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ORIGINAL ARTICLE

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Impact of metabolic dysfunction-associated steatotic liver disease on the efficacy of immunotherapy in patients with chronic hepatitis B-related hepatocellular carcinoma

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ABSTRACT

Objective: To investigate the impact of metabolic dysfunction-associated steatotic liver disease (MASLD) on the efficacy of immune checkpoint inhibitor (ICI)-based therapy in patients with chronic hepatitis B (CHB)-related hepatocellular carcinoma (HCC).

Methods: A total of 155 patients with CHB-related HCC who received ICI–based therapy (in the Department of Hepatology, Tianjin Second People's Hospital and Department of Hepatobiliary Oncology, Tianjin Medical University Cancer Institute & Hospital) between April 2021 and December 2023 were evaluated. Patients were divided into two groups: MASLD concurrent with CHB [MASLD-CHB] (n = 38), and CHB (n = 117).

Results: The median progression-free survival (PFS, 6.9 months *vs.* 9.3 months; P = 0.001), progressive disease (57.89% *vs.* 37.61%; P = 0.028), and disease control rate (42.11% *vs.* 62.39%; P = 0. 028) in the MASLD-CHB group were significantly worse than the CHB group. The median overall survival was not attained. The percentage of CD4+PD1+ (17. 56% *vs.* 8.89%; P < 0.001) and CD8+PD1+ T cells (10.50% *vs.* 7.42%; P = 0.005) in patient samples from the MASLD-CHB group were significantly higher than the CHB group. Concurrent MASLD [hazard ratio (HR) = 1.921; 95% CI, 1.138–3.245; P = 0.015] and alpha-fetoprotein levels after 3 months of treatment (HR = 2.412; 95% CI, 1.360–4.279; P = 0.003) were independent risk factors for PFS in all patients.

Conclusions: ICI-based therapy in patients with CHB-related HCC and concurrent MASLD resulted in poorer efficacy and shorter PFS compared to patients with CHB-related HCC alone.

KEYWORDS Metabolic dysfunction-associated steatotic liver disease; chronic hepatitis B; hepatocellular carcinoma; immunotherapy; efficacy

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the fourth leading cause of cancer-related deaths worldwide[1-3]. Therefore, HCC has become a major global public health challenge. Radical therapies, such as surgery or radiofrequency ablation, are ideal choices for treating tumor lesions in patients with early-stage HCC. However, at the initial visit greater than two-thirds of patients with HCC are in advanced stage and approximately 70% of patients have unresectable disease. Furthermore, approximately 61% of patients relapse within 5 years after radical surgery[4]. Studies conducted in China have shown that the 5-year survival rate among patients with HCC is only 14.1%[1]. In recent years, the emergence of immune checkpoint inhibitors (ICIs) has given hope to patients with advanced malignant tumors. ICIs include monoclonal antibodies targeting programmed cell death receptor-1 (PD-1), programmed death ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen-4[5].

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Studies have shown that ICIs achieve promising results in the treatment of malignant tumors, such as non-small cell lung cancer, melanoma, and bladder cancer. Unfortunately, reports on the effectiveness of ICIs in the treatment of HCC are limited. The objective response rate (ORR), however, has been reported to be only 15%–20%[6,7].

Greater than 80% of HCC cases in China are caused by chronic hepatitis B virus (HBV) infection. In recent years, however, non-alcoholic fatty liver disease (NAFLD) has emerged as an important cause of HCC due to lifestyle changes[8]. The global prevalence of NAFLD is currently approximately 32.4%[9]. Notably, the increase in liver cancer-related mortality can be largely attributed to NAFLD[10]. Interestingly, the definition of NAFLD does not fully match the criteria used by clinicians and researchers. Rienlla et al.[11] reached a consensus in 2023 after 4 rounds of a Delphi survey that concluded metabolic dysfunction-associated steatotic liver disease (MASLD) should replace the term, NAFLD. Metabolic syndrome contributes to the development of MASLD, which is associated with cardiometabolic disorders, such as obesity, insulin resistance, and hyperlipidemia. These factors lead to chronic inflammation, changes in lipid metabolism, and alterations in the immune microenvironment, ultimately promoting progression to HCC[12]. Studies have shown that approximately 15%–30% of patients with CHB have NAFLD, which may lead to a more rapid progression of the disease[13,14]. Researchers have confirmed through *in vivo* and *in vitro* experiments that patients with NAFLD/non-alcoholic steatohepatitis (NASH)-related HCC can develop immunotherapy resistance[15]. However, whether MASLD can reduce the efficacy of immunotherapy and affect the prognosis of CHB-related HCC remains unclear. Therefore, we determined the impact of MASLD on the efficacy of ICI-based therapy in patients with CHB-related HCC.

Materials and methods

Patients and inclusion/exclusion criteria

This study retrospectively analyzed 155 patients with CHB-related HCC who received ICI-based therapy at the Department of Hepatology, Tianjin Second People's Hospital and Department of Hepatobiliary Oncology, Tianjin Medical University Cancer Institute & Hospital between April 2021 and December 2023. The inclusion criteria were as follows: (1) patients diagnosed with CHB according to "Update on prevention, diagnosis, and treatment of chronic hepatitis B: American Association for the Study of Liver Diseases (AASLD) 2018 hepatitis B guidance" and diagnosed with HCC according to "AASLD guidelines for the treatment of hepatocellular carcinoma" of the AASLD[16,17]; (2) patients who received ICI-based therapy; (3) patients between 18 and 80 years of age; and (4) patients with intermediate or advanced HCC according to the Barcelona Clinic Liver Cancer (BCLC) Staging System. The exclusion criteria were as follows: (1) irregular use of immunotherapy; (2) ICI-based therapy less than three times; (3) inconsistent attendance at follow-up appointments for re-examination and evaluation of treatment effects; (4) declined to participate in the study; (5) declined signing informed consent; (6) prophylactic use of ICIs; and (7) history of cytoreductive surgery prior to immunotherapy. A total of 145 patients who were diagnosed by dynamic computed tomography (CT) or magnetic resonance imaging (MRI) met the inclusion criteria. Under the recommendations of the new consensus, MASLD uses affirmative diagnostic criteria with greater emphasis on the role of metabolic cardiovascular risk factors in fatty liver disease instead of adopting the previous exclusive diagnosis. MASLD was identified using ultrasound Doppler or dynamic CT when at least one of the cardiometabolic criteria was met and excluded other causes of steatosis according to "A multisociety Delphi consensus statement on new fatty liver disease nomenclature"[11]. The current study included 117 patients with CHB-related HCC (CHB group) and 38 patients with MASLD and CHB-related HCC (MASLD-CHB group). **Figure 1** shows the patient grouping flowchart.

