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Preoperative Aspartate Aminotransferase to White Blood Cell Count Ratio Predicting Postoperative Outcomes of Hepatocellular Carcinoma

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Abstract: Effective biomarkers for predicting prognosis of hepatocellular carcinoma (HCC) patients after hepatectomy is urgently needed. The purpose of this study is to evaluate the value of the preoperative peripheral aspartate aminotransferase to white blood cell count ratio (AWR) for the prognostication of patients with HCC.

Clinical data of 396 HCC patients who underwent radical hepatectomy were retrospectively analyzed. The patients were divided into the low-AWR group (AWR ≤ 5.2) and the high-AWR group (AWR >5.2); univariate analysis, Kaplan–Meier method analysis, and the multivariate analysis by Cox regression were conducted, respectively.

The results showed that AWR was associated with alpha-fetoprotein (AFP), tumor size, Barcelona clinic liver cancer (BCLC) stage, portal vein tumor thrombus (PVTT), and alanine aminotransferase (ALT) in HCC. AWR > 5.2, AFP > 100 ng/mL, size of tumor >6 cm, number of multiple tumors, B-C of BCLC stage, PVTT, and distant metastasis were predictors of poorer disease-free survival (DFS) and overall survival (OS). Except for recurrence, which was an independent predictor for OS only, AWR >5.2, size of tumor >6 cm, and PVTT were independent predictors of both DFS and OS.

We concluded that preoperative AWR > 5.2 was an adverse predictor of DFS and OS in HCC after hepatectomy, AWR might be a novel prognostic biomarker in HCC after curative resection.

Abbreviations: AFP = alpha-fetoprotein, ALT = alanine aminotransferase, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, DFS = disease-free survival, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HR = hazard ratio, NK = natural killer, NLR = neutrophil lymphocyte ratio, OS = overall survival, PVTT = portal vein tumor thrombus, ROC = receiver operating characteristic, Tregs = regulatory T cell.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers that pose severe threats on the health and lives of people around the world and has become a major cause of global morbidity and mortality. To date, surgical resection is considered as an important curative treatment for HCC, and the postoperative survival rate of HCC patients has improved over time. However, owing to the difficulty in early diagnosis, the presence of tumor invasiveness and metastasis, and recurrence, the clinical efficacy (prognosis) of HCC is still far from satisfactory. Although serum α-fetoprotein (AFP) levels have been extensively used for the diagnosis and prognostication of HCC, the sensitivity and specificity of AFP in HCC are limited. Therefore, the identification of more effective biomarkers for HCC diagnosis at very early stages and during postoperative monitoring is warranted.

Recent investigations have shown that peripheral blood cells can be used as predictors of prognosis in cancer patients. Peripheral blood white blood cells (leukocytes) mainly include neutrophils, lymphocytes, and monocytes. Several studies have determined that these subsets and subset-based indices could be utilized as prognostic factors. For instance, preoperative neutrophil count in peripheral blood has proposed as a prognostic predictor for poor survival in patients with metastatic melanoma[1] and non-metastatic upper urinary tract cancer.[2] High monocyte count is an independent factor of poor prognosis in patients with colorectal liver metastasis, cervical cancer, melanoma, and HCC.[1,3–5] A high preoperative elevated neutrophil lymphocyte ratio (NLR) is associated with short survival in patients with various malignancies (i.e., colorectal cancer, gastric cancer, breast cancer, and ovarian cancer),[6–10] as well as in HCC patients.[11–13] Nevertheless, studies reporting the prognostic role of leukocytes are limited. To date, no study has established the use of leukocytes and its related indices as prognostic factors for HCC patients.

The present study aimed to investigate the optimal value of aspartate aminotransferase to white blood cell count ratio (AWR) and to evaluate the correlation of preoperative AWR

with clinicopathologic features and prognosis in HCC patients who underwent curative resection.

RESULTS

An Optimal Cutoff Value for Elevated AWR

According to the receiver operating characteristic (ROC) curve, the optimal cutoff value of preoperative AWR that had a relatively high specificity was 5.2. The area under the ROC curves was 0.668, with a 95% confidence interval (95% CI) for the area between 0.626 and 0.732. A cutoff value of 5.2 presented a sensitivity of 67.9% and a specificity of 66.8%.

The Preoperative AWR in Patients with HCC and Its Relationship with Clinical Pathologic Characteristics

Table 1 shows the relationship between preoperative peripheral blood AWR and clinical pathologic characteristics. Two hundred sixty-five patients (66.92%) identified as high-AWR group showed an elevated AWR (>5.2), and 131 patients (33.08%) were identified as the low-AWR (≤5.2) group. Our

results showed that preoperative AWR was closely correlated to serum AFP level (> 100 ng/mL) ($\chi^2 = 7.712$; $P = 0.005$), tumor size (range, >6 cm) ($\chi^2 = 4.750$; $P = 0.029$), BCLC stage (B-C) ($\chi^2 = 5.344$; $P = 0.021$), PVTT ($\chi^2 = 5.434$; $P = 0.020$), and ALT (>38 U/L) ($\chi^2 = 46.863$; $P < 0.001$). No obvious correlations with age, gender, family history, hepatitis B surface antigen (HBsAg), combination of liver cirrhosis, number of tumors, wine-drinking, smoking, distant metastasis of tumors, and postoperative recurrence were observed ($P > 0.05$).

