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Effect of potent nucleos(t)ide analog on alpha fetoprotein changes and occurrence of hepatocellular carcinoma in patients with chronic hepatitis B

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Abstract

Background Successful antiviral therapy significantly decreases the incidence of hepatocellular carcinoma (HCC) in patients with chronic hepatitis B (CHB). Alpha-fetoprotein (AFP) in the serum is a valuable early indicator of HCC. However, it is unclear whether different antiviral medications have varying effects on AFP levels. The purpose of this study was to evaluate this issue in those treated with entecavir (ETV) versus tenofovir disoproxil fumarate (TDF).

Methods We prospectively enrolled treatment-naive CHB adults who commenced treatment with ETV or TDF. Their changes in biochemical, virological, and fibrosis parameters and the elevation of AFP or development of HCC during follow-up were analyzed.

Results A total of 1942 CHB patients were included (10–90% follow-up time 3–60 months), and 104 patients with elevated AFP (5.3%) and 27 patients with HCC development (1.4%) were identified during the follow-up. The difference in the cumulative incidence of AFP abnormalities and HCC was statistically significant between patients who received ETV or TDF therapy. Multivariate Cox regression showed that elevated liver stiffness with shear wave elastography (Hazard ratio (HR) = 1.05, 95% Confidence interval (CI) 1.03–1.08, $P < 0.001$) and abnormal AFP at baseline (HR = 1.00, 95% CI 1.00–1.00, $P < 0.001$) were independent risk factors for abnormal AFP in CHB patients, while shear wave elastography (HR = 1.07, 95% CI 1.02–1.12, $P < 0.001$) was also independent risk factor for HCC. Similar results were obtained after propensity score matching (PSM) analysis. The combination of shear wave elastography (SWE), mPage-B score, age and type 2 diabetes mellitus had an area under the curve of 0.838 ($P < 0.001$) in predicting the occurrence of HCC.

Conclusions Similar AFP elevation and HCC development rates were observed in CHB patients treated with ETV or TDF. Elevated SWE and abnormal AFP at baseline were independent risk factors for abnormal AFP in CHB patients.

Keywords Alpha fetoprotein, Antiviral therapy, Entecavir, HBV infection, Hepatocellular carcinoma, Tenofovir disoproxil fumarate

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Introduction

Hepatocellular carcinoma (HCC) ranks sixth among the most frequent malignant tumors globally and is the third most common cause of cancer-related deaths (after lung and gastric cancer) [1, 2]. Despite the continuous progress in prevention, screening, diagnosis and treatment, the incidence rate and mortality of HCC are still rising [2], with a mortality rate as high as 95%. Approximately 600,000 people die of HCC every year, and the 5-year survival rate is only 6.9% [3]. hepatitis B virus (HBV) infection is the most common cause of HCC, especially among Asians [2]. Worldwide, approximately 80% of HCC patients are infected with HBV [4]. Studies have confirmed that the risk of HCC in chronic hepatitis B (CHB) patients is 30 times higher than that in healthy people [2]. Entecavir, tenofovir or propofol tenofovir are the first-line treatments for patients with chronic hepatitis B [5]. Antiviral drugs can effectively reduce but not completely eliminate the risk of progressing to end-stage liver disease, including hepatocellular carcinoma [5, 6]. Therefore, optimizing the early identification of HCC in NA-treated patients is of great clinical significance.

Recent studies have shown that serum alpha fetoprotein (AFP) is an oncoprotein that contributes to the progression of HCC, and intracellular AFP acts as a signaling molecule that mediates multiple cellular processes [7]. AFP regulates the phenotype of HCC cells through activation of the AKT and CXCR4 signaling pathways to promote cell growth and metastasis [7]. AFP is currently the most widely used screening and diagnostic marker of HCC, and its use in HCC has been recommended by the APASL, EASL and AASLD guidelines [8–10]. AFP remains one of the most widely used biomarkers for hepatocellular carcinoma in clinical practice [11]. Earlier studies have demonstrated that 40% of patients with HBV infection-related liver cancer had significantly increased levels of serum AFP, indicating that AFP may be utilized as a diagnostic marker [12]. Since AFP may also exhibit abnormally elevated levels during hepatitis flares, AFP has been applied as a critical end-point for evaluating disease progression during antiviral therapy.

Recently, several long-term follow-up reports and meta-analyses have raised concerns about more effective HCC prevention with tenofovir disoproxil fumarate (TDF) than entecavir [13–16], whereas other previous studies have shown similar treatment outcomes measured by biochemical, virologic, immunological, and pathologic responses and survival [17]. However, the effect of different antiviral drugs on AFP levels and especially the predictive value of AFP elevation concomitant with normal levels of liver enzymes or virology marker regarding the risk of HCC remain unclear at present [5, 6].

This study evaluated the changes in serum AFP levels and the occurrence of HCC during different oral antiviral treatments in a Han Chinese population enrolled from a high prevalence area of HBV in China.

Methods

Study design and patients

This prospective cohort study was carried out at the Outpatient Department of Chronic Hepatitis B in the First Affiliated Hospital of Sun Yat-sen University, China from January 1, 2011, to December 31, 2021. The study protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University (ethics number 187) and all participants provided written informed consent.

We included patients consecutively admitted with CHB and met the following criteria: (1) age over 18 years; (2) complete anthropometric and laboratory test results available; (3) diagnosed with chronic HBV infection, chronic hepatitis B, or HBV-related HCC.

The diagnosis of chronic HBV infection was defined as HBsAg and/or HBV DNA positivity for more than 6 months [5, 18]. The diagnostic criteria for CHB were persistent/repeated elevation of alanine aminotransferase (ALT) or liver histology showing hepatitis and a chronic HBV infection diagnosis [18].

The diagnosis of HCC was confirmed by histological assessment [5]. Puncture biopsy of liver lesions was followed by their fixation and a description of their gross and microscopic characteristics, immunohistochemistry and molecular pathology. The use of standardized pathological examination was crucial in ensuring the precision of the pathological diagnosis, which can serve as a valuable guide for assessing the risk of recurrence, long-term prognosis, and development of personalized treatment strategies in clinical settings [19].

The pathological diagnosis of hepatitis B cirrhosis includes: (1) positive for HBsAg or negative for HBsAg but positive for anti-HBC, with a clear history of chronic HBV infection (previous HBsAg positive > 6 months) and exclusion of other causes; (2) liver biopsy and pathology consistent with the manifestations of liver cirrhosis [5, 19, 20]. The clinical diagnosis of hepatitis B cirrhosis includes: (1) HBsAg positivity and a clear history of chronic HBV infection; (2) Patients with non-cirrhotic portal hypertension were excluded if the following criteria were met: imaging examination showed signs of cirrhosis or portal hypertension; endoscopy showed esophageal and gastric varices; the hardness of the liver was consistent with that of liver cirrhosis; blood biochemical examination showed that the level of albumin was decreased (< 35 g/L) or the prothrombin time was prolonged (prolonged > 3 s); routine blood examination

showed that the platelet count was less than 100×10^9/L [5, 20, 21].