The Medical Ethics Committee of Tianjin Second People's Hospital approved the study protocol (Approval No. LL-BG-2024006), which conformed to the ethical guidelines of the Declaration of Helsinki amended in 2008.

Clinical and laboratory data

The study collected baseline characteristics and follow-up data of the enrolled patients, including gender, age, body mass index, hepatitis B virus deoxyribonucleic acid (HBV-DNA) (< 10 IU/mL), hepatitis B virus surface antigen (HBsAg) (< 0.05 IU/mL), hepatitis Be antigen (HBeAg) (< 10 IU/mL), alanine aminotransferase (ALT) (9–50 U/L), aspartate aminotransferase (AST) (9–40 U/L), albumin (ALB) (40–55 g/L), total bilirubin (TBIL) (≤ 20 μmol/L), total cholesterol (TC) (≤ 5.17 mmol/L), triglycerides (TG) (0.56–1.7 mmol/L), high-density lipoprotein (HDL) (0.96–1.15 mmol/L), low-density lipoprotein (LDL) (0–3.1 mmol/L), alpha-fetoprotein levels (AFP)

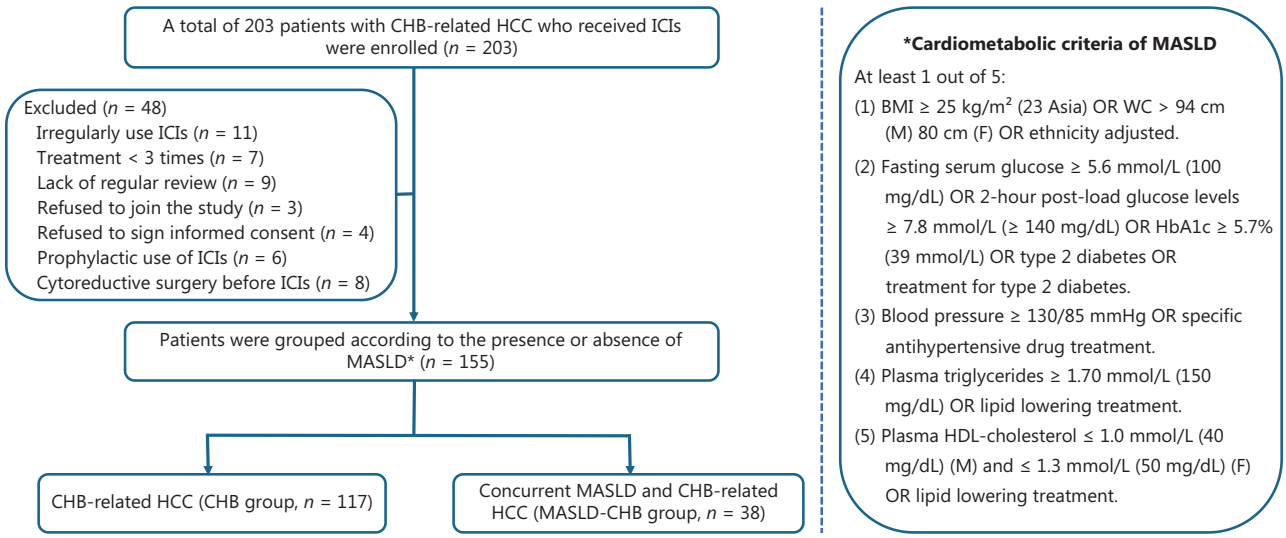


Figure 1 Flow chart of patient groups. CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; MASLD, metabolic dysfunction-associated steatotic liver disease; BMI, body mass index; WC, waist circumference; M, male; F, female; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoprotein; ICIs, immune checkpoint inhibitors.

(0–10 ng/mL); fibrosis 4 score (Fib-4), basic diseases, including hypertension and diabetes, Child-Pugh classification, AFP level after 3 months of treatment, diameter and the number of tumors, BCLC stages, extrahepatic and portal vein tumor metastases, immunotherapy drugs, and treatment methods. The "AASLD guidelines for the treatment of hepatocellular carcinoma" suggest that tumor size can indicate the degree of HCC progression[17]. A maximum tumor diameter of 5 cm can be used as the standard for stratifying tumor size using dynamic CT or MRI. The Fib-4 index was used to assess the degree of liver fibrosis, which was calculated using four clinical indicators (age, AST, ALT, and platelet count). A higher Fib-4 index value indicates a more severe degree of liver fibrosis. The Fib-4 index was determined using the Age × AST/ PLT count × √ALT formula.

Efficacy evaluation

The two groups of patients primarily received monoclonal antibodies targeting PD-1/PD-L1. The Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 was used to assess the complete response (CR), stable disease (SD), partial response (PR), progressive disease (PD), objective response rate (ORR) (ORR = CR + PR), and disease control rate (DCR) (DCR = CR + PR + SD) of solid tumors in the two groups of patients receiving ICI-based therapy[18]. The evaluation was performed every 6 weeks using dynamic CT or MRI. All patients had follow-up evaluations until 29 February 2024. At the end of the follow-up period, the overall survival (OS) and progression-free survival (PFS) in both groups were evaluated.

Assessment of immune status

The peripheral blood lymphocyte count and the ratio of PD-1-positive $CD4^+$ and $CD8^+$ T cells in both groups were analyzed by flow cytometry.

Statistical analysis

The data were analyzed using SPSS 26.0 statistical software. Measurement data that followed a normal distribution are expressed as $\bar{x} \pm S$. HBV-DNA was calculated logarithmically. Student's t-test was used to compare groups. Data that did not follow a normal distribution are represented as a median (IQR 25%–75%). The Wilcoxon rank-sum test was used to compare differences between groups. The chi-square test was used to compare differences in unordered classified data between groups. The Kaplan–Meier method was used to estimate PFS and the log-rank test was used to compare groups. The prognostic factors for PFS were analyzed using univariate and multivariate regression analyses. Variables with a $P < 0.10$ in univariate analysis were included in the multivariate analysis. Graphs were created using GraphPad Prism 8 software. Statistical significance was set at a $P < 0.05$.

