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Reappraisal of the diagnostic value of alpha-fetoprotein for surveillance of HBV-related hepatocellular carcinoma in the era of antiviral therapy

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Abstract

This study was designed to explore if antiviral treatment influences the performance of serum alpha-fetoprotein (AFP) for hepatocellular carcinoma (HCC) among the high-risk chronic HBV-infected patients. A total of 5936 patients who had evidence of chronic HBV infection were enrolled from four independent centres in this retrospective study, including 1721 chronic hepatitis B (CHB), 2286 liver cirrhosis (LC), 798 HCC within Milan criteria and 1131 HCC beyond Milan criteria patients. Stratified by whether they received treatment or not, the patients were further divided into antiviral and non-antiviral groups. Then, the performance of AFP for discriminating HCC was evaluated. Patients receiving antivirals had significantly lower median levels of AFP compared with the non-antiviral patients ($P < .001$), and there were significantly less patients with abnormal AFP levels in antiviral groups ($P < .001$). Antiviral therapy improved the AUROCs of AFP for discriminating HCC within Milan criteria. When

Abbreviations: +LR, positive likelihood ratio; −LR, negative likelihood ratio; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under receiver operating characteristic curve; CHB, chronic hepatitis B; CI, confidence interval; DOR, diagnostic odds ratio; HCC, hepatocellular carcinoma; IFN, interferon; IQR, interquartile range; LC, liver cirrhosis; NAs, nucleos(t)ide analogues; SD, standard deviation; SROC, summary receiver operating characteristic; ULN, upper limit of normal; US, Ultrasound.

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setting the cut-off values at 20 ng/mL and 100 ng/mL as surveillance and confirmatory tests respectively for HCC among patients receiving antiviral treatment, AFP exhibited a significantly higher sensitivity than those of 200 ng/mL and 400 ng mL, which are currently recommended by some guidelines, without compromising specificity. Further analysis in antiviral patients revealed that serum AFP had better performance for discriminating HCC within Milan criteria in ALT ≤ 1ULN patients than that in ALT > 1ULN patients. In conclusion, in the era of antiviral therapy, serum AFP's surveillance performance was substantially improved for HCC within Milan criteria among the high-risk population of CHB and LC patients.

KEYWORDS
alpha-fetoprotein, antiviral treatment, hepatitis B virus, hepatocellular carcinoma

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death worldwide.[1,2] Though some advances in therapeutic interventions for HCC have been made in the past decade, the 5-year overall survival of patients with HCC remains as low as 15%-17%, largely because more than two thirds of patients are diagnosed at advanced stages of disease and lose the opportunity for curative therapies.[3-5] Hence, the diagnosis of HCC at an early stage through HCC surveillance is crucial in patients who are at high-risk and deemed to be an effective way to obtain a reduction in HCC-related mortality.[2,6,7]

Serum AFP has been the most widely used biomarker for the diagnosis and surveillance of HCC. However, the clinical application of AFP has been challenged in recent years, due to its low sensitivity and non-specificity.[8-10] Serum AFP levels might not be elevated in at least one third of HCC patients but could be elevated in patients with active chronic viral hepatitis and cirrhosis but free of HCC.[8,11] Such elevation in chronic liver disease is found to be associated with hepatic regeneration induced by massive liver damage including severe inflammation, fibrosis and bridging hepatic necrosis.[12-14] In addition, our previous work revealed that HBV viral transcription co-regulator HBx could transcriptionally upregulate AFP gene expression.[15] Several reports have suggested that such 'falsely' increased serum levels of AFP in patients with active viral hepatitis were dramatically decreased after initiation of antiviral therapy,[13,16,17] and antiviral treatment could improve the diagnostic accuracy of AFP for HCC among patients with chronic HBV infection.[13,18] Though all these studies indicate the potential influence of antiviral therapy on AFP's performance for the detection of HCC among the high-risk chronic HBV-infected patients, the relatively small number of HCC patients in these studies might have limited the precise assessment for AFP.

In this multi-centre, cross-sectional real-world study, the performance of AFP for the surveillance/diagnosis of HCC among patients with CHB and liver cirrhosis (LC) who underwent antiviral treatment was reexamined, in comparison with treatment-naïve patients. Finally, better cut-off values of AFP as surveillance and confirmatory tests for HCC among patients with antiviral treatment were investigated and validated.