This study excluded the following patients: (1) pregnant or breastfeeding women; (2) patients with previous antiviral treatment; and (3) patients who had other malignant tumors or other serious diseases with organ dysfunction in addition to HCC. Follow-up and antiviral treatment were carried out according to the guidelines for the prevention and treatment of chronic hepatitis B and the patient's clinical condition and preference [22, 23]. Antiviral drugs included entecavir, tenofovir or other antiviral drugs, including tenofovir alafenamide fumarate (TAF) and telbivudine (LdT). The entecavir treatment group and TDF group had similar age, sex, body mass index (BMI) and history of hepatitis B.

Laboratory measurements

The study involved collecting venous blood samples from patients who had fasted for at least 8 h. The liver biochemical and metabolic parameters assessed included ALT, aspartate aminotransferase cellulase (AST), GGT, alkaline phosphatase, albumin (ALB), globulin (GLB), direct bilirubin (DBil), total bilirubin (TBil), platelets (PLT), INR, AFP, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb and HBV DNA. The detection limit for HBV DNA was set at 100 IU/mL [24].

The serum AFP levels were determined using an enzyme immunoassay (Beckman Coulter AU 5800 system). A cutoff value of 20 ng/mL was used to define normal AFP levels. Liver stiffness was measured using abdominal ultrasound shear wave elastography (SWE) [25, 26]. In previous studies, 2D shear wave elastography (2D-SWE) has been shown to be an effective noninvasive method for assessing liver fibrosis in patients with hepatitis B [26]. The 2D-SWE measurements, including the evaluation date and number of measurements, were recorded.

On the basis of previous research results both domestically and internationally, we used various commonly used liver cell carcinoma scoring models, including models such as REACH-B score [27], Page-B score [28], mPage-B score [29] and aMAP score [30], to evaluate the risk of developing liver cell carcinoma in HBV infected patients after antiviral therapy.

We collected data on whether the patients had concurrent non-alcoholic/ metabolic associated fatty liver disease (NAFLD/ MAFLD) or diabetes, as numerous studies have demonstrated that these conditions can accelerate the occurrence of hepatitis B-related liver cancer [2, 26]. The diagnosis of NAFLD was based on liver imaging, such as abdominal ultrasonography, that revealed steatosis. Patients without a history of alcohol consumption < 30 g/day in men or < 20 g/day in women, drug-induced liver disease, total parenteral nutrition, hepatolenticular degeneration, autoimmune hepatitis, or other specific diseases that may lead to fatty liver were included [31]. The diagnostic criteria for type 2 diabetes included typical symptoms of diabetes, such as fasting blood glucose ≥ 7.0 mol/L, random blood glucose ≥ 11.1 mol/L, 2-h blood glucose ≥ 11.1 mol/L on an oral glucose tolerance test, or glycosylated hemoglobin (HbA1c) ≥ 6.5% [31].

Clinical follow-up and treatment

The patients were treated with personalized antiviral treatment or underwent regular follow-up according to the hepatitis B prevention and treatment guidelines [22, 23]. Follow-up examinations were conducted at 1, 3, 6, 9, and 12 months, and then every 6 months thereafter, with a maximum deviation of one month from the scheduled time point. During each follow-up visit, the patients' anthropometric parameters, AFP levels, HBV-related virological indices, liver biochemical parameters, and abdominal ultrasound SWE results were re-measured.

If the AFP level increased during follow-up, patients were screened for HCC using liver ultrasound, dynamic contrast-enhanced CT or MRI. Patients who had not yet started antiviral treatment were recommended to start antiviral treatment, while those who have already received antiviral treatment recommend to use entecavir, TDF or TAF as additional antiviral treatment [6, 23].

Statistical analysis

Statistical analyses were conducted using IBM 24.0 SPSS Statistics software. Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Kruskal–Wallis or Pearson rank sum tests were used to compare variables between groups, and ANOVA tests were used for multiple comparisons between groups. To mitigate potential bias, propensity score matching statistical methods was applied to balance baseline features between groups. Cox regression analysis was used to estimate the potential risk factors associated with elevated AFP and HCC incidence.

Results

Baseline characteristics stratified with AFP status

Among the 1942 treatment naive HBV patients eventually included in the study, the detailed flow chart was supplied in Fig. 1, 227 cases (12%) had elevated AFP (greater than 20 ng/ml) at baseline, and 26 patients (11%) of them were diagnosed with HCC whereas 35 patients (2%) with normal AFP were diagnosed with HCC in the baseline normal AFP group with 1715 patients (98%) (Fig. 1). There were a total of 1680 patients in the baseline normal AFP group. During the 60 months follow-up