Results

Patients and tumor characteristics

This study included 155 eligible patients with CHB-related HCC (136 males and 19 females) between 18 and 80 years of age. All patients had cirrhosis as a result of hepatitis B virus infection. The tumor stage was BCLC B/C and the tumor burden was high. Of the 155 patients, 72 (46.5%) had portal vein tumor thrombi and 85 (54.8%) had extrahepatic metastases. No significant difference was noted in the distribution of these characteristics between the two groups. The MASLD-CHB group had significantly higher AFP levels after 3 months of treatment (P = 0.017) than the CHB group. No significant differences were detected in the ALT, AST, HBV DNA, and TBIL levels, lipid index, Child-Pugh score, or tumor diameter between the two groups (**Table 1**).

Immunotherapy drugs and combined therapy

Sintilimab and camrelizumab were the most commonly used PD-1/PD-L1 monoclonal antibodies in both groups. Combination therapy frequently involved target drugs, with bevacizumab the most commonly used drug. The post-treatment results using immunotherapeutic drugs or combination therapies did not differ significantly between the two groups (**Table 2**).

Efficacy evaluation and prognosis

The MASLD-CHB group of patients who received ICI-based therapy had a significantly worse median PFS (mPFS) (6.9 months *vs.* 9.3 months; P = 0.001), PD (57.89% *vs.* 37.61%; P = 0.028), PR (5.26% *vs.* 19.66%; P = 0.036), ORR (5.26% *vs.* 20.51%; P = 0.029), and DCR (42.11% *vs.* 62.39%; P = 0. 028) than patients of the CHB group; the median OS was not attained. The 3-, 6-, and 12-month OS rates were 100.0%, 94.2%, and 83.8%, respectively. Furthermore, the SD (36.84% *vs.* 41.88%; P = 0.583) were lower in the MASLD-CHB group than the CHB group (**Figures 2 and 3**).

Liquid circulating immune status

No statistically significant difference was detected in the $CD19^+$ B, $CD3^+$ T, $CD3^+CD4^+CD8^+$ T, $CD3^+CD4^+CD8^-$ T, $CD4^+/CD8^+$ T, and $CD3^-CD16^+CD56^+$ NK cell levels between the patient blood samples from the two groups (**Figure 4**). The percentages of $CD4^+$ $PD1^+$ (17. 56% *vs.* 8.89%; $P < 0.001$) and $CD8^+$ $PD1^+$ T cells (10.50% *vs.* 7.42%; P = 0.005) in the MASLD-CHB group were significantly higher in the CHB group (**Figure 5**).

Analysis of patient prognostic factors

Univariate Cox regression analysis showed that concurrent MASLD [hazard ratio (HR) = 2.221; 95% CI, 1.332–3.702; P = 0.002] and AFP levels after 3 months of immunotherapy (HR = 2.665; 95% CI, 1.536–4.624; $P < 0.001$) were factors significantly associated with PFS. Multivariate Cox regression analysis revealed that concurrent MASLD (HR = 1.921; 95% CI, 1.138–3.245; P = 0.015) and AFP levels after 3 months of immunotherapy (HR = 2.412; 95% CI, 1.360–4.279; P = 0.003) were independent risk factors for PFS in all patients (**Figure 6**). Patients with CHB-related HCC who had low AFP levels after 3 months of treatment had a longer mPFS (9.3 months *vs.* 5.5 months; P = 0.002) compared to patients with high AFP levels (**Figure 7**).

Discussion

Among various etiologies, CHB and MASLD are independent risk factors for HCC development. A recent study conducted in Asian countries and regions reported a comprehensive prevalence rate of 29.0% (13.5%–56.0%) for the co-existence of CHB and NAFLD, which was an increasing trend[19]. Chan et al.[20] followed 270 patients with CHB for an average of 79.9 months. Of the 107 patients with NAFLD and CHB, there was a 7.3-fold increased risk of CHB-related HCC. Similarly, Lee et al.[21] reported that overlapping NAFLD in 321 patients with CHB led to a 3-fold increase in the risk of HCC. These studies showed that NAFLD increased the incidence and progression of HCC in patients with an underlying CHB infection. Immunotherapy with ICIs, particularly PD-1/PD-L1 blockade therapy, extends the median OS to approximately 24 months[7,22]. However, only 15%–20% of patients are sensitive to immunotherapy[6,7]. Several studies have confirmed that patients with NAFLD/NASH-related HCC are resistant to immunotherapy[15]. Nevertheless, whether MASLD affects the efficacy of immunotherapy or changes the prognosis of patients with CHB-related HCC remains unclear. Therefore, identifying populations that may or may not be responsive to

Table 1 Baseline demographics and laboratory data of patients between the CHB and MASLD-CHB groups

Variables	CHB group (n = 117)	MASLD-CHB group (n = 38)	t/z/x^2	P
Gender			2.215	0.137
Male	106 (90.5%)	31 (81.6%)		
Female	11 (9.5%)	7 (18.4%)		
Age (years)	56.26 ± 24.35	57.58 ± 23.10	−0.740	0.461
BMI (kg/m^2)	24.35 ± 4.20	23.10 ± 3.27	1.230	0.222
Lg (HBV-DNA) (IU/mL)	1.72 ± 2.31	1.35 ± 1.76	−0.829	0.408
HBsAg (IU/mL)	250.00 (59.71, 250.00)	250.00 (65.80, 250.00)	−1.226	0.222
HBeAg			2.174	0.337
Negative	31 (26.5%)	12 (31.6%)		
Positive	86 (73.5%)	26 (68.4%)		
ALT (U/L)	30.50 (18.63, 45.48)	35.00 (16.70, 52.63)	0.210	0.834
AST (U/L)	37.30 (26.00, 55.35)	41.00 (27.43, 56.50)	−0.998	0.320
ALB (g/L)	39.00 ± 5.28	38.14 ± 5.98	0.833	0.406
TBIL (μmol/L)	15.80 (11.60, 21.80)	16.35 (11.33, 24.48)	−0.116	0.908
Lipid index				
TC (mmol/L)	4.14 ± 0.90	4.11 ± 1.00	0.138	0.890
TG (mmol/L)	1.05 ± 0.40	1.22 ± 0.65	−1.856	0.066
HDL (mmol/L)	1.12 ± 0.32	1.25 ± 0.44	−1.869	0.064
LDL (mmol/L)	2.61 ± 0.76	2.77 ± 0.96	−1.022	0.309
FIB-4 index	3.99 ± 3.62	4.69 ± 5.37	−0.908	0.365
Basic disease				
Hypertension	47 (40.2%)	13 (34.2%)	0.133	0.715
Diabetes	23 (19.7%)	11 (28.9%)	0.759	0.384
Child-Pugh			2.455	0.293
A	97 (82.9%)	28 (73.7%)		
B	20 (17.1%)	10 (26.3%)		
AFP level before treatment (ng/mL)			0.631	0.537
≤ 400	77 (65.8%)	27 (71.1%)		
> 400	40 (34.2%)	11 (28.9%)		
AFP level after 3 months of treatment (ng/mL)			6.874	0.017*
≤ 400	100 (85.5%)	25 (65.8%)		
> 400	17 (14.5%)	13 (34.2%)		
Diameter of tumor (mm)			0.310	0.578
< 50	51 (43.5%)	16 (42.1%)		