As we all know, as a relatively mature method, serum AFP level not only has been extensively used for diagnosis but also for prognostication of HCC. The association between AWR and serum AFP level is also shown in Table 1. 43.43% patients showed an elevated AWR (>5.2) and a higher serum AFP level (>100 ng/mL), whereas 23.49% patients had an elevated AWR (>5.2) and a lower serum AFP level (≤100 ng/mL). The percentage of patients with a lower AWR (≤5.2) and a higher serum AFP level (>100 ng/mL), and with a lower AWR (≤5.2) and a lower serum AFP level (≤ 100 ng/mL) was 16.67% and 16.41%, respectively. In patients with a lower serum AFP level (≤100 ng/mL), 58.9% of the patients had an elevated AWR (>5.2) and 41.1% showed a lower AWR (≤5.2). In patients with a higher serum AFP level (>100 ng/mL), 72.3% of the

TABLE 1. Correlation Between Clinicopathologic Variables and AWR in HCC

Clinical Characteristic	Variable	No. of Patients	AWR		x^2	*P* value
			≤5.2 N (%)	>5.2 N (%)		
Age (years)	≤ 55	272	94 (34.6)	178 (65.4)	0.857	0.355
	>55	124	37 (29.8)	87 (70.2)		
Gender	Female	49	17 (34.7)	32 (65.3)	0.066	0.798
	Male	347	114 (32.9)	233 (67.1)		
Family history	No	341	112 (32.8)	229 (67.2)	0.062	0.804
	Yes	55	19 (34.5)	36 (65.5)		
HBsAg	Negative	62	27 (43.5)	35 (56.5)	3.638	0.056
	Positive	334	104 (31.1)	230 (68.9)		
AFP (ng/mL)	≤ 100	158	65 (41.1)	93 (58.9)	7.712	0.005
	>100	238	66 (27.7)	172 (72.3)		
Tumor size (cm)	≤ 6	169	66 (39.1)	103 (60.9)	4.750	0.029
	>6	227	65 (28.6)	162 (71.4)		
Cirrhosis	No	34	12 (35.3)	22 (64.7)	0.082	0.774
	Yes	362	119 (32.9)	243 (67.1)		
Number of tumors	Single	269	93 (34.6)	176 (65.4)	0.843	0.359
	Multiple	127	38 (29.9)	89 (70.1)		
Wine drinking	No	191	62 (32.5)	129 (67.5)	0.064	0.800
	Yes	205	69 (33.7)	136 (66.3)		
Smoking	No	200	62 (31.0)	138 (69.0)	0.790	0.374
	Yes	196	69 (35.2)	127 (64.8)		
BCLC stage	0-A	188	73 (38.8)	115 (61.2)	5.344	0.021
	B-C	208	58 (27.9)	150 (72.1)		
PVTT	No	298	108 (36.2)	190 (63.8)	5.434	0.020
	Yes	98	23 (23.5)	75 (76.5)		
Distant metastasis	No	352	112 (31.8)	240 (68.2)	2.281	0.131
	Yes	44	19 (43.2)	25 (56.8)		
Recurrence	No	259	79 (30.5)	180 (69.5)	2.249	0.134
	Yes	137	52 (38.0)	85 (62.0)		
ALT (U/L)	≤38	218	104 (47.7)	114 (52.3)	46.863	<0.001
	>38	178	27 (15.2)	151 (84.8)		

AFP = alpha-fetoprotein, ALT = alanine aminotransferase, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, HBsAg = hepatitis B surface antigen, PVTT = portal vein tumor thrombus.
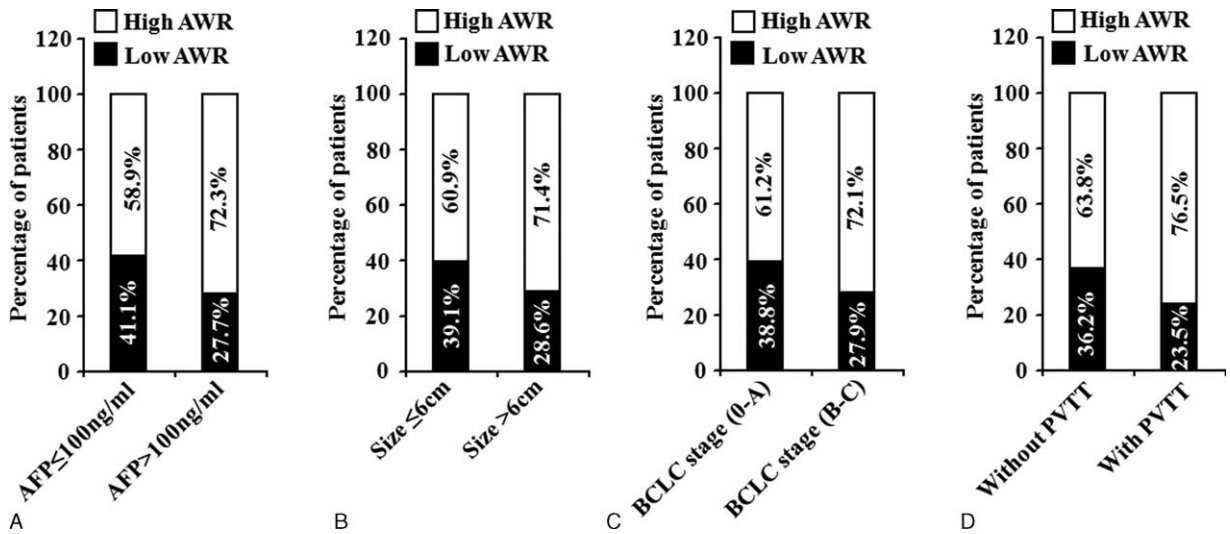
FIGURE 1. (A) Percentage distribution of patients with elevated AWR (>5.2) or lower AWR (≤5.2) in patients with different serum AFP levels (≤100 vs. >100 ng/mL). (B) Percentage distribution of patients with elevated AWR (>5.2) or lower AWR (≤5.2) in patients with different tumor sizes (≤6 vs. >6 cm). (C) Percentage distribution of patients with elevated AWR (>5.2) or lower AWR (≤5.2) in patients at different BCLC stages (0-A vs. B-C). (D) Percentage distribution of patients with elevated AWR (>5.2) or lower AWR (≤5.2) in patients with or without PVTT. AFP = alpha-fetoprotein, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, PVTT = portal vein tumor thrombus.