2 | PATIENTS AND METHODS

2.1 | Study population

This retrospective and cross-sectional study enrolled patients from four centres (the Fifth Medical Center of Chinese PLA General Hospital (PLAGH); the Third Affiliated Hospital of SUN YAT-SEN University; Peking University Shenzhen Hospital; and the Third Hospital of Hebei Medical University), during a period from 2010 to 2018. All patients enrolled in this trial were hepatitis B surface antigen (HBsAg) positive for at least 6 months. The details for each centre were shown in Figure S1. All patients had information on laboratory data including AFP test and other clinical characteristics with clear clinical records of receiving or not antiviral treatment. Patients with liver diseases due to co-infection with hepatitis C virus or other hepatitis, genetic and autoimmune disorders, primary biliary cirrhosis and sclerosing cholangitis were excluded. Pregnant patients were excluded. We also excluded patients with other malignant tumours which could lead to an aberrant increase of serum AFP levels and HCC patients if they had prior treatment of their tumours.

A total of 5936 patients were finally recruited in the study cohorts. As described in the flow chart (Figure S1), patients in each of the four study cohorts were comprised of CHB and chronic HBV infection-related LC and HCC, with or without antiviral treatment. Most of the patients were inpatient, a small part came from outpatient.

The enrolled patients were divided into the antiviral group or non-antiviral group according to their recent histories of antiviral therapy. The antiviral group was defined as patients who had received continuous antiviral therapy for at least 3 months when recruited, with either nucleos(t)ide analogues (NAs) and/or interferon (IFN). Patients who were treatment naïve or had interrupted antiviral therapy for more than 6 months were allocated into the non-antiviral group. For the patients with antiviral therapy, 2387 patients were treated with NAs only, 78 patients with IFN only and 137 patients with sequential or combined IFN and NAs therapies. The criteria for eligible antiviral treatment were: HBeAg-positive CHB with HBV DNA ≥ 20 000 IU/mL or HBeAg-negative CHB with HBV DNA ≥ 2000 IU/mL, treatment should start if with persistent ALT ≥ 2ULN, regardless of the degree of fibrosis. Patients with persistently detectable HBV DNA and either: (a) persistent ALT level changes from 1ULN to 2ULN with moderate liver necroinflammation or fibrosis or more serious or, (b) persistently normal ALT, but older than 30 years, or with family history of cirrhosis or HCC and extrahepatic manifestations or, (c) compensated or decompensated cirrhosis, regardless of ALT levels, should also be treated.[19,20]

2.2 | Diagnosis and staging of HCC

The diagnosis of HCC was established based on histopathological confirmation, or detection of a positive lesion with recommended imaging techniques and contrast agents [multiphasic computed tomography (CT) and dynamic contrast-enhanced magnetic resonance imaging (MRI), contrast-enhanced ultrasonography (CEUS)]. This, in combination with hypervascularity in late arterial phase and washout on portal venous and/or delayed phases, was identified as the typical hallmarks of HCC.[21,22]

Patient with HCC within Milan criteria (HCC-WMC) was defined as follows: 1 nodule ≤ 5 cm or 2 to 3 nodules, each ≤ 3 cm in diameter, without gross vascular invasion or extrahepatic metastases.[23] HCC patient who did not meet the Milan criteria were defined as HCC beyond Milan criteria (HCC-BMC).

Liver cirrhosis was diagnosed by using a combination of clinical, laboratory and imaging features. Liver biopsies were not routinely performed. There could not be clinical and imaging (US, CT or MRI) evidence demonstrating a hepatic mass in patients with chronic liver disease at enrolment. Patients of CHB and LC with an aberrant AFP exceeding normal at enrolment were confirmed with a CT or MRI that showed no lesion indicative of HCC within recent months.

2.3 | Laboratory testing of AFP and other variables

Alpha-fetoprotein levels were measured in local laboratories at each of the four clinical centres by using an automated electrochemiluminescence immunoassay (Roche Diagnostics). The upper limit of normal (ULN) ranges for AFP values among these centres varied between 8.1 and 13.4 ng/mL. The lower limit was all the same at 0 ng/mL, and the upper limit of detection was 1210 ng/mL. The cut-off values of 20 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL were analysed to elucidate the clinical utility of AFP. The investigations for HCC are currently recommended with a value of AFP above 20 ng/mL. The values 200 ng/mL and 400 ng/mL are frequently used as confirmatory tests for HCC diagnosis, which are a necessary supplement to imaging to determine the presence of focal solid lesions in the liver.[21,24-26]

Liver-related biochemical testing, routine blood testing and other tests were determined in local laboratories of each centre using commercially available kits.

All clinical and laboratory data from patients were collected following the same criteria above. Individual unusual values were reviewed to verify the accuracy of data.