Table 1 Continued

Variables	CHB group (n = 117)	MASLD-CHB group (n = 38)	t/z/x^2	P
≥ 50	66 (56.4%)	22 (57.9%)		
Number of tumors			7.934	0.440
≤ 3	52 (44.4%)	11 (28.9%)		
> 3	65 (55.6%)	27 (71.1%)		
BCLC stage			0.296	0.571
B	45 (38.5%)	12 (31.6%)		
C	72 (61.5%)	26 (68.4%)		
Extrahepatic metastasis			0.643	0.419
Yes	61 (52.1%)	24 (63.2%)		
No	56 (47.9%)	14 (36.8%)		
Portal vein tumor thrombi			0.214	0.627
Yes	54 (46.2%)	18 (47.4%)		
No	63 (53.8%)	20 (52.6%)		

*P < 0.05 was considered statistically significant. CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease; BMI, body mass index; HBV-DNA, hepatitis B virus deoxyribonucleic acid; HBsAg, hepatitis B virus surface antigen; HBeAg, hepatitis Be antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AFP, alpha-fetoprotein; FIB-4, fibrosis 4 Score; BCLC, Barcelona Clinic Liver Cancer.

Table 2 Application of PD-1/PD-L1 monoclonal antibody and other treatment methods in the CHB and MASLD-CHB groups

	CHB group (n = 117)	MASLD-CHB group (n = 38)	x^2	P
PD-1/PD-L1 monoclonal antibody				
Sintilimab	48 (41.1%)	21 (55.2%)	2.365	0.124
Camrelizumab	30 (25.6%)	8 (21.1%)	0.220	0.639
Tislelizumab	15 (12.8%)	1 (2.6%)	3.217	0.073
Toripalimab	11 (9.4%)	2 (5.3%)	0.257	0.612
Atezolizumab	13 (11.1%)	6 (15.8%)	0.584	0.445
Combination therapy				
Molecular-targeted drugs	108 (92.3%)	35 (92.1%)	1.870	0.171
TACE	78 (66.7%)	21 (55.3%)	0.031	0.861
Non-radical ablation	34 (29.1%)	8 (21.1%)	0.653	0.419

CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease; PD-1, programmed cell death receptor-1; PD-L1, programmed death ligand-1; TACE, transhepatic arterial chemotherapy and embolization.

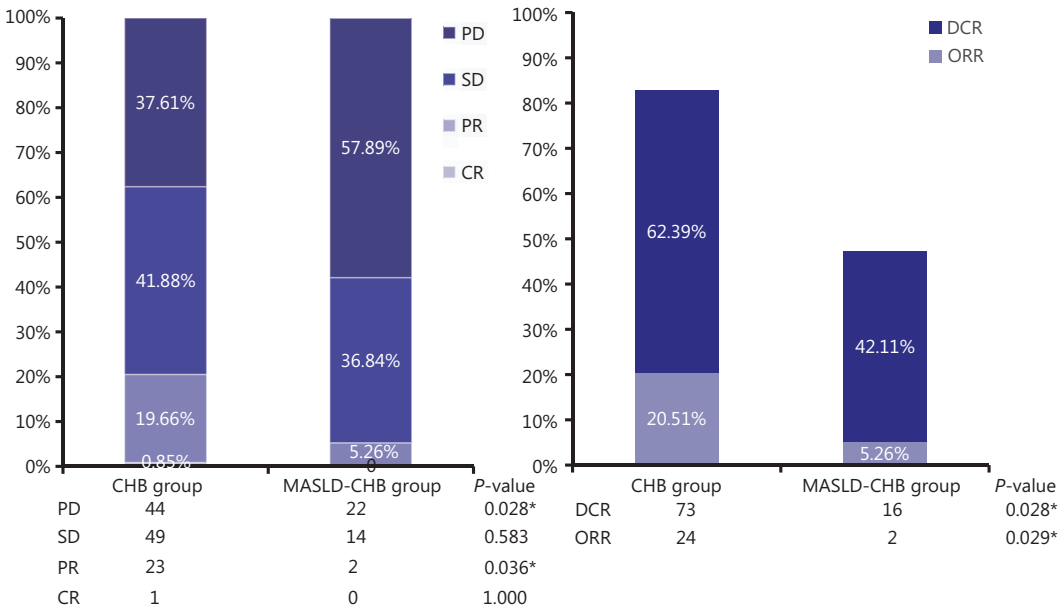


Figure 2 Evaluation of immunotherapy efficacy between the CHB and MASLD-CHB groups. CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease; *P < 0.05 was considered statistically significant.