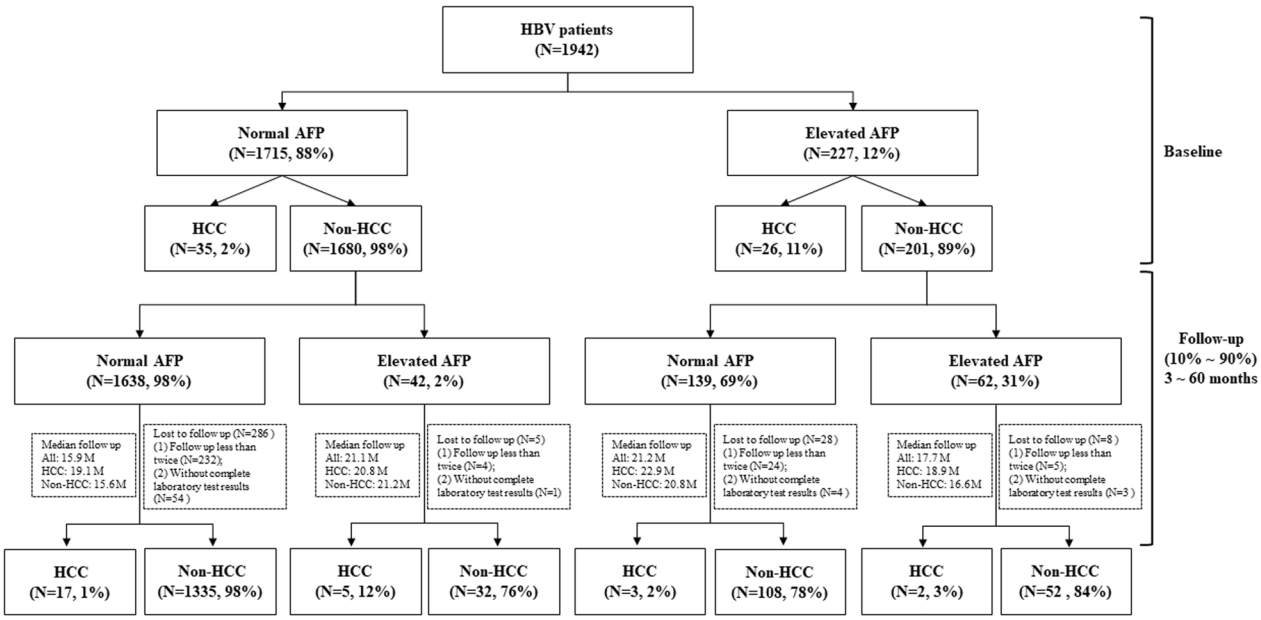

Fig. 1 Flow diagram of participant recruitment and screening

period, 42 patients experienced elevated AFP levels, and 1638 patients maintained normal AFP levels throughout the entire follow-up period. 1638 patients underwent 60 months of follow-up, and ultimately 22 patients developed HCC. Among them, 17 patients did not detect abnormal elevation of AFP levels, while 5 patients detected abnormal AFP levels (Fig. 1). There was a total of 201 patients in the baseline abnormal AFP group. During a follow-up period of 60 months, the AFP levels of 139 patients gradually returned to normal levels. As of the end of follow-up, a total of 3 patients in this group were monitored for HCC. There were 62 patients with persistent abnormal AFP levels throughout the follow-up period, and ultimately 2 patients detected the occurrence of HCC (Fig. 1).

According to the baseline AFP level after follow-up and/or whether they had HCC, they were divided into four groups: HCC patients with normal AFP (n = 35), non-HCC patients with normal AFP (n = 1680), HCC patients with abnormal AFP (n = 26) and non-HCC patients with abnormal AFP (n = 201) (Table 1). There were significant differences in age, sex, liver enzymology, AFP, SWE, REACH-B score, Page-B score, mPage-B score, aMAP score, HBV virological indices and antiviral treatment among the four groups (Table 1). HCC patients with abnormal AFP had higher TB (20.2 μmol/L vs. 14.2 μmol/L), DB (4.8 μmol/L vs. 2.9 μmol/L), LSM with SWE (15.6 kPa vs. 6.1 kPa) and liver cirrhosis proportions (84.6% vs. 17.2%) than non-HCC patients with normal AFP (all $P < 0.001$, Table 1). Within the non-HCC group, the AFP abnormal group had an older age and higher male proportion, liver enzymology index, liver metabolism index, platelet, SWE and liver cirrhosis than the AFP normal group, as well as higher HBV DNA levels (all $P < 0.01$, Table 1). There were similar trends in REACH-B score, Page-B score, mPage-B score and aMAP score among the four groups of patients. Compared with non-HCC patients with normal AFP, the group of HCC patients with normal AFP, the group of HCC patients with abnormal AFP, and the group of non-HCC patients with abnormal AFP all had higher REACH-B score (7.3 vs. 9.4 vs. 10.9 vs. 10.0), Page-B score (14.4 vs. 20.4 vs. 22.5 vs. 18.8), mPage-B score (7.7 vs. 12.8 vs. 13.2 vs. 10.4), and AMAP score (46.7 vs 58.6 vs 60.3 vs 54.4) (all $P < 0.001$, Table 1).

ETV or TDF treatment and AFP surveillance and HCC risks

Among 1942 patients included in this study, their past anti hepatitis B virus treatment was counted at the first outpatient reception in our hospital. There were 1322 patients who had not received antiviral treatment before, and 610 patients who had received anti -viral treatment. In the follow-up diagnosis and treatment process of our hospital, 28 patients were lost after 3 months of inclusion in the study and were not included in the subsequent study data analysis. Among the remaining 1914 patients, the majority chose to receive antiviral treatment, including 759 patients (40%) chose to take entecavir (ETV) for long-term antiviral therapy, while 360 patients (18%) selected tenofovir disoproxil fumarate (TDF); 268

Table 1 Baseline characteristics of hepatitis B patients with normal AFP and elevated AFP before follow-up treatment in our hospital