This study was approved by the ethics committee of Peking University Health Science Center. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.4 | Statistical analyses

Statistical analyses were performed by SPSS 24.0 software and GraphPad Prism version 5.0. Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) according to the data's distribution and were analysed by t test or Mann-Whitney's tests, as appropriate. Chi-square test was applied to compare the rates of the categorical variables. For the analyses of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the percentage of missing values was 2.1% and 6.8% respectively. The area under receiver operating characteristic curve (AUROC) with 95% confidence interval (CI) were used to analyse the performance of AFP in discriminating HCC from at-risk patients, and summary receiver operating characteristic (SROC) curves were conducted to further compare the diagnostic odds ratio (DOR) through meta-analysis of multi-centre data by RevMan5.3 software. Sensitivity, specificity, positive likelihood ratio (+LR) and negative likelihood ratio (−LR) of different cut-off values of AFP levels were calculated. All tests of significance were two-tailed, and $P < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

The clinical characteristics of patients enrolled in this study are summarized in Table 1. Compared with their counterpart, the non-antiviral group of patients, the mean ages in the antiviral group were significantly older in patients with CHB (37.39 ± 10.30 vs

TABLE 1 Clinical characteristics of patients enrolled in the study

Variable	CHB			LC		
	Antiviral (n = 604)	Non-antiviral (n = 1117)	*P* value	Antiviral (n = 1319)	Non-antiviral (n = 967)	*P* value
Age (y)	43.00 ± 10.83	37.39 ± 10.30	<.001	49.83 ± 10.76	49.68 ± 12.37	.761
Male, n (%)	485 (82.3)	877 (78.5)	.385	1018 (77.2)	729 (75.4)	.319
HBeAg[a] (+/−)	227/354	543/456	<.001	352/840	359/535	<.001
HBV DNA[a] (+/−)	139/428	943/80	<.001	230/999	741/174	<.001
ALT (U/L)	26 (17, 40)	156 (39, 580)	<.001	26 (19, 38)	48 (28, 118)	<.001
AST (U/L)	24 (20, 36)	82 (31, 279)	<.001	33 (25, 49)	58 (36, 114)	<.001
AFP (ng/mL)	2.49 (1.69, 3.84)	5.06 (2.47, 22.32)	<.001	2.69 (1.64, 5.35)	7.92 (3.15, 37.87)	<.001
	HCC-WMC			HCC-BMC		
	Antiviral (n = 414)	Non-antiviral (n = 384)	*P* value	Antiviral (n = 265)	Non-antiviral (n = 866)	*P* value
Age(y)	52.47 ± 10.07	53.47 ± 10.35	.165	54.31 ± 9.80	51.12 ± 11.49	<.001
Male, n (%)	341 (82.4)	318 (82.8)	.868	231 (87.2)	772 (89.2)	.374
HBeAg[a] (+/−)	105/302	106/269	.437	58/177	215/519	.171
HBV DNA[a] (+/−)	81/258	244/79	<.001	103/126	621/82	<.001
ALT (U/L)	29 (21, 42)	37 (24, 56)	<.001	32 (23, 49)	49 (32, 79)	<.001
AST (U/L)	31 (24, 45)	37 (27, 62)	<.001	44 (31, 73)	72 (42, 133)	<.001
AFP (ng/mL)	12.36 (3.68, 109.06)	28.25 (5.38, 280.08)	<.001	85.68 (5.97, 1210.0)	807.05 (28.70, 1210.0)	<.001

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMC, beyond Milan criteria; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; LC, liver cirrhosis; WMC, within Milan criteria.

[a]Information about some patients was missing.

43.00 ± 10.83, $P < .001$) and in HCC-BMC (51.12 ± 11.49 vs 54.31 ± 9.80, $P < .001$). Interestingly, compared with the non-antiviral patients, patients receiving antivirals always exhibited significantly lower median values of AFP, not only in patients with CHB (2.49 vs 5.06, $P < .001$), LC (2.69 vs 7.92, $P < .001$), but also with HCC-WMC (12.36 vs 28.25, $P < .001$) or HCC-BMC (85.68 vs 807.05, $P < .001$) and no matter if there were concurrent cirrhosis or not (Table S1, for details). Meanwhile, a noticeably larger number of patients was classified as HCC-WMC in the antiviral group than in the non-antiviral group (61.0% vs 30.7%, $P < .001$), according to the Milan criteria. Further analysis also showed that no significant differences of AFP levels between the NAs treated group and IFN treated group was observed (Table S2 and S3, for details).

3.2 | Antiviral therapy brought serum AFP level down in chronic HBV-infected patients at all disease-progression stages

As showed in Figure 1A, the proportion of patients with abnormal serum AFP levels between those with and without antiviral treatment was compared in each stage from CHB, LC, to HCC-WMC and HCC-BMC. Compared with the untreated group, the proportions of patients with aberrantly elevated serum AFP levels were always much lower in the antivirals treated group, at all meaningfully elevated AFP levels (Figure 1A). In other words, antiviral therapy significantly declined the fraction of patients with abnormal AFP (>ULN in this study) ($P < .001$). The proportions of abnormal AFP levels in antiviral groups were significantly lower compared with non-antiviral groups in patients of CHB (7.1% vs 35.3%), LC (13.9% vs 45.2%), HCC-WMC (52.9% vs 65.4%) and HCC-BMC (70.9% vs 84.4%), respectively. These results indicated that the decline of AFP levels was related to antiviral treatment, indiscriminate of if the patients had HCC-free CHB and LC, or HCC-WMC and HCC-BMC.