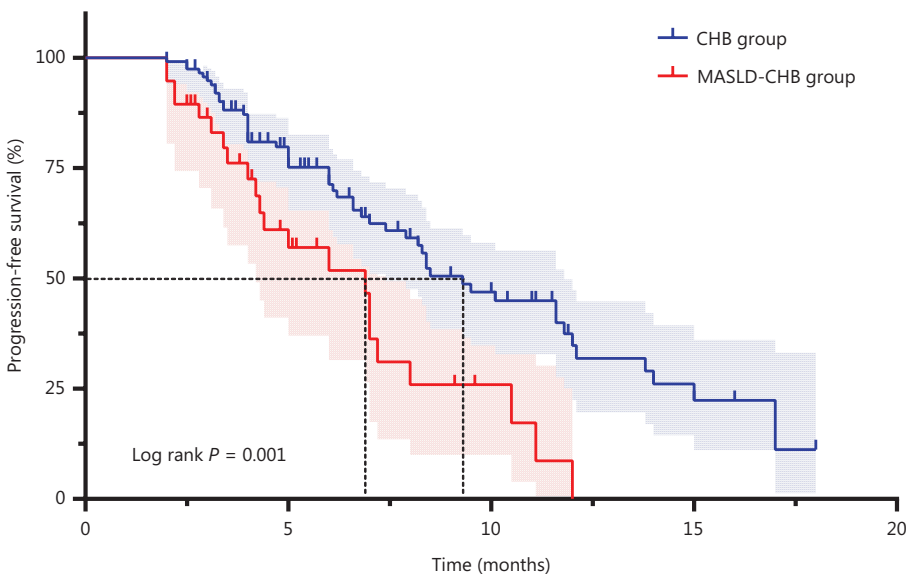


Figure 3 Kaplan–Meier curve for PFS of the CHB and MASLD-CHB groups. CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease; PFS, progression-free survival.

immunotherapy is essential. This approach helps avoid unnecessary immune-related adverse effects and optimizes the allocation of medical resources. In the current study patients in the MASLD-CHB group treated with ICI-based therapy were shown to have a significantly reduced PFS of approximately 2.4 months compared to the CHB group. The PD rate was significantly higher in the MASLD-CHB group than the CHB group with respect to local tumor control. Patients