	Normal AFP (N = 1715)		Elevated AFP (N = 227)		*P* value	Post-hoc					
	HCC	Non-HCC	HCC	Non-HCC		1# vs. 2#	1# vs. 3#	1# vs. 4#	2# vs. 3#	2# vs. 4#	3# vs. 4#
Patients, n (%)	35 (2.0)	1680 (98.0)	26 (11.5)	201 (88.5)							
Age, years	50.1 ± 9.1	40.1 ± 12.6	55.0 ± 12.3	44.0 ± 12.4	< 0.001	< 0.001	0.128	0.008	< 0.001	< 0.001	< 0.001
Male, n (%)	30 (85.7)	1105 (65.8)	21 (80.8)	162 (80.6)	< 0.001	0.014	0.606	0.473	0.109	< 0.001	0.983
BMI, kg/m^2	23.2 (21.2,25.3)	22.2 (20.0,24.6)	22.2 (20.1,23.6)	23.1 (22.0,23.9)	0.188						
History of HBV, years	14.5 (8.0,20.0)	10.0 (6.0,20.0)	12.5 (10.0,20.0)	10.0 (5.0,20.0)	0.381						
NAFLD, n (%)	2 (5.7)	259 (15.4)	2 (7.7)	21 (10.4)	0.076						
T2DM, n (%)	2 (5.7)	51 (3.0)	1 (4.0)	7 (3.5)	0.811						
Hepatitis B Virology											
Log10 HBVDNA, IU/mL	3 (2,5)	4 (2,6)	4 (2,5)	6 (4,7)	< 0.001	0.703	0.998	0.001	0.740	< 0.001	0.003
Log10 HBsAg quantification, IU/mL	3 (3,3)	3 (3,4)	3 (2,3)	3 (3,4)	0.002	0.036	0.470	0.014	0.004	0.197	0.002
HBeAg-positive, n (%)	6 (17.1)	713 (42.4)	12 (46.2)	97 (48.3)	< 0.001	0.003	0.014	0.001	0.704	0.115	0.840
Biochemistry											
ALT, U/L	45 (33,61)	36 (24,58)	47 (33,72)	87 (45,296)	< 0.001	0.354	0.397	< 0.001	0.769	< 0.001	< 0.001
AST, U/L	35 (29,50)	31 (25,47)	62 (47,119)	87 (50,197)	< 0.001	0.917	0.175	< 0.001	0.086	< 0.001	< 0.001
GGT, U/L	32 (26,61)	26 (18,45)	122 (37,257)	85 (50,141)	< 0.001	0.860	0.336	0.080	0.153	< 0.001	0.717
Alkaline phosphatase, U/L	87 (78,101)	76 (65,90)	122 (83,165)	99 (79,121)	< 0.001	0.118	< 0.001	0.189	< 0.001	< 0.001	0.001
ALB, U/L	43.2 (39.3,46.7)	44.8 (42.3.46.9)	40.4 (37.7,42.5)	41.6 (37.6,44.2)	< 0.001	0.080	0.893	0.906	0.074	< 0.001	0.950
GLB, U/L	30.9 (28.8,32.9)	29.1 (26.4,32.1)	32.1 (30.5,36.0)	33.2 (29.0,36.0)	0.013	0.702	0.528	0.403	0.226	0.002	0.980
Total bilirubin, umol/L	12.9 (12.1,14.7)	14.2 (10.9,19.3)	20.2 (15.0,38.6)	19.4 (15.6,33.4)	< 0.001	0.196	0.041	0.001	< 0.001	< 0.001	0.563
Direct bilirubin, umol/L	2.9 (2.4,3.7)	2.9 (2.1,4.2)	4.8 (3.6,8.1)	5.6 (3.8,11.0)	< 0.001	0.029	0.203	0.012	< 0.001	< 0.001	< 0.001
PLT, 10^9/L	159 (108,191)	204 (164,247)	127 (82,145)	155 (111,187)	< 0.001	< 0.001	0.820	0.411	0.001	< 0.001	0.641
INR	1.0 (0.9,1.1)	1.0 (0.9,1.1)	1.1 (1.0,1.1)	1.2 (1.1,2.0)	0.943						
LSM with SWE, kPa	13.8 (7.4,15.6)	6.1 (5.3,8.0)	15.6 (13.2,17.9)	13.1 (7.5,16.0)	< 0.001	< 0.001	0.022	0.286	< 0.001	< 0.001	0.049
AFP, ng/mL	3.8 (2.8,6.6)	2.8 (2.0,4.3)	95.1 (33.4,414.2)	47.7 (25.4,124.0)	0.008	0.999	0.420	0.199	0.278	0.001	0.873
Cirrhosis, n (%)	25 (71.4)	289 (17.2)	22 (84.6)	158 (78.6)	< 0.001	< 0.001	0.402	0.348	< 0.001	< 0.001	0.799
REACH-B score	9.4 ± 2.7	7.3 ± 3.4	10.9 ± 3.3	10.0 ± 3.2	< 0.001	< 0.001	0.088	0.315	< 0.001	< 0.001	0.215
Page-B score	20.4 ± 4.7	14.4 ± 6.0	22.5 ± 5.1	18.8 ± 6.2	< 0.001	< 0.001	0.219	0.177	< 0.001	< 0.001	0.005
mPage-B score	12.8 ± 3.1	7.7 ± 3.8	13.2 ± 3.3	10.4 ± 3.9	< 0.001	< 0.001	0.668	0.002	< 0.001	< 0.001	0.001
aMAP score	58.6 ± 8.1	46.7 ± 9.2	60.3 ± 7.0	54.4 ± 8.7	< 0.001	< 0.001	0.506	0.024	< 0.001	< 0.001	0.003
Medication											
None, n(%)	16 (45.7)	1178 (70.1)	11 (42.3)	127 (63.2)	< 0.001	0.002	0.791	0.051	0.002	0.044	0.040
Nucleoside analogues, n(%)	19 (54.3)	502 (29.49)	15 (57.7)	74 (36.8)	< 0.001	0.002	0.791	0.051	0.002	0.044	0.040
ETV, n(%)	12 (63.2)	252 (50.2)	6 (40.0)	48 (64.9)	0.061	0.267	0.179	0.890	0.436	0.018	0.072

Table 1 (continued)

	Normal AFP (N = 1715)		Elevated AFP (N = 227)		*P* value	Post-hoc					
	HCC	Non-HCC	HCC	Non-HCC		1# vs. 2#	1# vs. 3#	1# vs. 4#	2# vs. 3#	2# vs. 4#	3# vs. 4#
TDF, n(%)	0 (0.0)	33 (6.6)	0 (0.0)	6 (8.1)							
TAF, n(%)	0 (0.0)	3 (0.6)	0 (0.0)	1 (1.4)							
Others (LAM, LdT, ADV), n(%)	7 (36.8)	214 (42.6)	9 (60.0)	19 (25.7)	0.018	0.616	0.179	0.333	0.181	0.006	0.009

Data are median (first quartile, third quartile), n (%), or mean ± SD (standard deviation)

1- Normal AFP with HCC group; 2- Normal AFP without HCC group; 3- Elevated AFP with HCC group; 4- Elevated AFP without HCC group

AFP alpha fetoprotein, *HCC* hepatic cell carcinoma, *BMI* body mass index, *HBV* hepatitis B virus, *NAFLD* non-alcoholic fatty liver disease, *T2DM* type 2 diabetes mellitus, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyl transferase, *ALB* albumin, *GLB* globulin, *TB* total bilirubin, *DB* direct bilirubin, *PLT* platelet, *INR* international normalized ratio, *SWE* shear wave elastography, *ETV* entecavir, *TDF* tenofovir disoproxil fumarate, *TAF* tenofovir alafenamide fumarate, *LAM* lamivudine, LdT telbivudine, *ADV* adefovir dipivoxil

patients (14%) chose to receive other antiviral drugs, including TAF, lamivudine (LAM), telbivudine (LdT) and adefovir dipivoxil (ADV); and 527 patients (28%) chose not to take antiviral treatment temporarily and were followed up regularly (Table 2). In terms of their initial AFP level and their antiviral treatment choice of ETV or TDF, they were divided into six groups: patients with normal AFP without antiviral treatment (n = 527); patients with normal AFP treated with antiviral therapy in addition to ETV and TDF (n = 268); patients with normal AFP treated with ETV (n = 598); patients with abnormal AFP treated with ETV (n = 161); patients with normal AFP treated with TDF (n = 324) and patients with abnormal AFP treated with TDF (n = 36) (Table 2). In patients with normal AFP, there were significant differences in PLT, SWE, cirrhosis, HBV DNA and HBsAg quantification between the ETV treatment group and the TDF treatment group (Table 2). In patients with abnormal AFP, there were significant differences in age, ALT, AST, GLB, TB, DB, SWE and HBsAg quantification between the ETV treatment group and the TDF treatment group (Table 2). Six groups of patients were evaluated using different liver cancer model scores, and the results showed multiple similarities between the REACH-B score, Page-B score, mPage-B score, and aMAP score among the six groups of patients (Table 2).