Then, we retrospectively investigated the dynamic changes of AFP levels in a small group of patients from Cohort A (Figure 1C). None of the 54 patients in this cohort were diagnosed with HCC during the observation, and all had AFP levels ≥ 20 ng/mL at the time of enrolment. The patients were given regular antiviral treatment including 51 patients with entecavir (ETV) and the other three with IFN, telbivudine (LdT) or adefovir dipivoxil (ADV), respectively. To those patients, AFP, ALT and AST were tested and recorded about every 3 months. The decline of ALT and AST validated the efficacy of the antivirals in this cohort. It was worthwhile to note that a dramatic decline of AFP levels was also observed at the third month of antiviral therapy, which was in parallel with the dynamic changes of ALT and AST levels (Figure 1B,C).

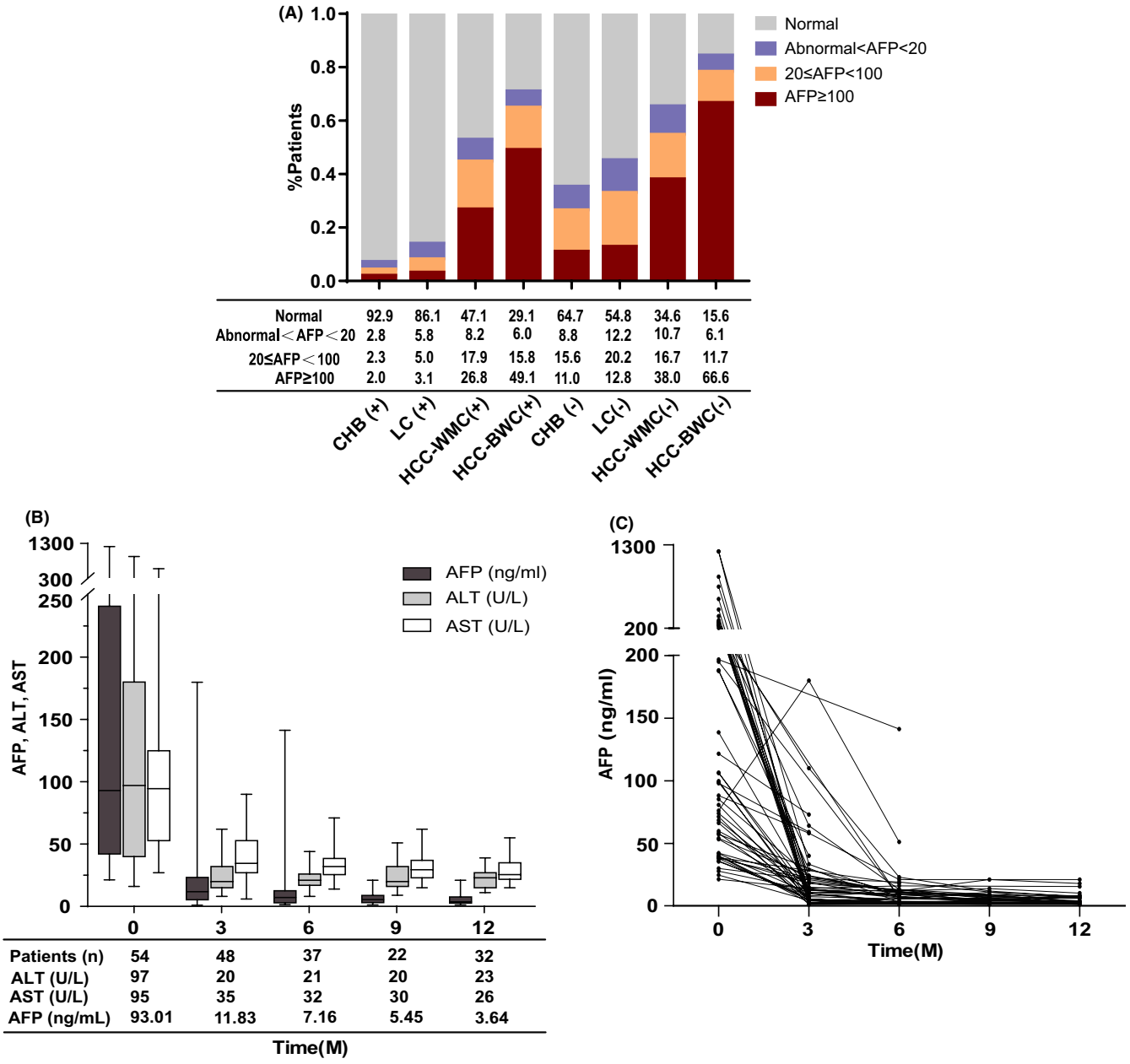

FIGURE 1 (A) Percentage of patients in each subgroup at enrolment separated by AFP value. (+), antiviral group; (−), non-antiviral group; BMC, beyond Milan criteria; WMC, within Milan criteria. (Abnormal refers to elevated above the normal upper limit of local laboratory of each centre, which varied from 8.1 ng/mL to 13.4 ng/mL). (B) AFP, ALT and AST levels at different time points during 12 mo of antiviral therapy. (C) Changes of AFP levels during 12 mo of antiviral therapy in chronic hepatitis B