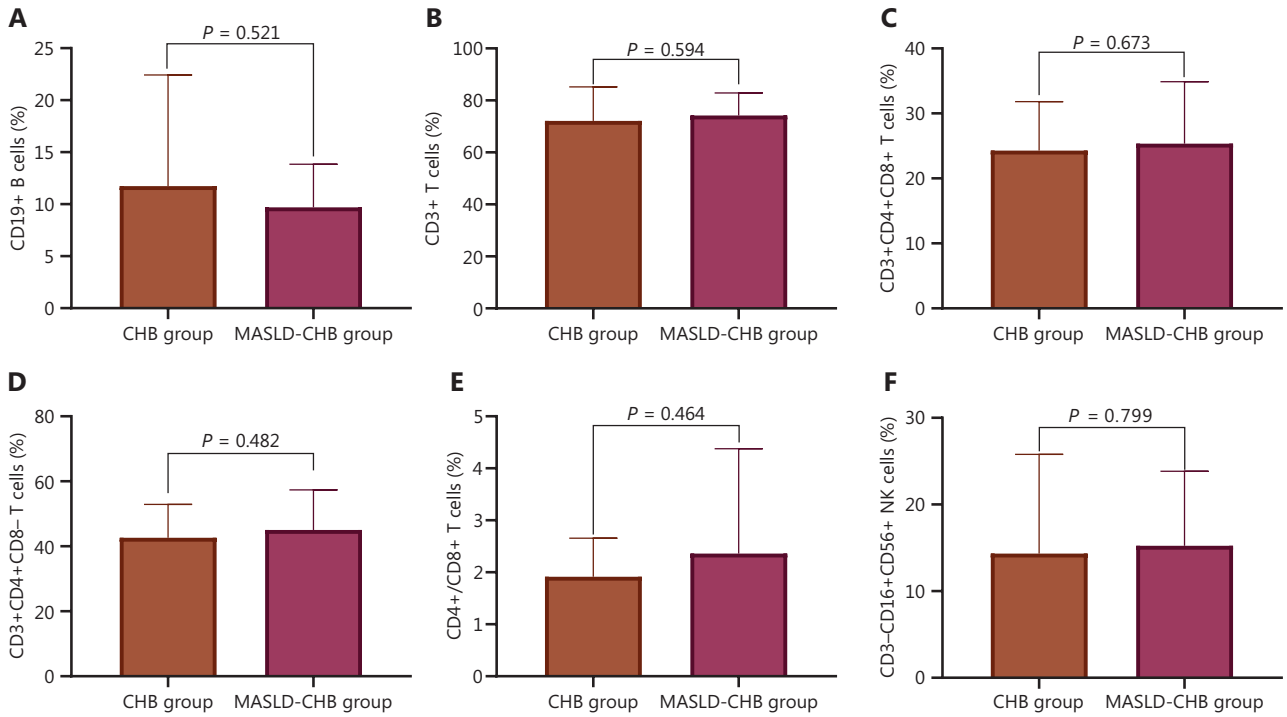


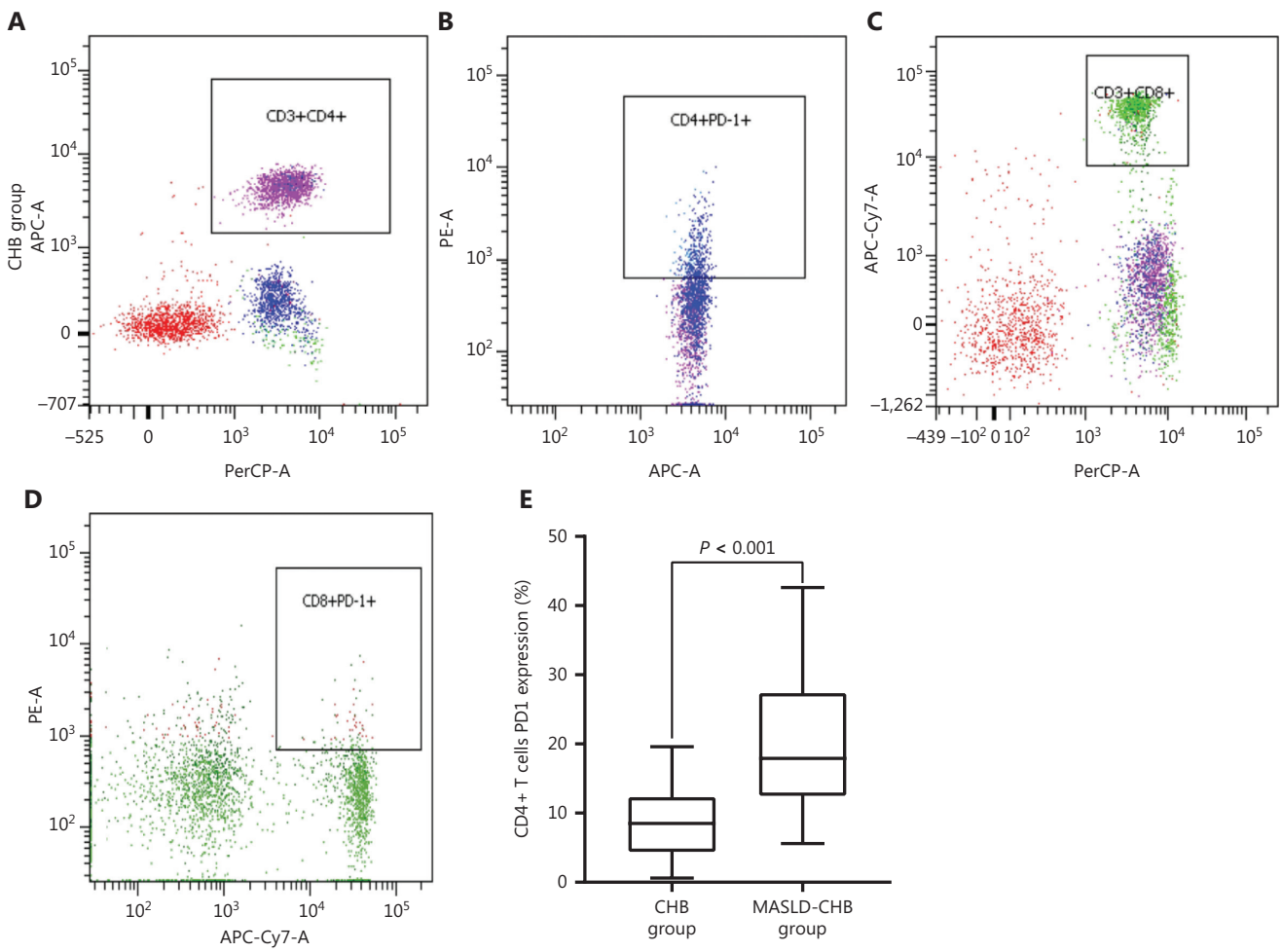
Figure 4 Peripheral blood lymphocyte counts between the CHB and MASLD-CHB groups. CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease.

in the MASLD-CHB group who received ICI-based therapy had a significantly inferior PR, ORR, and DCR than the CHB group. Although there was no significant difference in CR and SD between the two groups, a lower proportion of patients belonged to the MASLD-CHB group than the CHB group. Furthermore, multivariate Cox regression analysis indicated that concurrent MASLD and AFP levels 3 months after immunotherapy were independent risk factors for PFS in all patients with CHB-related HCC.

NAFLD/NASH affects the efficacy of ICIs for HCC treatment, as demonstrated by Pfister et al.[15]. This cohort study analyzed 130 patients with HCC who were treated with PD-1 immunotherapy, including 13 patients with NAFLD, to determine the effects of underlying liver diseases on PD-1/PD-L1 immunotherapy. HCC patients with NAFLD-related conditions had a poorer prognosis, with a median survival time of 5.4 months, compared to non-NAFLD patients, who had an average survival time of 11 months. Additionally, another cohort of 118 patients with HCC who received PD-1/PD-L1 immunotherapy was analyzed, 11 of whom had concurrent NAFLD. Patients with NAFLD-related HCC had a significantly lower median survival time compared to other patients (8.8 months *vs.* 17.7 months). Although the sample size of patients with NAFLD-related HCC was small, poor efficacy of anti-PD-1 treatment was demonstrated. A recent meta-analysis of 8 randomized controlled trials involving 3739 patients confirmed that the efficacy of ICIs in treating non-viral hepatitis-related HCC was inferior to viral hepatitis-related cases[23]. Currently, research involving the MASLD effects on the therapeutic efficacy and progression of CHB-related HCC is limited. The current study was conducted according to the MASLD definition. The impact on the efficacy of ICI-based treatment in patients with CHB-related HCC was investigated. Our findings showed that MASLD co-existing with CHB compromised the survival benefit derived from ICIs-based treatment compared to CHB-related HCC alone. In addition, MASLD co-existing with CHB negatively affected local tumor control and remission.

Resistance to immunotherapy in patients with CHB-related HCC and MASLD may be due to the harsh tumor microenvironment (TME). The HCC TME is a complex and spatially organized entity that includes non-parenchymal liver resident cells, tumor cells, immune cells, and cancer-associated fibroblasts[24,25]. The TME in patients with immunocompetent HCC

is characterized by rich infiltration of activated helper $CD4^+$ and cytotoxic $CD8^+$T cells and is more likely to respond to ICIs. T cells quickly eliminate mutated tumor cells that are recognized by the immune system and are therefore crucial in the antitumor response. However, tumor cells with high PD-L1 expression can bind to PD-1 on the surface of T cells that causes a mistaken identification of tumor cells as autologous, which inhibits the ability of the immune system to kill tumor cells[26]. ICIs disrupt the interaction between abnormally high levels of PD-L1 on tumor cell surfaces and PD-1 on immune cell surfaces using small molecules or monoclonal antibodies. This process enables restoration of killing activity in tumor-infiltrating lymphocytes and facilitates the effective elimination of tumor cells. Additionally, liver tumors exhibit an intermediate mutational burden and utilize different immune evasion mechanisms[27]. Recent studies using single-cell ribonucleic acid sequencing have shown significant interpatient variability in HCC cells and demonstrated a more consistent repetitive gene expression signature within the TME[28]. A study using mass spectrometry and flow cytometry detected NAFLD- and other etiologic-related HCC in the blood of patients. The results showed a significant increase in the number of $CD8^+$ $PD\text{-}1^+$ T-cells in NAFLD-related HCC patients[15]. Notably, the tumors did not respond to anti-PD-1/PD-L1-based therapy and were conducive to liver fibrosis and tumor metastasis after treatment. Resident $CD8^+PD^-1^+$ T cells may impair the proliferative ability of liver cells, leading to inadequate immune surveillance and subsequent liver tissue damage in patients with NAFLD-related HCC following anti-PD-1/PD-L1 immunotherapy. In the current study the percentage of $CD8^+PD1^+$T cells was significantly higher in blood samples from patients with MASLD and co-existing CHB-related HCC than the



