0.0035	---		0.34 (0.18, 0.65)	0.0011	0.34 (.012, 0.91)	0.0327
AJCC stage		1.90 (1.54, 2.36)	<0.0001	1.84 (1.47, 2.30)	<0.0001	2.96 (0.98, 8.97)	0.0546	7.13 (1.35, 37.83)	0.0210
Child-Pugh	B vs. A	2.38 (0.88, 6.44)	0.0869	---		0.30 (0.06, 1.43)	0.3197	---	
classification	C vs. A	---				---			
ALBI grade	2 vs. 1	1.49 (1.08, 2.06)	0.0495	---		0.46 (0.13, 1.63)	0.4814	---	
	3 vs. 1	1.48 (0.37, 6.02)				---			
WFA+-M2BP*		2.18 (1.46, 3.26)	0.0001	1.87 (1.23, 2.83)	0.0033	0.88 (0.25, 3.14)	0.8418	1.96 (0.34, 11.40)	0.4562

HR, hazard ratio; CI, confidence interval; ALT, alanine aminotransferase; PT, prothrombin time; INR, international normalized ratio; ALBI, albumin-bilirubin. *The cut-off values of WFA+-M2BP for recurrence were used 0.75 and 3.81 for hepatectomy and transplant, respectively.

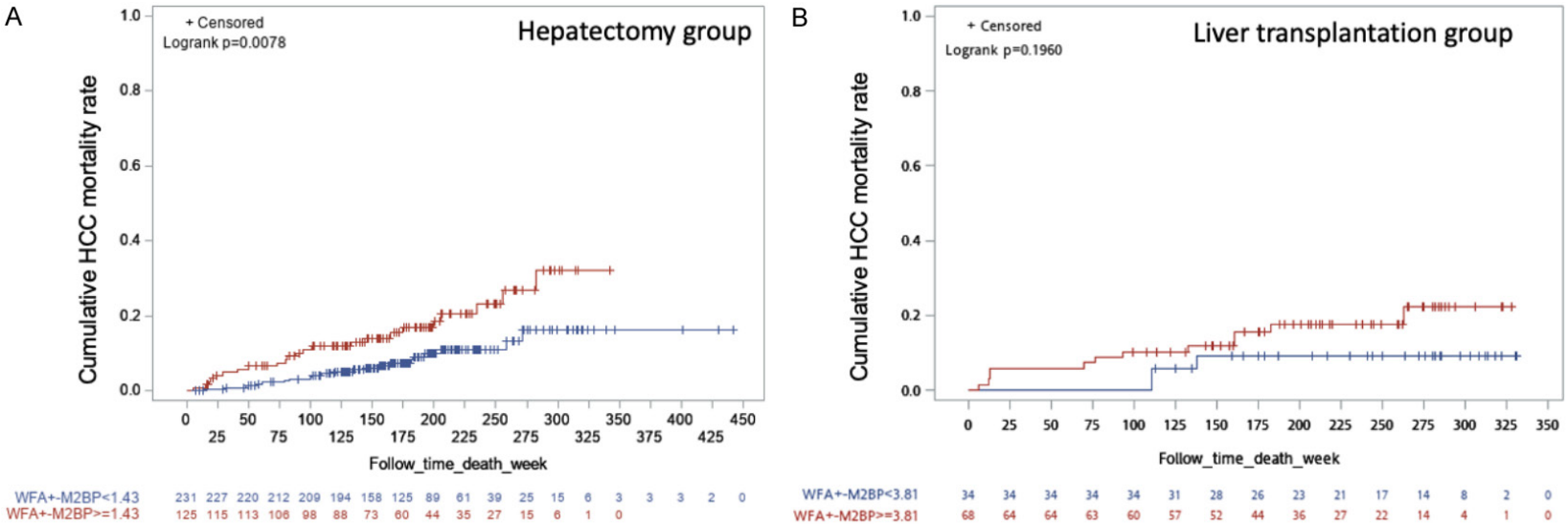

Figure 4. Clinical significance of serum WFA+-M2BP in patient's survival in the two surgical groups after calculating the optimal cut-off level. A higher WFA+-M2BPGi cut-off value (WFA+-M2BP COI ≥1.43) had a higher mortality risk (P=0.0078) in the hepatectomy group than the lower WFA+-M2BP value (A). There was no statistical significance observed in liver transplantation group by cut of levels WFA+-M2BP levels (COI =3.81) (P>0.05, B).