3.3 | Antiviral treatment improved the performance of serum AFP in identifying HCC in early stage within Milan criteria

To validate the influence of antiviral therapy on HCC surveillance and/or diagnostic efficacy of serum AFP, the AUROCs between the antiviral group and non-antiviral group were compared in cohorts at each centre. In general, no significant differences in the AUROCs of serum AFP were observed between the antiviral and the non-antiviral groups for total HCC patients (Table S4, for details). Then, the HCC patients were sub-grouped into HCC-WMC and HCC-BMC. The AUROC of serum AFP in the antiviral group was much higher compared with the non-antiviral group for the discrimination of HCC-WMC in each centre (Table 2): Cohort A (0.776 vs 0.701, $P = .012$), Cohort B (0.784 vs 0.693, $P = .030$), Cohort C (0.815 vs 0.719, $P = .028$) and Cohort D (0.849 vs 0.711, $P = .010$). Noticeably, such improvement of AFP for detecting HCC brought by antiviral treatment disappeared in patients with HCC-BMC groups in all cohorts (Table S5, for details). But the optimal cut-off values of serum AFP in antiviral groups were all lower compared to those in non-antiviral groups, with any stage of HCC (Table 2, Table S4 and S5).

TABLE 2 Comparison of diagnostic performance for serum AFP discriminating HCC within Milan criteria between the antiviral and non-antiviral groups

HCC-WMC	AUROC (95% CI)	Cut-off value	Sen (%)	Spe (%)	+LR	−LR	*P* value
Cohort A							
Antiviral	0.776 (0.749,0.800)	5.47	60.19	83.12	3.57	0.48	.012
Non-antiviral	0.701 (0.662,0.738)	16	55.50	74.94	2.21	0.59	
Cohort B							
Antiviral	0.784 (0.743,0.822)	3.1	86.25	56.78	2.00	0.24	.030
Non-antiviral	0.693 (0.660,0.725)	8.33	74.65	56.15	1.70	0.45	
Cohort C							
Antiviral	0.815 (0.780,0.847)	9.7	62.50	90.71	6.73	0.41	.028
Non-antiviral	0.719 (0.685,0.752)	114.14	45.00	91.19	5.11	0.60	
Cohort D							
Antiviral	0.849 (0.802,0.889)	6.15	79.07	80.50	4.05	0.26	.010
Non-antiviral	0.711 (0.663,0.755)	61.85	47.62	83.53	2.89	0.63	

Abbreviations: +LR, positive likelihood ratio; −LR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; HCC, hepatocellular carcinoma; Sen, sensitivity; Spe, specificity; WMC, within Milan criteria.