patients presented an elevated AWR (>5.2) and 27.7% showed a lower AWR (≤5.2) (Figure 1A). These results indicated that a high AWR (>5.2) had a positive correlation with a high AFP level (>100 ng/mL).

The associations between AWR and tumor size, BCLC stage, and PVTT were also further analyzed. In patients with a tumor size of ≤6 cm, 60.9% of the patients had an elevated AWR (>5.2) and 39.1% showed a lower AWR (≤5.2). In patients with a tumor size of >6 cm, the percentage of patients with an elevated AWR (>5.2) and a lower AWR (≤5.2) was 71.4% and 28.6%, respectively (Figure 1B). In terms of BCLC stage, 61.2% of the patients with an elevated AWR (>5.2) and 38.8% of the patients with a lower AWR (≤5.2) were at the 0-A stage, whereas 72.1% patients with an elevated AWR (>5.2) and 27.9% of patients with a lower AWR (≤5.2) were at the B-C

stage (Figure 1C). Similarly, in patients without PVTT, 63.8% of the patients had an elevated AWR (>5.2) and 36.2% showed a lower AWR (≤5.2). In patients with PVTT, the percentage of patients with an elevated AWR (>5.2) and a lower AWR (≤5.2) was 76.5% and 23.5%, respectively (Figure 1D). These results also demonstrated that a high AWR was positively correlated with tumor size (>6 cm), BCLC stage (B-C), and PVTT.

Association of AWR or Clinical Pathologic Index between Postoperative DFS and OS

Kaplan–Meier survival analysis showed that an AWR >5.2 was associated with a shorter disease-free survival (DFS) (Figure 2A) and OS (Figure 2B). Univariate analysis revealed a distinct association between clinical parameters and
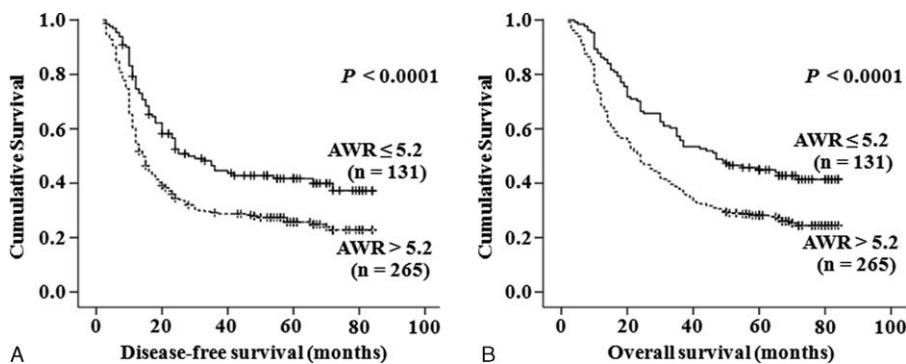
FIGURE 2. Kaplan–Meier curves analysis of disease-free survival and overall survival in 396 HCC patients after hepatectomy. The patients were divided into two groups, AWR ≤5.2 and AWR >5.2, based on the optimal cutoff value of AWR. The solid line represents an AWR ≤5.2, whereas the dashed line represents an AWR > 5.2. (A) DFS rate of patients with AWR >5.2 was shorter than those with an AWR ≤5.2 (*P* <0.0001, log-rank test). (B) OS rate of patients with an AWR >5.2 was also shorter than those with an AWR ≤5.2 (*P* < 0.0001, log-rank test). AWR = aspartate aminotransferase to white blood cell count ratio, DFS = disease-free survival, HCC = hepatocellular carcinoma, OS = overall survival.

TABLE 2. Association of AWR, Clinical Parameters, and Disease-Free Survival/Overall Survival