Fifty-one patients with ETV and twenty-one patients with TDF were excluded because they were followed up less than twice and were not included in the follow-up analyses. A total of 708 patients with ETV and 339 patients with TDF were included in the analyses, with an average follow-up of 19.7 months (Table 3). Patients using ETV or TDF were divided into six groups according to their AFP level during follow-up or whether they were complicated with HCC: HCC patients treated with ETV (n = 24), AFP abnormal patients treated with ETV (n = 64), AFP normal patients treated with ETV (n = 620), HCC patients treated with TDF (n = 2), AFP abnormal patients treated with TDF (n = 16) and AFP normal patients treated with TDF (n = 321) (Table 3). There were significant differences in age, history of HBV, liver enzymology, liver metabolism, PLT, AFP, SWE, cirrhosis, REACH-B score, Page-B score, mPage-B score, and aMAP score and HBV virological indices among the six groups (Table 3). There were no significant differences in age, history of HBV, liver enzymes or virological index between the HCC groups treated with ETV or TDF (Table 3). There were significant differences in age, TB, DB, SWE, AFP, REACH-B score, Page-B score, mPage-B score, and aMAP score between the AFP abnormal groups treated with ETV or TDF. There were significant differences in age, history of HBV, GGT, PLT, SWE, HBV DNA, HBeAg positivity, HBsAg quantification and Page-B score, mPage-B score, and aMAP score between the AFP normal groups treated with ETV or TDF (Table 3).

For non-HCC patients with elevated baseline AFP, after 12 months of ETV or TDF treatment, their AFP cumulative normalization rate was 32% vs. 45% ($P < 0.05$). After 60 months of follow-up, the cumulative normalization rate reached 97% vs. 99%, without a significant difference (Fig. 2A). For non-HCC patients with abnormal baseline AFP, the cumulative rate of new cases of HCC was 19% vs. 1% ($P = 0.282$) in the ETV and TDF treatment groups, respectively (Fig. 2B). A similar incidence of abnormal AFP or HCC development in the ETV and TDF treatment groups was observed (8% vs. 2%, $P = 0.681$, for the former, Fig. 2C; and 8% vs. 2%, $P = 0.168$ for the latter, Fig. 2D) in non-HCC patients with normal baseline AFP.

The dynamic changes in ALT and HBV DNA levels, HBeAg conversion, HBsAg levels, and LFS with SWE were compared among patients with normal AFP treated with ETV (n = 64), abnormal AFP treated with ETV (n = 620), normal AFP treated with TDF (n = 16) and abnormal AFP treated with TDF (n = 321). ALT levels and HBV DNA levels in the four groups decreased with antiviral treatment, and there were no significant differences among their decreasing trends ($P > 0.05$, Fig. 3A and Fig. 3B). There was a significant difference in the HBeAg-negative accumulation rate between patients with abnormal AFP treated with TDF and those with normal AFP or abnormal AFP treated with ETV ($P < 0.05$, Fig. 3C).

There were also significant differences in the HBeAg-negative accumulation rate between patients with normal AFP treated with TDF and patients with abnormal AFP treated with ETV ($P < 0.001$, Fig. 3C). There was a significant difference in the level of HBsAg between the AFP normal patients treated with TDF and the other three groups ($P < 0.001$, Fig. 3D). There were significant differences in the SWE levels among the four groups ($P < 0.001$, Fig. 3E).

Predictive factors of elevated AFP and HCC development

Univariate Cox regression analysis indicated that elevated SWE, abnormal baseline AFP, Log10 HBV DNA, high REACH-B score, Page-B score, mPage-B score, and aMAP score were independent risk factors for abnormal AFP in CHB patients (Table 4). After multivariate adjustment, elevated SWE (adjusted HR (aHR) = 1.05, 95% CI 1.03–1.08, $P < 0.001$) and abnormal AFP at baseline (aHR = 1.00 95% CI 1.00–1.00, $P < 0.001$) were independent risk factors for abnormal AFP in CHB patients (Table 4).

Using HCC development as an outcome, we found that age ≥ 60 years, elevated SWE, high REACH-B score,

Table 2 Comparison of the first examination results between patients with antiviral drugs and patients without antiviral drugs during the follow-up in our hospital