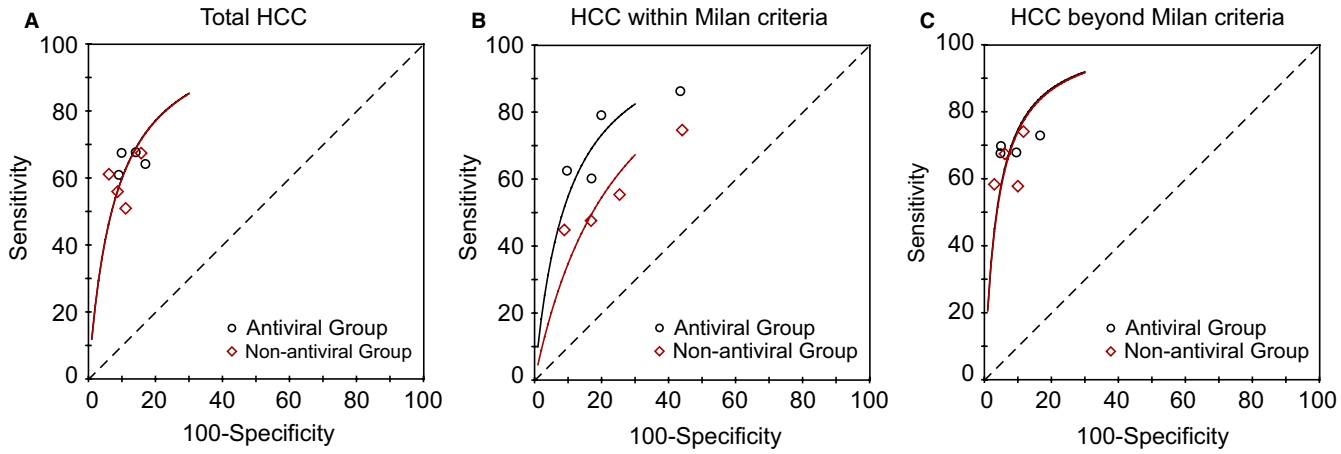

FIGURE 2 Comparison of the SROC curves of AFP between the antiviral group and non-antiviral group in different stages of HCC. (A) The SROC curves for total HCC. (B) The SROC curves for HCC within Milan criteria. (C) The SROC curves for HCC beyond Milan criteria

To further validate the above findings, SROC curves were plotted according to the optimal cut-off value, sensitivity and specificity of each subgroup. SROC curves for total HCC, HCC-WMC and HCC-BMC are shown in Figure 2A-C, respectively. Next, the data from the four centres were analysed by RevMan5.3 software, to obtain the diagnostic odds ratio (DOR). As shown in Figure 2, the 13.07 (95% CI, 8.89-19.21) of the antiviral group was similar to the 13.26 (95% CI, 8.40-20.92) of the non-antiviral group in total HCC. Such similarity was also observed in HCC-BMC with 25.48 (95% CI, 13.85-46.89) for the antiviral group and 24.41(95% CI, 14.45-41.24) for the non-antiviral group, respectively. For HCC-WMC, however, the DOR in the antiviral group was significantly higher than that in the non-antiviral group, with the former being 10.35 (95% CI, 6.79-15.77) and the latter only 4.73 (95% CI, 3.17-7.06).

Lastly, the data from the four centres were aggregated and analysed. The results also showed that the antiviral treatment could significantly improve the performance of serum AFP for discriminating HCC-WMC (0.787 vs 0.689, $P < .001$), which was not observed for HCC-BMC (Table S6, for details).

3.4 | Comparison of sensitivity, specificity, +LR, −LR of different cut-off values of serum AFP between antiviral and non-antiviral groups

As shown in Table 3, Tables S7 and S8, when the cut-off values increased from 20 ng/mL to 100 ng/mL, 200 ng/mL and then to 400 ng/mL, the sensitivities gradually declined, meanwhile, the specificities and +LR were increasingly elevated. Compared with the non-antiviral group, the values of specificity and +LR of the antiviral group for each cohort had a remarkable elevation at the cut-off value of AFP of 20 ng/mL. Such as in cohort A, the specificity increased

TABLE 3 Sensitivity, specificity, +LR and −LR of different AFP levels for the detection of HCC within Milan criteria between antiviral and non-antiviral groups

Cohort	AFP ng/mL	Antiviral Group				Non-antiviral Group			
		Sen (%)	Spe (%)	+LR	−LR	Sen (%)	Spe (%)	+LR	−LR
Cohort A	20	38.39	93.41	5.83	0.66	52.36	77.49	2.33	0.61
	100	23.70	97.11	8.20	0.79	36.13	91.05	4.04	0.70
	200	19.43	98.38	12.01	0.82	26.18	93.35	3.94	0.79
	400	13.74	99.08	14.86	0.87	16.23	96.93	5.29	0.86
Cohort B	20	51.25	89.83	5.04	0.54	54.93	67.63	1.70	0.67
	100	31.25	96.89	10.06	0.71	35.21	85.62	2.45	0.76
	200	17.50	97.74	7.74	0.84	25.35	91.29	2.91	0.82
	400	13.75	98.31	8.11	0.88	12.68	95.71	2.96	0.91
Cohort C	20	47.50	96.11	12.22	0.55	57.50	71.31	2.00	0.60
	100	27.50	97.84	12.73	0.74	45.00	89.90	4.46	0.61
	200	18.75	99.35	28.94	0.82	37.50	94.71	7.09	0.66
	400	10.00	99.57	23.15	0.90	26.25	98.08	13.65	0.75
Cohort D	20	58.14	90.87	6.37	0.46	59.52	67.05	1.81	0.60
	100	32.56	97.10	11.21	0.69	38.10	87.28	3.00	0.71
	200	25.58	97.93	12.33	0.76	30.95	92.77	4.28	0.74
	400	18.60	98.76	14.95	0.82	21.43	97.11	7.41	0.81

Abbreviations: +LR, positive likelihood ratio; −LR, negative likelihood ratio; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; Sen, sensitivity; Spe, specificity.