Clinical Characteristics	Category	No. of Patients	Disease-Free Survival (months)			Overall Survival (months)		
			Mean	95% CI	P value	Mean	95% CI	P value
AWR	≤5.2	131	44.03	38.14−49.92	<0.001	50.57	45.27−55.87	<0.001
	>5.2	265	30.94	27.13−34.77		36.51	32.83−40.19	
Age (years)	≤55	272	34.81	30.86−38.75	0.629	40.07	36.30−43.84	0.335
	>55	124	36.29	30.45−42.12		43.57	38.19−48.93	
Gender	Female	49	42.72	32.47−52.98	0.118	48.84	39.59−58.08	0.065
	Male	347	34.30	30.87−37.73		40.10	36.84−43.37	
Family history	No	341	34.23	30.75−37.71	0.158	40.31	37.01−43.61	0.185
	Yes	55	41.63	32.40−50.86		46.47	37.78−55.17	
HBsAg	Negative	62	36.10	27.99−44.21	0.496	42.28	34.61−49.94	0.705
	Positive	334	35.02	31.45−38.59		40.95	37.58−44.33	
AFP (ng/mL)	≤100	158	40.17	34.83−45.51	0.011	47.01	42.17−51.86	0.003
	>100	238	31.95	27.88−36.02		37.28	33.34−41.22	
Tumor size (cm)	≤6	169	49.81	44.65−54.97	<0.001	56.43	51.94−60.93	<0.001
	>6	227	24.58	20.94−28.21		29.82	26.24−33.39	
Cirrhosis	No	34	33.90	22.60−45.21	0.598	39.92	29.58−50.25	0.818
	Yes	362	35.37	31.96−38.79		41.29	38.05−44.53	
Number of tumors	Single	269	39.39	35.32−43.46	<0.001	45.63	41.87−49.38	<0.001
	Multiple	127	26.42	21.31−31.53		31.80	26.72−36.88	
Wine drinking	No	191	34.79	30.04−39.54	0.737	40.85	36.32−45.37	0.855
	Yes	205	35.66	31.16−40.17		41.45	37.22−45.68	
Smoking	No	200	35.51	30.88−4014	0.687	41.80	37.43−46.17	0.690
	Yes	196	34.82	30.20−39.44		40.52	36.14−44.89	
BCLC stage	0-A	188	47.03	42.04−52.02	<0.001	53.95	49.61−58.30	<0.001
	B-C	208	24.74	20.99−28.48		29.62	25.87−33.37	
PVTT	No	298	40.64	36.74−44.54	<0.001	47.49	43.94−51.04	<0.001
	Yes	98	19.22	14.70−23.75		21.90	17.38−26.40	
Distant metastasis	No	352	36.92	33.39−4045	0.007	42.77	39.45−46.09	0.002
	Yes	44	21.60	14.83−28.37		28.30	21.02−35.58	
ALT (U/L)	≤38	218	37.71	33.14−42.27	0.123	43.57	39.32−47.83	0.068
	>38	178	32.34	27.71−36.96		38.21	33.76−42.66	
Recurrence	No	259				36.22	32.07−40.37	<0.001
	Yes	137				48.78	44.50−53.07	

AFP = alpha-fetoprotein, ALT = alanine aminotransferase, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, CI = confidence interval, HBsAg = hepatitis B surface antigen, PVTT = portal vein tumor thrombus.

both DFS and OS (Table 2). Mean DFS in patients with AWR ≤5.2 was 44.03 months (95% CI: 38.14−49.92) compared with 30.94 months (95% CI: 27.13−34.77) in patients with AWR >5.2 ($P < 0.001$). Mean OS in the AWR ≤5.2 group and AWR >5.2 group was 50.57 months (95% CI: 45.27−55.87) and 36.51 months (95% CI: 32.83−40.19), respectively ($P < 0.001$). In addition to the high-AWR group (AWR >5.2), serum AFP level >100 ng/mL, size of tumor >6 cm, multiple tumor number, B-C of BCLC stage, combination of PVTT, and distant metastasis were also associated with a shorter DFS and OS ($P < 0.05$), and recurrence was associated with a shorter OS ($P < 0.001$) (Table 2).

Independent Predictors of DFS and OS in the Stepwise Multivariate Cox Proportional Hazards Model

The Cox proportional hazards model was used to examine the association between clinicopathologic factors and DFS/OS after surgical resection of HCC (Table 3). After adjusting other confounding factors, except the recurrence factor for OS, seven associated factors (high AWR, serum AFP level >100 ng/mL, size of tumor >6 cm, multiple tumor number, B-C of BCLC stage, combination of PVTT, and distant metastasis) were analyzed for DFS and OS using the stepwise multivariate Cox proportional hazards model. Three factors were significant in the Cox proportional hazards model. The hazard ratio (HR), 95% CI, and P values of the three independent predictors are listed in Table 3. A stepwise multivariate Cox proportional hazards model revealed that a high AWR (HR: 1.481; 95% CI: 1.128−1.946; $P = 0.005$), size of tumor >6 cm (HR: 2.153; 95% CI: 1.604−2.889; $P < 0.001$), and combination of PVTT (HR: 1.465; 95% CI: 1.059−2.027; $P = 0.021$) were independent predictors for DFS (Table 3). High AWR (HR: 1.505; 95% CI: 1.146−1.977; $P = 0.003$), size of tumor >6 cm (HR: 2.243; 95% CI: 1.670−3.012; $P < 0.001$), and combination of PVTT (HR: 1.722; 95% CI: 1.235−2.401; $P = 0.001$) were independent predictors for OS, and recurrence was an independent predictor for OS only (Table 3).