	None (N = 527)	Others (N = 268)	ETV(N = 759)		TDF(N = 360)		*P* value
	AFP ≤ 20(ng/mL)	AFP ≤ 20(ng/mL)	AFP ≤ 20(ng/mL)	AFP > 20(ng/mL)	AFP ≤ 20(ng/mL)	AFP > 20(ng/mL)	
Patients	527	268	598	161	324	36	
Age, years	38.1 ± 10.0	36.6 ± 10.7 ^{NS}a ‡	38.5 ± 9.0 ^{NS}ab	37.5 ± 8.8 ^{NS}abc	36.8 ± 10.1 ^{NS}abcd	37.1 ± 10.1 ^{NS}abcde	0.098
Male, n (%)	344 (65.3)	183 (68.3) ^{NS}a	437 (73.1) ^{NS}ab	120 (74.5) ^{NS}abc	223 (68.8) ^{NS}abcd	27 (75.0) ^{NS}abcde	0.057
BMI, kg/m^2	22.2 (19.9.,25.0)	21.8 (19.4,24.1) *a	22.4 (20.6,24.6) ^{Ns}a, *b	23.0 (21.4,23.9) ^{NS}abc	22.2 (19.7,24.2) ^{NS}abd, *c	23.2 (22.0,24.1) ^{NS}abcde	0.028
History of HBV, years	10 (7,20)	10 (8,15) ^{NS}a	10 (7,20) *a, **b	10 (6,20) ^{NS}abc	10 (7,20) ^{NS}abd, *c	10 (6,20) ^{NS}abcde	0.005
NAFLD, n (%)	90 (17.1)	30 (11.2) ^{NS}a	80 (13.4) ^{NS}ab	16 (9.9) ^{NS}abc	49 (15.1) ^{NS}abcd	3 (8.3) ^{NS}abcde	0.088
T2DM, n (%)	11 (2.1)	7 (2.6) ^{NS}a	31 (5.2) ^{NS}ab	8 (5.0) ^{NS}abc	12 (3.7) ^{NS}abcd	2 (5.6) ^{NS}abcde	0.091
Hepatitis B Virology							
Log10 HBVDNA, IU/mL	2 (2,5)	4 (2,7) **a	4 (2,5) *a, **b	5 (3,7) **ac, *b	5 (2,7) **ac, *b, ^{NS}d	6 (5,7) **ac, *b, ^{NS}de	< 0.001
Log10 HBsAg quantification, IU/mL	3 (2,4)	4 (3,4) **a	3 (3,4) *a, **b	3 (3,4) *ab, ^{NS}c	4 (3,5) **acd, *b	4 (3,4) **a, *c, ^{NS}bde	< 0.001
HBeAg-positive, n (%)	127 (24.1)	171 (63.8) **a	278 (46.5) **ab	79 (49.1) *ab, ^{NS}c	190 (58.6) **ac, ^{NS}b, *d	19 (52.8) **a, ^{NS}bcde	< 0.001
Biochemistry							
ALT, U/L	28 (20,39)	44 (26,80) *a	35 (25,57) *a,^{NS}b	84 (46,370) **abc	39 (26,71) *a, ^{NS}bc, **d	170 (56,572) **abcde	< 0.001
AST, U/L	26 (22,34)	33 (27,63) *a	32 (25,48) *a,^{NS}b	95 (58,217) **abc	34 (25,58) *a, ^{NS}bc, **d	137 (54,336) **abce, *d	< 0.001
GGT, U/L	21 (16,37)	26 (18,42) ^{NS}a	29 (20,52) ^{NS}ab	92 (54,174) ^{NS}abc	25 (19,43) ^{NS}abcd	89 (54,118) ^{NS}abcde	0.089
Alkaline phosphatase, U/L	71 (62,85)	82 (67,101) ^{NS}a	75 (66,90) *a,^{NS}b	102 (81,134) **abc	74 (65,83) ^{NS}abc, **d	97 (75,110) *ade, ^{NS}bc	< 0.001
ALB, U/L	45.0 (43.0,47.5)	44.8 (42.7,46.7) ^{NS}a	44.5 (42.3.46.8) *a,^{NS}b	41.6 (37.2,44.0) **abc	44.7 (42.1,46.5) ^{NS}abc, **d	41.5 (39.1,44.3) *a, ^{NS}bcde	< 0.001
GLB, U/L	29.3 (26.5,32.3)	29.9 (28.0,33.7) ^{NS}a	28.8 (26.1,31.9) ^{NS}ab	33.6 (30.4,36.6) *ac, ^{NS}b	29.2 (26.2,32.1) ^{NS}abc, *d	32.9 (28.4,35.0) ^{NS}abcde	0.014
Total bilirubin, umol/L	13.5 (10.2,17.3)	13.5 (10.4,18.9) ^{NS}a	15.0 (11.8,20.1) ^{NS}ab	20.3 (16.2,30.6) **abc	14.2 (10.9,18.8) ^{NS}abc, **d	18.9 (16.1,26.7) *abcde	< 0.001
Direct bilirubin, umol/L	2.7 (3.1,3.7)	2.9 (2.0,4.2) ^{NS}a	3.0 (2.3,4.8) ^{NS}ab	6.1 (3.9,10.6) **abc	2.9 (2.1,4.2) ^{NS}abc, **d	4.7 (3.8,10.8) *abcde	< 0.001
PLT, 10^9/L	224 (190,259)	199 (167,263) ^{NS}a	193 (151,233) **ab	147 (108,178) **abc	215 (164,250) *ab, **cd	168 (114,200) *ab, ^{NS}cd, **e	< 0.001
LSM with SWE, kPa	5.7 (4.9,6.3)	6.7 (5.8,8.0) *a	6.8 (5.6,9.3) **ab	15.0 (6.9,15.6) **abc	6.0 (5.2,8.1) *a, ^{NS}b, **cd	12.9 (8.8,17.0) **abce, *d	< 0.001
AFP, ng/mL	2.3 (1.7,3.4)	3.0 (2.0,4.7) ^{NS}a	3.1 (2.3,5.1) ^{NS}ab	61.0 (35.8,216) **ac, *b	2.7 (2.0,3.8) ^{NS}abc, *d	79.5 (32.8,147.8) ^{NS}abcde	0.006
Cirrhosis, n(%)	29 (5.5)	33 (12.3) *a	179 (29.9) **ab	125 (77.6) **abc	72 (22.2) **ad, *bc	30 (83.3) **abce, ^{NS}d	< 0.001
REACH-B score	5.7 ± 3.0	7.4 ± 3.0 **a	8.2 ± 3.5 **ab	10.6 ± 3.1 **abc	8.0 ± 3.0 **ad, *b, ^{NS}c	9.1 ± 2.8 **a, *bde, ^{NS}c	< 0.001
Page-B score	12.1 ± 5.6	13.0 ± 5.6 ^{NS}a	16.9 ± 5.9 **ab	20.2 ± 5.9 **abc	14.2 ± 5.5 **acd, *b	16.7 ± 5.1 **ab, *de, ^{NS}c	< 0.001
mPage-B score	7.0 ± 3.4	6.1 ± 3.7 *a	9.6 ± 3.6 **ab	11.5 ± 3.8 **abc	6.6 ± 3.5 **cd, ^{NS}ab	6.1 ± 3.7 **bd, *ae, ^{NS}c	< 0.001
aMAP score	44.7 ± 8.2	43.3 ± 8.6 ^{NS}a	51.1 ± 8.9 **ab	56.7 ± 8.7 **abc	43.9 ± 8.5 **cd, ^{NS}ab	50.8 ± 6.1 **abde, ^{NS}c	< 0.001

Data are median (first quartile, third quartile), n (%), or mean ± SD (standard deviation)

P values were for the ANOVA analysis across the groups, *$P < 0.05$, ** $P < 0.001$

‡ a—compared with None group, b—compared with Others group, c—compared with ETV and AFP ≤ 20 (ng/mL) group, d—compared with ETV and AFP > 20 (ng/mL) group, e—compared with TDF and AFP ≤ 20 (ng/mL) group, NS— non significant

ETV entecavir, *TDF* tenofovir disoproxil fumarate, *AFP* alpha fetoprotein, *HCC* hepatic cell carcinoma, *BMI* body mass index, *HBV* hepatitis B virus, *NAFLD* non-alcoholic

Table 2 (continued)

fatty liver disease, *T2DM* type 2 diabetes mellitus, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyl transferase, *ALB* albumin, *GLB* globulin, *TB* total bilirubin, *DB* direct bilirubin, *PLT* platelet, *INR* international normalized ratio, *SWE* shear wave elastography, Others *LAM* lamivudine; *LdT* telbivudine; *ADV* adefovir dipivoxil

Page-B score, mPage-B score, aMAP score and T2DM were independent risk factors for HCC in CHB patients in the univariate Cox regression analysis (Table 5). Multivariate Cox regression showed that increased SWE (aHR = 1.07, 95% CI 1.02–1.12, $P < 0.001$) was still a risk factor for HCC in CHB patients (Table 5).

We additionally investigated the potential of the variables identified by the Cox model to serve as predictors of HCC. We compared and analyzed four different liver cancer scoring models and calculated their corresponding area under the curve (AUCs), REACH-B score, Page-B score, mPage-B score and aMAP score with results of 0.758, 0.784, 0.824 and 0.820 ($P < 0.001$, Fig. 4A). Results demonstrated that age, SWE, mPage-B score, and T2DM could forecast the development of HCC (Fig. 4B), with corresponding areas under the curves (AUCs) of 0.634, 0.809, 0.817 and 0.526 (Fig. 4B). The amalgamation of these factors led to an AUC of 0.838 ($P < 0.001$, Fig. 4B).

Analysis of propensity score matching between ETV and TDF treatment groups

To enhance the comparability of the study subjects regarding clinical features and mitigate the impact of potential confounding factors, we conducted a propensity score matching analysis. This analysis was designed to balance the baseline characteristics between the two treatment groups. We selected covariates that demonstrated a strong correlation with AFP levels and the occurrence of HCC. The covariates ultimately included in our study were age, gender, history of HBV, BMI, whether or not having NAFLD or diabetes, baseline HBV DNA level, ALT and AST levels, cirrhosis and baseline AFP levels. Both treatment groups employed the logit model and established a matching caliper of 0.1 to calculate the propensity scores. As a result, we successfully identified 318 pairs of cases with similar baseline characteristics. Following the PSM analysis, the comparison between the two treatment groups revealed disparities in baseline HBV DNA levels, HBsAg quantification levels, HBeAg-positive rates, AST and bilirubin levels. No significant differences were observed in other respects. (Supplementary Table S1).