TABLE 4 Comparison of AUROCs for serum AFP discriminating HCC within Milan criteria between ALT ≤ 1ULN and ALT > 1ULN in antiviral group

	AUROC (95% CI)	Cut-off value	Sen (%)	Spe (%)	+LR	−LR	*P* value
Subgroups							
ALT ≤ 1ULN	0.809 (0.790,0.827)	7.32	57.81	90.27	5.94	0.47	<.001
ALT > 1ULN	0.689 (0.647,0.728)	6.11	71.82	60.96	1.84	0.46	

Abbreviations: +LR, positive likelihood ratio; −LR, negative likelihood ratio; AUROC, area under receiver operating characteristic curve; HCC, hepatocellular carcinoma; Sen, sensitivity; Spe, specificity; ULN: upper limit of normal.

from 77.49% to 93.41% and the + LR increased from 2.74 to 6.72 at the 20 ng/mL cut-off value (Table S7, for details). When at the cut-off values of 100 ng/mL, 200 ng/mL and 400 ng/mL, such appreciable increase of specificity and +LR remained. Whereas in the same situation, the corresponding sensitivity was decreased to some extent. It is noteworthy that the specificity of a cut-off value of 100 ng/mL in the antiviral group was almost the same as that of the cut-off value of 400 ng/mL in the non-antiviral group for each cohort in total HCC, HCC-WMC and HCC-BMC, and almost without compromising the corresponding sensitivity and + LR which was even better in HCC-WMC. For example, 97.11% and 96.93% respectively in cohort A. At the same time, when compared to the cut-off values of 200 ng/mL and 400 ng/mL of the same cohort in the antiviral group, the sensitivity of 20 ng/mL and 100 ng/mL was remarkably elevated without compromising the corresponding specificity and +LR, such as from 27.81% and 33.77% to 45.70% and 60.93% in cohort B (Table S7, for details). But in the non-antiviral group, the specificity had an obvious reduction as the cut-off value of AFP changed from 400 ng/mL to 100 ng/mL for each cohort. For example, from 95.71% to 85.62% in cohort B. Furthermore, the sensitivity and +LR of AFP at 100 ng/mL for HCC-WMC in the antiviral group were found even better than those of 400 ng/mL in the non-antiviral group (Table 3), and this phenomenon was not observed in total HCC or HCC-BMC (Table S7 and S8, for details).

3.5 | The influence of ALT normalization after antiviral therapy on the performance of serum AFP for discriminating HCC within Milan criteria

The liver inflammation would be a major confounding factor for AFP level and should be adjusted by the ALT level. Therefore, the patients in the antiviral group were further divided into ALT ≤ 1ULN and ALT > 1ULN groups. As expected, serum AFP had significant higher

AUROC for discriminating HCC-WMC in ALT ≤ 1ULN patients than that in patients with ALT > 1ULN (0.809 vs 0.689, $P < .001$) (Table 4). The cut-off values of serum AFP at 20 ng/mL and 100 ng/ml in ALT ≤ 1ULN patients had higher sensitivity, specificity and +LR than those in ALT > 1ULN patients (Table S9, for details). These results further suggested that serum AFP had better performance for discriminating HCC-WMC in ALT normalized patients after receiving antiviral therapy.

4 | DISCUSSION

In this real world-based and multi-centre study, the influence of antiviral therapy on AFP levels in patients with CHB, LC and HCC was investigated, and the sequential impact of antiviral treatment on the HCC discriminating performance of AFP was evaluated. The results suggested that antiviral therapy would significantly increase the AUROCs of AFP for the discrimination of HCC patients at early stage within Milan criteria (HCC-WMC), among the high-risk CHB and LC patients. Accordingly, a lower optimal cut-off value of serum AFP was suggested for the discrimination of HCC-WMC.

As a confirmatory test to discriminate HCC from other solid lesions of the liver imaged by US, the frequently used cut-off values of AFP are 200 ng/mL and 400 ng/mL.[24-26] Meanwhile, the 400 ng/mL remains to be the diagnostic cut-off value for HCC in the newest guideline of primary liver cancer in China, which accounts for more than half of newly diagnosed HCC cases annually worldwide.[21,22] But all these recommended values had been established on data coming from treatment-naïve patients, and the influence of antiviral treatment was not taken into consideration of the high-risk population with chronic viral hepatitis and relevant chronic liver diseases. In our antiviral population, 100 ng/mL showed a higher sensitivity compared with 200 ng/mL and 400 ng/mL, without compromising specificity. But such change of cut-off value of serum AFP should not be expanded to the non-antiviral treated individuals.

In the recently updated Asian-Pacific guidelines on the management of HCC, 200 ng/mL was recommended as the cut-off value of AFP for surveillance when used in combination with US, because AFP with cut-off value of 200 ng/mL showed a better combined +LR than that of 20 ng/mL (5.85 vs 2.45).[27] However, the same guideline also recommended that the cut-off value of AFP can be set at a lower value in populations with hepatitis virus suppression or eradication. In support of this recommendation, results in our study showed that the cut-off value of 20 ng/mL of AFP in the antiviral group had equivalent or higher +LR compared with the 200 ng/mL in the non-antiviral group, which was observed in each of the four centres. Our study provides new evidence for the recommendation on the lower cut-off value in populations with hepatitis virus suppression or eradication. It is predictable that more HCC patients within Milan criteria would be found in a timely manner by setting a relative lower cut-off value of serum AFP, providing possible curative treatment and survival benefit to these patients.