TABLE 3. Cox Multivariate Proportional Hazards Model of Independent Predictors on Disease-Free and Overall Survival

Variable	Disease-Free Survival		Overall Survival	
	Hazard Ratio (95%CI)	*P* Value	Hazard Ratio (95%CI)	*P* value
AFP ng/mL (>100 vs. ≤100)	1.164 (0.902−1.502)	0.242	1.157 (0.896−1.494)	0.263
Tumor size (cm) (>6 vs. ≤6)	2.153 (1.604−2.889)	<0.001	2.243 (1.670−3.012)	<0.001
Number of tumors (multiple vs. single)	1.083 (0.773−1.518)	0.644	1.106 (0.783−1.563)	0.567
BCLC stage (B-C vs. 0-A)	1.423 (0.970−2.088)	0.071	1.478 (0.996−2.126)	0.051
PVTT (yes vs. no)	1.465 (1.059−2.027)	0.021	1.722 (1.235−2.401)	0.001
Distant metastasis (yes vs. no)	1.181 (0.811−1.720)	0.386	1.237 (0.845−1.810)	0.274
AWR (>5.2 vs. ≤5.2)	1.481 (1.128−1.946)	0.005	1.505 (1.146−1.977)	0.003
Recurrence (yes vs. no)			1.336 (1.038−1.720)	0.025

AFP = alpha-fetoprotein, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, CI = confidence interval, PVTT = portal vein tumor thrombus.

Subgroup Analysis According to AFP level, tumor size, and BCLC stage

To further evaluate the prognostic role of preoperative AWR in different subgroups, we stratified patients according to AFP level (≤ 100 vs. >100 ng/mL), tumor size (≤6 vs. >6 cm), and BCLC stage (0-A vs. B-C). Our results showed that both DFS and OS in patients with a preoperative AWR >5.2 were significantly shorter than those of patients with an AWR ≤5.2 in all of the above subgroups patients regardless of the AFP level, tumor size, or BCLC stage (Figures 3 and 4), indicating that a preoperative AWR >5.2 could serve as a powerful prognostic factor for HCC patients within different risk groups.

DISCUSSION

In the present study, we investigated the prognostic role of the preoperative peripheral white blood cell-based index, AWR, in patients with HCC. We analyzed a large cohort of HCC patients (N = 396) who underwent liver resection, and our results revealed that a preoperative AWR >5.2 was an independent predictor of DFS and DFS in HCC patients after hepatectomy. To rule out empirical selection bias, we used ROC curve analysis to determine the optimal cutoff value of preoperative AWR to predict HCC prognosis following hepatectomy. To our knowledge, we are the first to investigate the prognostic significance of the preoperative AWR in HCC patients; therefore, this report presents a novel finding.

The incidence rate of PVTT in HCC patients was 20% to 70%, and the incidence rate of PVTT examined by microscopy from surgical specimens in HCC patients was as high as 90%. PVTT incidence rate from surgical specimens in HCC patients with tumor size <2 cm was as high as 40.5%. PVTT is the main type of intrahepatic metastasis of HCC, thus posing a critical challenge to the diagnosis and treatment of HCC.[14]

Correlation analysis showed that a high AWR demonstrated a positive correlation with AFP (>100 ng/mL) and PVTT. On one hand, this suggested that in combination with AFP, AWR could significantly improve the positive diagnosis accuracy for HCC. On the other hand, this result may improve our understanding of the correlation between PVTT and poor HCC prognosis. A high AWR might therefore contribute to the progression and intrahepatic metastasis of HCC by PVTT.

Intriguingly, subgroup analysis demonstrated that elevated preoperative AWR (>5.2) predicted poorer prognosis in HCC patients than a lower preoperative AWR (≤5.2), regardless of the AFP level, tumor size, or BCLC stage. This finding highlighted the significance of elevated preoperative AWR (>5.2) as a robust prognostic predictor, which will be available for a larger cohort of postoperative HCC patients, independent of the AFP level (≤100 vs. >100 ng/mL), tumor size (≤6 vs. >6 cm), or BCLC stage (0-A vs. B-C). Therefore, patients with a higher preoperative AWR (>5.2) even in the lower AFP level, small HCC, or early BCLC stage (0-A) subgroups should still be closely monitored because they are highly likely to undergo recurrence. This will facilitate in the early prediction, timely prevention, and generation of favorable clinical outcomes.

Several studies have reported an increased number of CD4$^+$CD25$^+$ regulatory T cells (Tregs) in the peripheral blood of patients with various types of cancer,[15–19] including HCC.[20,21] Tregs, which is a subset of CD4$^+$ T cells, have been demonstrated to play a critical role in maintaining self-tolerance, as well as suppression of antitumor immune responses.[22] Therefore, it is possible that the higher number of Tregs in the peripheral blood of HCC patients hampers the body's immune response against tumor, which in turn promotes HCC progression.

A recent study reported a marked decrease in the number and impaired function of peripheral natural killer (NK) cells in HCC patients. As the major component of innate immunity, NK cells play a crucial role in antitumor immunity. Interestingly, the reduction in number and functional impairment in NK cells was determined to be associated with an increase in the number of suppressive Tregs in HCC patients. Therefore, the increase in the number of peripheral Tregs might have inhibited NK cell antitumor immune responses in HCC patients.[23]