We further conducted univariate and multivariate Cox regression analysis on the two groups of patients after propensity score matching, and the results showed that elevated SWE (aHR = 1.15, 95% CI 1.10–1.21, $P < 0.001$) and abnormal AFP at baseline (aHR = 1.01, 95% CI 1.00–1.18, $P < 0.001$) were independent risk factors for abnormal AFP in CHB patients (Supplementary Table S2). Furthermore, increased SWE (aHR = 1.26, 95% CI 1.12–1.42, $P < 0.001$) was still a risk factor for HCC in CHB patients (Supplementary Table S3).

Discussion

In the clinic, early diagnosis of HCC through CHB treatment monitoring is very important for high-risk patients. AFP is the most widely acknowledged biomarker for the diagnosis and monitoring of HCC. An abnormal AFP during surveillance would attract the attention of clinicians to confirm HCC development or HBV replication breakthrough. Notably, our results showed that patients with CHB treated with TDF and ETV had significantly different AFP relapse and HCC occurrence rates despite similar HBV DNA suppression rates and LSM with SWE improvements. TDF was associated with a lower rate of occurrence of both abnormal AFP and HCC than ETV. Similar predictors, including age, SWE, REACH-B score, Page-B score, mPage-B score and aMAP score, contributed independently to the AFP increase and HCC development during antiviral therapy.

Our study showed that antiviral therapy can effectively reduce AFP levels. A recent multicenter study of 5936 patients showed similar results to our study. They believed that antiviral therapy could reduce the serum AFP level in patients with chronic HBV infection at all stages of disease progression. Antiviral therapy could improve the accuracy of serum AFP in the early diagnosis of HCC. In patients with ALT normalization after antiviral therapy, the serum AFP level in patients with chronic HBV infection was higher than that in patients with ALT normalization. Another study of 149 CHB patients by Jeng WJ showed that the early decrease in HBsAg in CHB patients treated with entecavir depended on the levels of AFP and ALT, and the predictive value of the AFP level was better than that of the ALT level and HBV genotype [32]. The AFP level of CHB patients ≥ 100 ng/ml, baseline HBsAg level and genotype B infection were independent factors for a significant decrease in HBsAg after 6 months of ETV treatment [32].

Similarly, long-term use of nucleoside analogs in patients with compensated cirrhosis due to chronic hepatitis B cannot completely eliminate the risk of hepatocellular carcinoma (HCC). A study of 258 patients in Italy showed that in patients with compensated cirrhosis due to chronic hepatitis B who received long-term treatment with tenofovir or entecavir, AFP increased to more than

Table 3 Characteristics after ETV/TDF therapy strategies with the development of abnormal AFP and HCC

	ETV (N = 708)			TDF (N = 339)			*P* value
	HCC	AFP > 20 (ng/mL)	AFP ≤ 20 (ng/mL)	HCC	AFP > 20 (ng/mL)	AFP ≤ 20 (ng/mL)	
Patients, n (%)	24 (3.4)	64 (9.0)	620 (87.6)	2 (0.6)	16 (4.7)	321 (94.7)	
Age, years	53.7 ± 11.4	45.6 ± 11.7	44.8 ± 12.3	62.0 ± 5.7	37.0 ± 8.1	35.6 ± 10.2	< 0.001
Male, n (%)	20 (83.3)	53 (82.8)	451 (72/7)	1 (50.0)	10 (62.5)	215 (67.0)	0.063
BMI, kg/m^2	23.0 (21.0,24.1)	23.3 (22.6,23.9)	22.8 (20.8,24.8)	22.5 (22.5,22.6)	23.4 (22.5,25.0)	22.6 (19.9,24.2)	0.587
History of HBV, years	10 (10,20)	10 (5,20)	10 (6,15)	7.5 (5,10)	20 (12,20)	10 (6.5,20)	0.034
NAFLD, n (%)	2 (8.3)	6 (9.4)	85 (13.7)	0 (0.0)	3 (18.8)	48 (15.0)	0.750
T2DM, n (%)	2 (8.3)	3 (4.7)	29 (4.7)	0 (0.0)	1 (6.3)	10 (3.1)	0.773
Hepatitis B Virology							
Log10 HBVDNA, IU/mL	5 (3,6)	5 (4,7)	4 (2,6)	4 (2,5)	5 (3,7)	5 (2,7)	< 0.001
Log10 HBsAg, IU/mL	3 (2,3)	3 (3,4)	3 (3,4)	3 (3,3)	3 (3,3)	4 (3,5)	< 0.001
HBeAg-positive, n (%)	11 (45.8)	30 (46.9)	255 (41.1)	0 (0.0)	7 (43.8)	192 (59.8)	< 0.001
Biochemistry							
ALT, U/L	44 (24,68)	84 (45,283)	43 (27,86)	36 (27,44)	100 (48,267)	47 (27,85)	< 0.001
AST, U/L	56 (33,71)	95 (48,246)	39 (28,67)	37 (32,42)	72 (38,176)	36 (26,69)	< 0.001
GGT, U/L	51 (33,185)	93 (58,162)	38 (24,78)	36 (31,41)	77 (22,110)	28 (19,56)	< 0.001
Alkaline phosphatase, U/L	101 (80,145)	109 (82,148)	80 (67,98)	97 (91,103)	81 (62,87)	75 (66,88)	< 0.001
ALB, U/L	39.1 (30.3,43.3)	41.9 (38.9,44.8)	43.7 (40.9,46.0)	45.7 (44.3,47.0)	41.9 (40.0,44.3)	44.7 (41.7,46.4)	0.100
GLB, U/L	36.0 (29.6,39.5)	31.7 (28.3,37.0)	29.8 (26.7,33.8)	31.9 (27.0,36.7)	29.9 (26.0,33.2)	29.4 (26.8,32.9)	< 0.001
Total bilirubin, umol/L	21.9 (13.4,38.9)	19.6 (14.1,32.0)	16.5 (12.2,22.0)	11.2 (8.2,14.2)	12.9 (10.8,24.1)	15.3 (11.5,20.2)	< 0.001
Direct bilirubin, umol/L	6.4 (3.4,12.9)	8.0 (3.9,12.5)	3.6 (2.5,5.5)	2.4 (1.8,3.0)	3.5 (2.1,9.1)	3.1 (2.2,4.5)	< 0.001
PLT, 10^9/L	114 (77,164)	160 (125,189)	173 (130,217)	116 (71,160)	176 (134,190)	214 (168,255)	< 0.001
INR	1.11 (1.08,1.14)	1.85 (1.13,2.02)	1.02 (0.96,1.12)	1.53 (1.03,2.02)	1.09 (0.98,1.20)	1.00 (0.95,1.06)	0.774
LSM with SWE, kPa	16.3 (9.5,26.3)	12.9 (7.6,15.5)	7.7 (5.9,13.5)	11.7 (8.1,15.3)	7.3 (4.8,18.1)	6.1 (5.2,9.2)	< 0.001
AFP, ng/mL	9.6 (2.9,32.8)	46.4 (22.2,214.5)	4.6 (2.7,21.2)	9.8 (4.6,15.0)	18.0 (2.7,118.5)	2.9 (2.1,7.0)	0.002
Cirrhosis, n (%)	23 (95.8)	45 (70.3)	193 (31.1)	2 (100.0)	8 (50.0)	81 (25.2)	< 0.001
REACH-B score	11.5 ± 3.3	10.5 ± 3.2	8.3 ± 3.5	10.5 ± 0.7	8.4 ± 3.4	7.9 ± 3.1	< 0.001
Page-B score	17.1 ± 4.1	14.3 ± 4.9	12.7 ± 5.3	17.5 ± 7.8	10.6 ± 5.9	8.9 ± 4.9	< 0.001
mPage-B score	13.5 ± 3.0	10.4 ± 3.9	9.6 ± 3.7	13.0 ± 4.2	7.6 ± 3.6	6.6 ± 3.4	< 0.001
aMAP score	60.9 ± 7.2	54.4 ± 9.4	51.3 ± 8.9	60.0 ± 11.3	47.7 ± 7.6	44.2 ± 8.3	< 0.001