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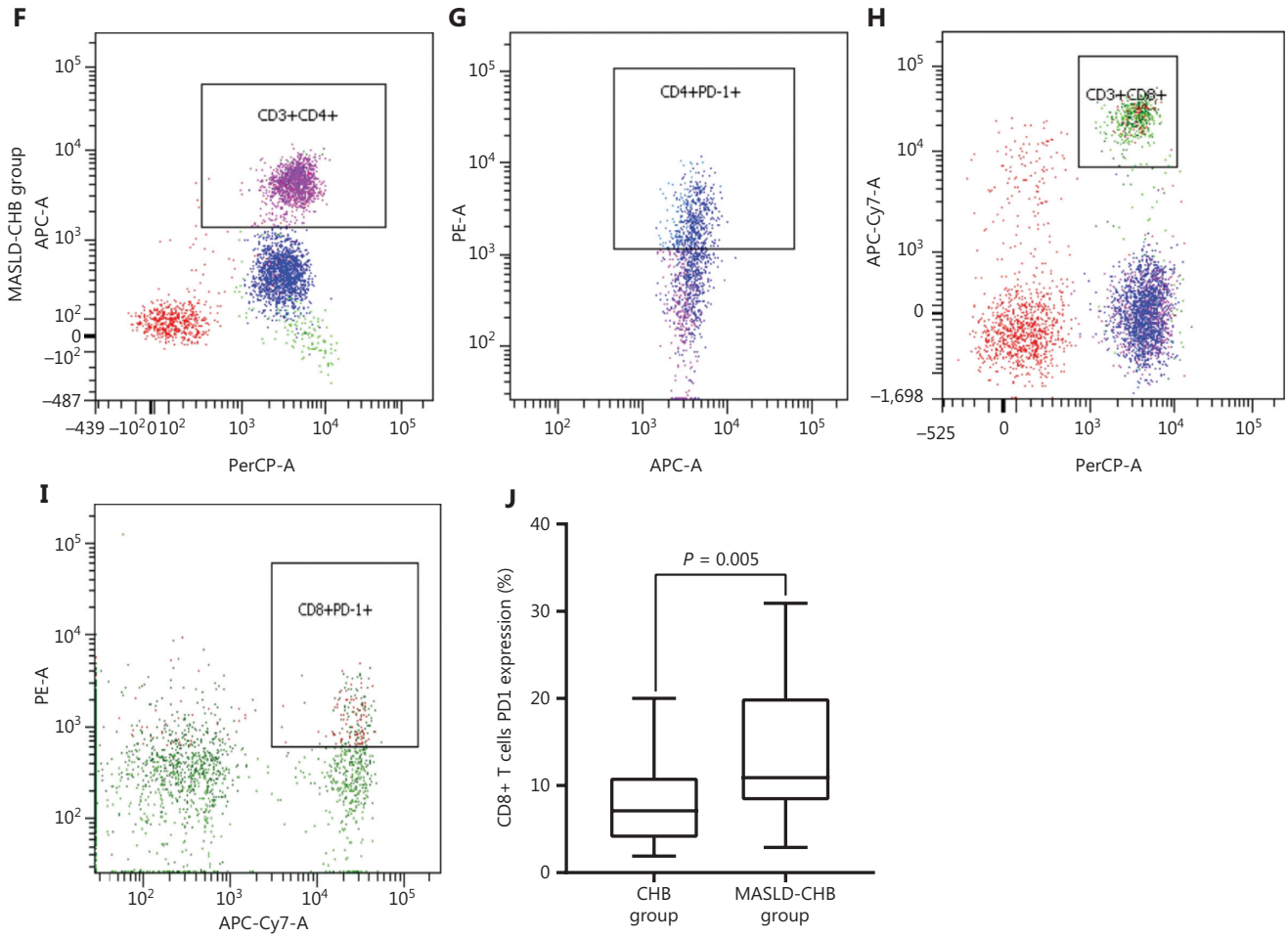

Figure 5 PD-1 expression of $CD4^+$ and $CD8^+$ T cells between the CHB and MASLD-CHB groups. CHB, chronic hepatitis B; MASLD, metabolic dysfunction-associated steatotic liver disease; PD-1, programmed cell death receptor-1.

CHB group. The percentage of $CD4^+PD1^+$T cells was also increased in the MASLD-CHB group. Therefore, MASLD may cause a small proportion of activated helper $CD4^+$ and cytotoxic $CD8^+$T cells in the HCC TME to become immunoreactive. Additionally, a large number of immunosuppressive cells lead to immunotherapy resistance owing to the high expression of PD-1 by $CD4^+$ and $CD8^+$T cells.

Concurrent MASLD and AFP levels after 3 months of immunotherapy affected patient outcomes. Both were identified as independent risk factors for an inferior PFS in patients with CHB-related HCC after immunotherapy. AFP is the most commonly used serum marker in clinical practice and has a critical role in the diagnosis of HCC and therapeutic effect monitoring. High AFP levels may be attributed to pregnancy, chronic or active liver disease, gonadal embryonic tumors, and digestive tract tumors. Provided that all of the aforementioned causes have been excluded, serum AFP levels ≥ 400 ng/mL strongly suggest the presence of HCC[29]. Some studies have shown that high serum AFP levels in patients with HCC are closely associated with an increased risk of HCC progression and a reduced survival rate[30,31]. Another study involving 78,743 patients with HCC showed that the AFP level is an independent risk factor for poor outcomes regarding HCC tumor grade, TNM stage, tumor size, and overall patient survival[32]. The number of patients with high AFP levels in the MASLD-CHB group increased from 29.9% before treatment to 35.3% after 3 months of treatment. This increase was significantly greater than that observed in the CHB group (14.5%). When using 400 ng/mL as the AFP cut-off value, the patients were divided into 2 groups and PFS was analyzed. Patients with CHB-related HCC and low AFP levels after 3 months of treatment had a prolonged mPFS (approximately 3.8 months) compared