A previous study has also shown that the significant increase in the number of peripheral Tregs in HCC patients resulted in the suppression of the activation, proliferation, and function of CD8$^+$ T cells.[21] CD8$^+$ T cells, which are an indispensable component of adaptive immunity, play a pivotal role in antitumor immunity. The immunosuppression of CD8$^+$ T cells by Tregs might therefore be responsible for HCC progression.[21] Taken together, the increase in the number of peripheral Tregs in HCC patients resulted in the immunosuppression of NK cell-mediated innate antitumor immune response and CD8$^+$ T cell-mediated adaptive antitumor immune, thereby promoting HCC progression and contributing to poor survival of HCC patients. Besides Tregs in HCC, an elegant study by Amedei et al[24] revealed that Th17 (CD4$^+$ IL-17$^+$) cells, a newly identified subset of CD4$^+$ T cells, were
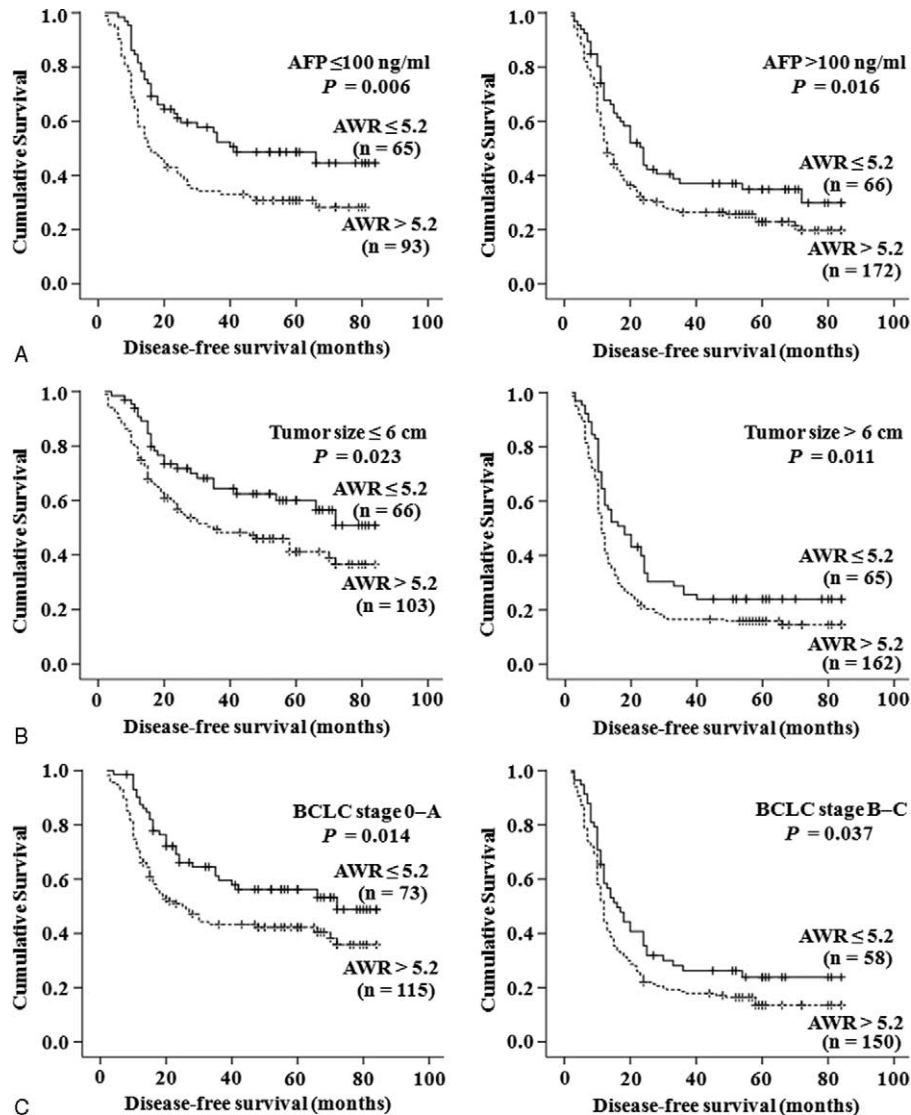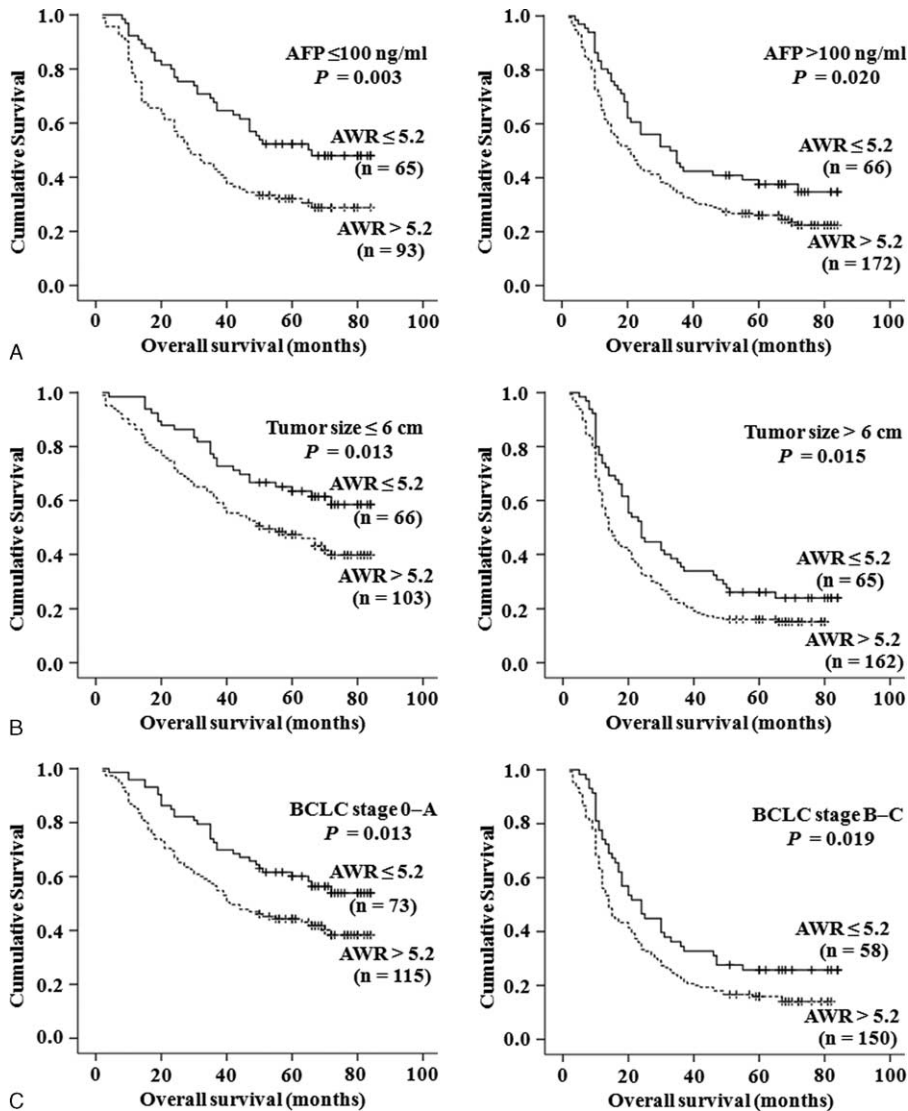
FIGURE 3. Subgroup analysis of DFS of 396 HCC patients after hepatectomy with an AWR ≤5.2 and an AWR >5.2 according to AFP level, tumor size, and BCLC stage. (A) In the AFP level ≤100 ng/mL subgroup of patients, the DFS rate of patients with an AWR >5.2 was significantly shorter than those with an AWR ≤5.2 (P = 0.006, log-rank test). In the AFP level >100 ng/mL subgroup patients, the DFS rate of patients with an AWR >5.2 was also significantly shorter than those with an AWR ≤5.2 (P = 0.016, log-rank test). (B) In the tumor size ≤6 cm subgroup of patients, the DFS rate of patients with an AWR >5.2 was significantly shorter than those with an AWR ≤5.2 (P = 0.023, log-rank test). In the tumor size >6 cm subgroup of patients, the DFS rate of patients with an AWR >5.2 was also significantly shorter than those with an AWR ≤5.2 (P = 0.011, log-rank test). (C) In the BCLC stage (0-A) subgroup of patients, the DFS rate of patients with an AWR >5.2 was significantly shorter than those with an AWR ≤5.2 (P = 0.014, log-rank test). In the BCLC stage (B-C) subgroup of patients, the DFS rate of patients with an AWR >5.2 was also significantly shorter than those with an AWR ≤5.2 (P = 0.037, log-rank test). AFP = alpha-fetoprotein, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, DFS = disease-free survival, HCC = hepatocellular carcinoma.