Table 4. Univariate and multivariable Cox regression for HCC death by different surgical types

Variable	Type	Hepatectomy				Transplant			
		Univariate		Multivariable		Univariate		Multivariable	
		HR (95% CI)	*P*-value	adj. HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	adj. HR (95% CI)	*P*-value
Age		1.03 (1.00, 1.06)	0.0383	1.03 (1.00, 1.06)	0.0660	1.02 (0.94, 1.10)	0.6618	1.05 (0.97, 1.14)	0.2328
Gender	M vs F	3.22 (1.00, 10.41)	0.0507	3.62 (1.10, 11.94)	0.0346	1.23 (0.42, 3.60)	0.7052	1.13 (0.34, 3.70)	0.8443
ALT		1.002 (1.00, 1.004)	0.2089	---		1.001 (0.998, 1.004)	0.6257	---	
AFP		1.00 (1.00, 1.00)	0.0234	---		1.01 (1.00, 1.02)	0.0340	---	
Total bilirubin		1.58 (1.01, 2.45)	0.0432	---		0.88 (0.67, 1.17)	0.3906	---	
Hepatitis	B(+)C(-)	0.71 (0.30, 1.65)	0.7498	---		2.26 (0.26, 19.32)	0.0345	2.70 (0.30, 24.21)	0.0328
[vs. B(-)C(-)]	B(-)C(+)	0.96 (0.39, 2.39)				2.31 (0.28, 19.23)		1.94 (0.22, 17.09)	
	B(+)C(+)	0.61 (0.08, 4.99)				13.40 (1.39, 129.22)		14.10 (1.41, 140.9)	
PTINR		58.17 (4.51, 750.34)	0.0018	---		0.73 (0.12, 4.47)	0.7344	---	
Fibrosis score		1.05 (0.83, 1.31)	0.7051	---		0.47 (0.29, 0.78)	0.0033	0.28 (0.14, 0.55)	0.0002
AJCC stage		2.68 (1.76, 4.06)	<0.0001	2.10 (1.39, 3.19)	0.0005	2.10 (0.85, 5.20)	0.1103	---	
Child-Pugh	B vs. A	4.93 (1.18, 20.50)	0.0284	---		---		---	
classification	C vs. A	---							
ALBI grade	2 vs. 1	3.12 (1.70, 5.73)	0.0002	2.56 (1.35, 4.88)	0.0089	0.82 (0.22, 3.08)	0.9535	---	
	3 vs. 1	8.19 (1.90, 35.38)		4.24 (0.91, 19.73)		0.83 (0.19, 3.71)			
WFA+-M2BP*		2.21 (1.22, 3.99)	0.0088	1.47 (0.77, 2.81)	0.2379	2.33 (0.66, 8.28)	0.1908	3.75 (0.80, 17.59)	0.0943

ALBI: Albumin-Bilirubin; *The cut-off values of WFA+-M2BP for death were used 1.43 and 3.81 for hepatectomy and transplant, respectively.

In the liver transplantation group, only dual B and C virus infection (HR 14.10, 1.41-140.9), and liver fibrosis status (HR 0.28, 0.14-0.55) were the most important factors affecting patient's survival in the multivariable analysis. WFA+-M2BP level was associated with patient's survival in the multivariable analysis, with a borderline significance (HR 3.75, 0.80-17.59, P=0.0943) (**Table 4**).

Discussion

In real-world practice, the early identification of advanced liver fibrosis and cirrhosis is extremely important because of the increasing risk of ascites, variceal bleeding, hepatic encephalopathy, and HCC [8]. It warns us to initiate antiviral treatment to cease the progression of fibrosis and decrease the risk of HCC [26, 27]. Therefore, a serum marker with high accuracy and specificity for diagnosing liver fibrosis is important for selecting the appropriate medical treatment. In the present study, we provided strong evidence that WFA+-M2BP is a reliable serum marker to predict the liver fibrosis (**Figure 1**). Actually, correlation between WFA+-M2BP and liver fibrosis has been documented in many studies. However, the results were mostly compared with specimens of liver biopsy that sampling errors might happen [3]. Reference of hepatic fibrosis obtained from surgical specimens is the most reliable and accurate method for correlation studies. The present study represents the first study combining specimens of both hepatectomy and transplantation. The results were expected to lead to a more acute interpretation of WFA+-M2BP as a fibrotic marker.

Table 2 shows the risk factors for patients' prognosis after surgery (hepatectomy and liver transplantation). We found that advanced liver fibrosis and advanced HCC stages were associated with HCC recurrence. In addition, we also found that ALBI grade was an extremely important risk factor for patient's survival after surgery, which is consistent with the reports of previous studies [28, 29]. Furthermore, we also provided the clinical data to demonstrate that patients who underwent partial hepatectomy had a higher HCC recurrence rate but had a similar survival rate with those who underwent liver transplantation. Resection and liver transplantation are both curative treatments for early-stage HCC based on the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL) guidelines [19, 20]. Patients who received surgical resection mostly belong to early stages of HCC. Higher recurrence of HCC is expected in patients after hepatectomy due to backgrounds of fibrotic liver when compared with liver transplant patients. As there is relatively short follow up period in the present study (median 186.5 weeks for survival), multimodality of HCC therapies might rescue recurrent patients from death in a short period of time. Therefore, we are unable to see the difference of HCC related death between patients with surgical resection and liver transplantation in the present study. Longer follow up is needed. Clinical doctors should provide information about the benefits and disadvantages of hepatectomy and transplantation to patients [30].