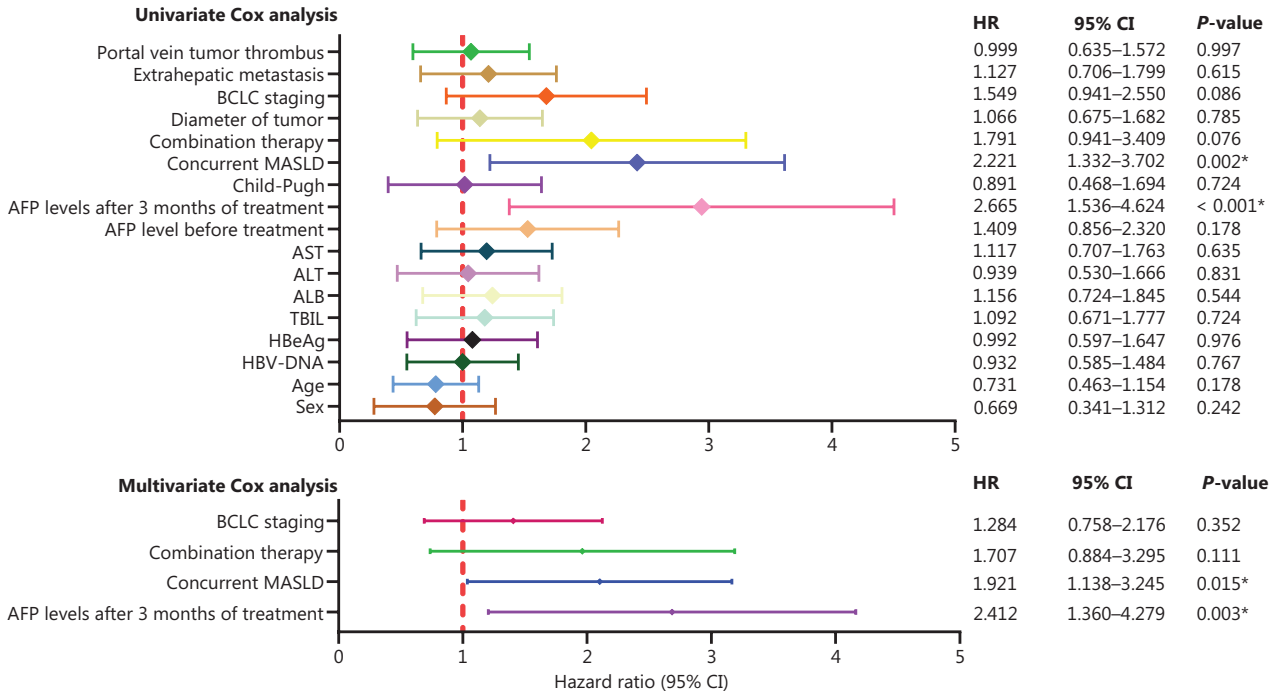

Figure 6 Univariate and multivariate analyses of factors affecting PFS in CHB-related HCC. CHB, chronic hepatitis B; PFS, progression-free survival; HCC, hepatocellular carcinoma; MASLD, metabolic dysfunction-associated steatotic liver disease; HBV-DNA, hepatitis B virus deoxyribonucleic acid; HBeAg, hepatitis Be antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; *P < 0.05 was considered statistically significant.

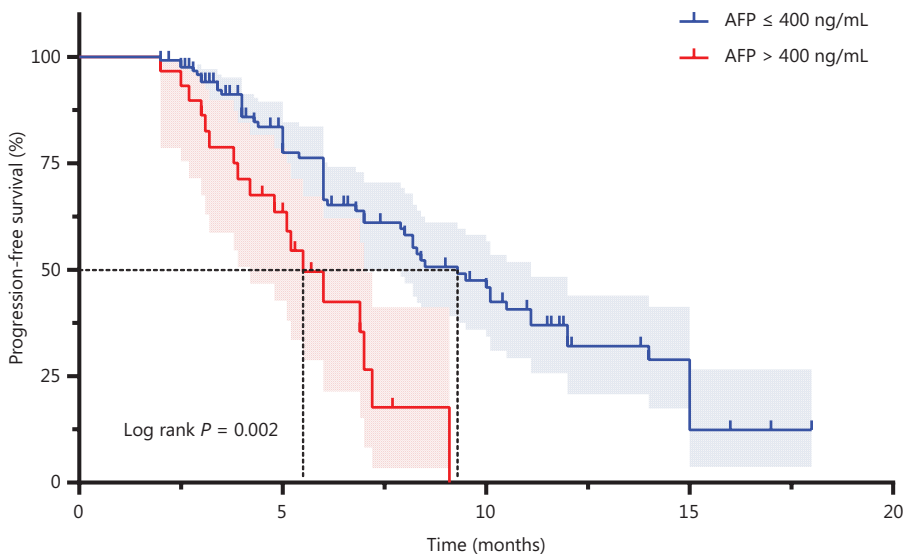

Figure 7 Kaplan–Meier curve for PFS after stratification by high or low levels of AFP. PFS, progression-free survival; AFP, alpha-fetoprotein.

to patients with high AFP levels. In patients with CHB-related HCC, a persistently high AFP level after 3 months of treatment indicated a diminished survival time and a poorer prognosis. In addition, the follow-up time was too short to determine the OS rates but at 3, 6, and 12 months the OS rates were 100.0%, 94.2%, and 83.8%, respectively.

This study had several limitations. First, the sample size was small and the follow-up time was relatively short. Increasing the number of patients and extending the follow-up period may yield somewhat different results. Second, the variety of ICIs and combined treatment methods were complex, which may have affected the results. Third, the number of patients in the two groups was unbalanced, which may have affected the results, although nearly all demographic and laboratory data baseline levels were consistent, and more patients from additional centers for further discussion are needed. Finally, the study enrolled only individuals of ethnic Han descent and did not include other races. Corollary studies may be conducted on a larger scale and for a longer period of time to circumvent these issues. Despite some caveats surrounding the current study, the data are robust and the results are encouraging. Furthermore, we plan to conduct a multi-center, large-sample study to investigate the impact of renaming NAFLD to MASLD on immunotherapy.

Conclusions

In conclusion, concurrent MASLD appeared to result in poorer efficacy and shorter PFS when patients with CHB-related HCC were treated with ICI-based therapy. This finding might also be attributed to the increase in $CD4^+PD1^+$ and $CD8^+PD1^+$ T cells in peripheral blood. Moreover, in addition to concurrent MASLD, the AFP level after 3 months of ICI-based therapy was an independent risk factor for PFS in patients with CHB-related HCC. Therefore, we need to consider how to screen the dominant population and optimize the treatment plan when formulating an immunotherapy plan for patients with concurrent MASLD and CHB-related HCC.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Liang Xu, Wei Lu, Ningning Zhang, and Wentao Kuai.
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Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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