present in *Helicobacter pylori*-infected patients with gastric adenocarcinoma. Th17 cells response elicited by *H pylori* protein drove a gastric inflammatory response, which contributed to the gastric carcinoma development. The findings of Amedei et al shed light on the relevance between the *H. pylori* infection and gastric cancer. In agreement with this result, recent evidence[25] demonstrated that the frequencies of Th17 cells were significantly increased in the peripheral blood from GC patients, the circulating Th17 cells increased as tumor stage advanced, and the increased Th17 cells correlated with a lower

rate of patient' overall survival. These results indicated that immune responses orchestrated by Th17 subsets may play an important role in controlling the development of cancers.

Inflammation is critical in promoting tumor progression.[26–28] Chronic hepatitis B virus (HBV) infection is the most important factor that is related to HCC development.[29,30] In China, the vast majority (about 95%) of HCC patients are infected with HBV and it is often coupled with liver cirrhosis. Because of hypersplenism, liver cirrhosis is frequently accompanied by severe leukocytopenia in the peripheral

FIGURE 4. Subgroup analysis of overall survival of 396 HCC patients after hepatectomy with an AWR ≤5.2 and an AWR >5.2 according to AFP level, tumor size, and BCLC stage. (A) In the AFP level ≤100 ng/mL subgroup of patients, the OS rate of patients with an AWR >5.2 was significantly shorter than those with an AWR ≤5.2 ($P = 0.003$, log-rank test). In the AFP level >100 ng/mL subgroup of patients, the OS rate of patients with an AWR >5.2 was also significantly shorter than those with an AWR ≤5.2 ($P = 0.020$, log-rank test). (B) In the tumor size ≤6 cm subgroup of patients, the OS rate of patients with an AWR >5.2 was significantly shorter than those with an AWR ≤5.2 ($P = 0.013$, log-rank test). In the tumor size >6 cm subgroup of patients, the OS rate of patients with an AWR >5.2 was also significantly shorter than those with an AWR ≤5.2 ($P = 0.015$, log-rank test). (C) In the BCLC stage (0-A) subgroup of patients, the OS rate of patients with an AWR >5.2 was significantly shorter than those with an AWR ≤5.2 ($P = 0.013$, log-rank test). In the BCLC stage (B-C) subgroup of patients, the OS rate of patients with an AWR >5.2 was also significantly shorter than those with an AWR ≤5.2 ($P = 0.019$, log-rank test). AFP = alpha-fetoprotein, AWR = aspartate aminotransferase to white blood cell count ratio, BCLC = Barcelona clinic liver cancer, HCC = hepatocellular carcinoma, OS = overall survival.

blood.[31] Leukocytopenia is associated with immunosuppression and is believed to contribute to progression of HCC. On the contrary, serum liver biochemistry enzyme indices increased because of liver damage, as exemplified by the AST and ALT levels, ultimately leading to an increase in the AWR. In summary, the present study provides mechanisms underlying the close correlation between preoperative AWR and poor HCC prognosis.