The serum WFA+-M2BP has been used in clinical practice in previous studies. It is a useful predictor of HCC development in patients with various liver diseases [31-34]. Elevated serum WFA+-M2BP is a strong positive predictor of HCC development in various viral hepatitis [31-34]. The prognostic role of WFA+-M2BP in HCC patients after curative treatment has also been investigated [16, 18, 35]. The results always indicated that elevated serum WFA+-M2BP predicted poor outcome of HCC patients after curative treatment. When compared with previous studies, the present study provided additional role of serum WFA+-M2BP in the prognosis of HCC recurrence after curative hepatectomy, in addition to other traditionally prognostic factors, such as gender, viral hepatitis status, as well as AJCC tumor staging. A similar result was also reported by Kim and Toyoda studies [16, 18]. Our study examines the clinical application and validates the importance of WFA+-M2BP in the real-world setting without any modification.

In terms of patients' survival after curative resection of HCC, several studies reported that serum WFA+-M2BP level was also an important prognostic factor of survival after a curative hepatic resection for HCC [17, 18, 35]. In line with previous studies, the present study demonstrated that WFA+-M2BP was another important factor affecting patient's survival after hepatectomy in the univariate analysis. However, it is not an independent factor when

compared with other important factors such as ABLI and AJCC stages. The ALBI grade is an important factor for predicting patient's survival after various treatments [36, 37]. This result was compatible with those of a larger study conducted by Ho et al., which demonstrated that ALBI grade could predict HCC recurrence in patients with HCC undergoing surgical resection [38]. Therefore, the HCC stage must be carefully evaluated before the initiation of treatment and that the most appropriate treatment must be selected for patients [39, 40]. The difference of the prognostic role of WFA+-M2BP on HCC survival might be attributed to different study population and statistical methods. Usually, a multivariable adjustment is needed to get the final conclusion in a retrospective study.

We selected the UCSF criteria for liver transplantation as it has an equivalent survival rate and benefits a greater number of patients compared with the Milan criteria [22]. The serum and specimens were stored during the operation. As a result, the data on patients' serum laboratory results, liver fibrosis status, and HCC pathology were collected accurately. There have been two studies dealing with the issue of WFA+-M2BP and liver transplantation [41, 42]. All of the studies used WFA+-M2BP as a fibrotic marker to evaluate graft fibrosis after transplantation. They concluded that WFA+-M2BP is an accurate, non-invasive detection method for evaluating fibrosis after transplantation. The aim and design of the present study is quite different from the previous ones. Actually, it is the first and novel study to see if pre-transplant serum WFA+-M2BP levels could predict HCC recurrence and survival after transplantation. You can see that the levels of WFA+-M2BP in transplant patients were much higher than that of hepatectomy patients (**Table 1**). It might be attributed to the very high percentages of advanced liver fibrosis in transplant patients. By the Cox regression hazard model, elevated WFA+-M2BP levels did not associate with HCC recurrence, but predict survival after transplantation with a borderline significance in multivariable analysis (**Tables 3** and **4**). It might indicate since liver transplantation allowed the total removal of an old cirrhotic or advanced fibrotic liver; hence, the effect of liver fibrosis and WFA+-M2BP would both decrease [43]. Instead, viral hepatitis status and AJCC staging still played an important role on the outcomes of HCC related transplants. Notably, original fibrotic stages were negatively correlated with both HCC recurrence and survival after transplantation (**Tables 3** and **4**). It is quite interesting to notice that levels of WFA+-M2BP did not completely reflect the original degrees of liver fibrosis in transplant patients. The difference of mechanism needs further elucidation in the future.

However, our study has several limitations. First, our study only included 460 patients. However, the number of curative hepatectomy and liver transplantation patients is not relatively small as the patients should meet the UCSF criteria (solitary tumor ≤6.5 cm, or ≤3 nodules with the largest lesion size of ≤4.5 cm and a total tumor diameter of ≤8 cm) or the AASLD or EASL clinical practice guidelines for diagnosing early-stage HCC without radiofrequency ablation [20, 21, 39, 44]. On the contrary, this was a retrospective study and not a prospective study. In the future, a prospective study should be conducted to further validate the role of WFA+-M2BP.

Conclusions

Serum WFA+-M2BP is a reliable marker for liver fibrosis in the present study. It is also reliable marker to predict prognosis of HCC after surgical resection. However, the prognostic role of WFA+-M2BP in HCC related transplants is equivocal, which is quite different from that of surgical resection.

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Disclosure of conflict of interest

None.

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