It has been well reported that the elevation of AFP is strongly associated with ALT and AST the biomarkers reflecting hepatic inflammation and damage, but the underlying mechanism of such association is poorly understood.[8,12,18,28] One interpretation is that the AFP gene in primitive hepatocytes is re-expressed during regeneration induced by severe liver necroinflammation and damage,[11,29] conducted by the increase of oval cells in liver regeneration and repair.[30,31] Meanwhile, AFP gene expression is upregulated by the HBV viral transcription co-regulator HBx which means that HBV infection could directly lead to the elevation of AFP.[15] In our study, we did demonstrate that a dramatic decline in AFP levels occurred at the third month after antiviral therapy initiation, which was in parallel with the dynamic changes of ALT and AST levels. Since an efficient antiviral therapy could improve the inflammation damage via suppressing HBV viral replication, it is reasonable to postulate that decreased HBV viral load and remission would, to some extent, downregulate the expression and secretion of HCC-unrelated AFP in CHB patients. In line with this, the significant improvement of serum AFP performance for discriminating HCC-WMC was more obvious in those antiviral treated patients who achieved ALT normalization.

It should be emphasized that our current study has several limitations. Although the study is a multi-centre and large-scale study based on the real world and adheres to the same quality control specification of data which would increase the statistical power and reliability of results, uncontrollable differences might exist such as the similar but still different proportions of CHB, LC and HCC patients between the different centres and the possible selection bias of patients. Moreover, this is a retrospective study that could have been influenced by unmeasured potential biases, the cut-off value 100 ng/mL of AFP mentioned above might be only representative of a tendency to reduction induced by antiviral therapy and needed to be further validated. It would be of difference to evaluate the discriminating performance of AFP for HCC in the presence of known HCC compared with HCC detection in a prospective cohort of patients with chronic liver disease.

Different from the European and American guidelines suggesting surveillance using US, with or without AFP, every 6 months,[2,32] the surveillance strategy of the Asian-Pacific and China suggests the combined use of US and serum AFP measurement biannually. In addition, AFP is also an auxiliary test in combination with imaging for the diagnosis of HCC in China.[22,27] The reasons leading to these differences might reflect the differences of epidemiology, risk factors for populations and levels of economic development in these different regions. For example, the largest attributable fraction is caused by HBV in East Asia and China, whereas only 20% of cases can be attributed to HBV infection in the Western world.[33] Recently, Kristina and colleagues through a meta-analysis had stated that the sensitivity of combined use of US with AFP for early-stage HCC was significantly higher than US-alone (63% vs 45%, $P = .002$).[34] Similarly, a phase 3 biomarker study from Korea also demonstrated that the sensitivity increased from 48.6% of US-alone to 88.6% by addition of AFP without markedly decreasing specificity (from 96.4% to

82.7%), in which the majority of patients had HBV-related cirrhosis with suppressed viral loads and normal ALT levels.[35] So it appears to be particularly important to make a reappraisal of AFP in the current era of antiviral treatment for HBV infection, before new biomarkers with higher performance for detecting HCC appear.

In conclusion, antiviral treatment of patients with HBV infection-related liver disease can lower serum AFP levels which decreases the HCC-unrelated elevation of AFP levels, and thus, the surveillance performance of serum AFP for early-stage HCC within Milan criteria was substantially improved among the high-risk population of CHB and LC patients, in the era of antiviral therapy.

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CONFLICTS OF INTEREST

All authors have no conflict of interests to declare.

AUTHOR'S CONTRIBUTIONS

Xiangjun Qian and Shuhong Liu: acquisition of data, analysis and interpretation of data, drafting of the manuscript and statistical analysis; Huiling Long, Siyu Zhang, Jiyuan Zhou, Jiao Gong and Tao Zhou: acquisition of data; Xiaotong Yan, Mingjie Yao, Xiajie Wen, Xiangwei Zhai: statistical analysis; Jianwen Wang and Ting Zhang: support of experimental technology; and Qiang Xu, Xiangmei Chen, Guoxin Hu, Jie Wang and Zhiliang Gao: administrative, technical or material support; Yuemin Nan, Junhui Chen, Bo Hu, Jingmin Zhao: material support, study supervision, critical revision of the manuscript for important intellectual content. Fengmin Lu: study concept and design, obtained funding, study supervision and critical revision of the manuscript for important intellectual content. All authors of this research have approved the final version of the article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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