Most importantly, AWR, as a novel predictive tool in clinical practice, presents major advantages of simplicity and

objectivity. The AWR was derived from two readily available laboratory results, namely AST level and white blood cell count, which are routine tests performed in the clinical; therefore, no additional tests are required to determine an AWR. This approach is therefore relatively simple, objective, and inexpensive.

In conclusion, the results of the present study suggest that elevated preoperative AWR is an independent predictor of poor HCC prognosis after hepatic resection. HCC patients with an elevated AWR should therefore be closely monitored and

timely postoperative therapeutic intervention should be conducted to improve their quality of life. The combined use of the existing prognostic biomarkers with the AWR will offer incremental predictive information, as well as substantially enhance the sensitivity and specificity of the prognostication of HCC patients. The present study showed that a tumor size >6 cm and PVTT were also independent prognostic factors. It is likely that when we employ the AWR for the prognostication of HCC patients, these two factors should be taken into consideration to improve the accuracy of the prognoses.

One limitation of the present study was that we evaluated the prognostic role of AWR just by retrospectively analyzing the clinical data of 396 patients from our center. Validation of the prognostic value of AWR in an independent clinical trial involving other centers is therefore warranted.

METHODS

Patients Selection and Clinical Data Collection

The present study involved a total of 396 HCC patients who underwent hepatic resection at the Affiliated Hospital of Guilin Medical University (Guilin, China) from March 1997 to May 2008. These subjects were confirmed to have HCC based on clinical, serological, ultrasonography (US), computerized tomography, magnetic resonance imaging, and pathologic examination, following the Primary Liver Cancer Clinical Diagnosis and Staging Criteria (Ministry of Health, Beijing, China). Clinicopathologic characteristics of these patients, including AWR, age, gender, family history, hepatitis B surface antigen (HBsAg), AFP, size and number of tumors, combined liver cirrhosis, wine drinking, smoking, Barcelona clinic liver cancer (BCLC) stage, portal vein tumor thrombus (PVTT), distant metastasis, recurrence, and alanine aminotransferase (ALT), were collected and presented in Table 1. Preoperative AWR was calculated using the following formula: (AST value/leukocyte count) $\times 10^9$/U.

The present study was conducted as a retrospective analysis of a prospectively collected computerized database in a single medical institution. Among these, 396 patients who met the inclusion criteria were enrolled in the present study. Patients were obviated if they: (1) diagnosed with cholangiocarcinoma or were not primary patients with HCC; (2) died during the perioperative period; (3) did not have detailed clinical data; (4) had clinical evidence of infection, immune system disease, or hematologic disease or used hematology-influenced drugs within 1 month before the initiation of the study; (5) lost contact during the follow-up; or (6) were HIV positive.

The written informed consent was obtained from each patient according to the Declaration of Helsinki. This study protocols were approved by the Hospital Ethics Committee of Guilin Medical University and the methods were carried out in accordance with the approved guidelines.

Follow-Up

Our research group investigated HCC patients by conducting long-term follow-up after surgery, including serum AFP testing and US examination every 2 months and chest radiography every 6 months during the first two postoperative years and at 3- to 6-month intervals thereafter. Computerized tomography or magnetic resonance imaging scans were performed when recurrence was suspected based on abnormal results of the AFP test or US examination. The mean postoperative follow-up time was 36.0 months (median: 20.0 months; range: 2.0–84.0 months). DFS was measured from the date of surgery to the date of recurrence, metastasis, death, or last follow-up. Overall survival (OS) was measured from the date of surgery to the date of death or last follow-up.

Selection of Cutoff Score

To avoid a predetermined cut point, receiver operating characteristic (ROC) curve analysis was applied to define the optimal cutoff score for preoperative AWR. The score was selected as the optimal cutoff value that was closest to the point with maximum joint sensitivity and specificity. Other clinicopathologic parameters used were dichotomized: age ($\leq$ 55 vs. >55 years), gender (female vs. male), family history (no vs. yes), HBsAg (negative vs. positive), AFP level ($\leq$100 vs. >100 ng/mL), tumor size ($\leq$6 vs. >6 cm), cirrhosis (no vs. yes), tumor number (single vs. multiple), wine-drinking (no vs. yes), smoking (no vs. yes), BCLC stage (0-A vs. B-C), PVTT (no vs. yes), distant metastasis (no vs. yes), ALT ($\leq$38 vs. > 38U/L), and recurrence (no vs. yes). Subsequently, the clinicopathologic and prognostic significance of the AWR level in HCC was investigated.

Statistical Analysis

SPSS13.0 (SPSS Inc., Chicago, IL) and MedCalc statistical software version 11.3.0.0 (MedCalc Software, Broekstraat 52 Mariakerke, Belgium) were used for data analysis. The Pearson χ^2 test was used to compare qualitative variables. Univariate analysis was first performed to determine the significance of variables using the logistic regression model for the response rate and the Cox regression model for DFS and OS. Survival curves were estimated by Kaplan–Meier analysis, and the log-rank test was used to examine differences in survival distributions between groups. Subsequently, the candidate variables from the univariate analysis with $P < 0.05$ were subjected to multivariate analysis. Cox proportional hazards regression model was used to determine the independent prognostic factors. A value of $P < 0.05$ was considered statistically significant.

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