	Post-hoc														
	1# vs. 2#	1# vs. 3#	1# vs. 4#	1# vs. 5#	1# vs. 6#	2# vs. 3#	2# vs. 4#	2# vs. 5#	2# vs. 6#	3# vs. 4#	3# vs. 5#	3# vs. 6#	4# vs. 5#	4# vs. 6#	5# vs. 6#
Patients, n (%)															
Age, years	0.003	< 0.001	0.332	< 0.001	< 0.001	0.636	0.049	0.008	< 0.001	0.037	0.008	< 0.001	0.004	0.001	0.635
Male, n (%)															
BMI, kg/m^2															
History of HBV, years	0.043	0.057	0.153	0.704	0.010	0.500	0.430	0.195	0.640	0.355	0.279	0.027	0.215	0.478	0.096
NAFLD, n (%)															
T2DM, n (%)															
Hepatitis B Virology															
Log10 HBVDNA, IU/mL	0.354	0.046	0.406	0.155	0.413	< 0.001	0.246	0.396	0.724	0.781	0.001	< 0.001	0.154	0.269	0.265

Table 3 (continued)

	Post-hoc														
	1# vs. 2#	1# vs. 3#	1# vs. 4#	1# vs. 5#	1# vs. 6#	2# vs. 3#	2# vs. 4#	2# vs. 5#	2# vs. 6#	3# vs. 4#	3# vs. 5#	3# vs. 6#	4# vs. 5#	4# vs. 6#	5# vs. 6#
Log10 HBsAg quantification, IU/mL	0.001	0.006	0.478	0.003	< 0.001	0.045	0.057	0.629	0.012	0.119	0.114	< 0.001	0.045	0.016	0.404
HBeAg-positive, n (%)	0.930	0.646	0.207	0.897	0.179	0.375	0.190	0.823	0.056	0.238	0.833	< 0.001	0.231	0.086	0.202
Biochemistry															
ALT, U/L	0.001	0.273	0.890	< 0.001	0.183	< 0.001	0.189	0.271	< 0.001	0.637	< 0.001	0.427	0.095	0.583	0.001
AST, U/L	< 0.001	0.744	0.778	0.026	0.804	< 0.001	0.132	0.588	< 0.001	0.696	0.010	0.827	0.215	0.712	0.009
GGT, U/L	0.922	< 0.001	0.128	0.128	< 0.001	< 0.001	0.126	0.100	< 0.001	0.685	0.164	0.008	0.393	0.891	0.035
Alkaline phosphatase, U/L	0.001	< 0.001	0.058	< 0.001	< 0.001	< 0.001	0.421	0.001	< 0.001	0.697	0.721	0.357	0.625	0.630	0.925
ALB, U/L															
GLB, U/L	0.053	< 0.001	0.413	0.008	< 0.001	0.001	0.855	0.155	0.001	0.678	0.910	0.501	0.723	0.629	0.764
Total bilirubin, umol/L	0.056	0.044	0.291	0.116	0.026	< 0.001	0.081	< 0.001	< 0.001	0.630	0.755	0.448	0.726	0.687	0.928
Direct bilirubin, umol/L	< 0.001	0.657	0.665	0.768	0.442	< 0.001	0.079	< 0.001	< 0.001	0.752	0.999	0.311	0.766	0.832	0.776
PLT, 10^9/L	0.305	0.065	0.870	0.258	< 0.001	0.268	0.596	0.683	< 0.001	0.453	0.888	< 0.001	0.508	0.172	0.066
INR															
LSM with SWE, kPa	0.032	< 0.001	0.151	< 0.001	< 0.001	< 0.001	0.451	0.001	< 0.001	0.470	0.531	< 0.001	0.638	0.277	0.103
AFP, ng/mL	0.020	0.988	0.996	0.995	0.987	< 0.001	0.433	0.045	< 0.001	0.999	0.996	0.996	0.998	0.999	0.995
Cirrhosis, n (%)	0.011	< 0.001	0.768	0.001	< 0.001	< 0.001	0.361	0.124	< 0.001	0.036	0.109	0.059	0.180	0.016	0.028
REACH-B score	0.183	< 0.001	0.674	0.004	< 0.001	< 0.001	0.990	0.031	< 0.001	0.360	0.888	0.104	0.414	0.284	0.565
Page-B score	0.035	< 0.001	0.915	< 0.001	< 0.001	0.018	0.392	0.009	< 0.001	0.186	0.106	< 0.001	0.073	0.019	0.222
mPage-B score	0.001	< 0.001	0.858	< 0.001	< 0.001	0.109	0.317	0.006	< 0.001	0.186	0.028	< 0.001	0.046	0.012	0.266
aMAP score	0.004	< 0.001	0.890	< 0.001	< 0.001	0.010	0.369	0.007	< 0.001	0.158	0.108	< 0.001	0.061	0.011	0.127

Data are median (first quartile, third quartile), n (%), or mean ± SD (standard deviation)

1- ETV group and HCC occurred during follow-up; 2- ETV group and AFP > 20 (ng/mL) during follow-up; 3- ETV group and AFP ≤ 20 (ng/mL) during follow-up; 4- TDF group and HCC occurred during follow-up; 5- TDF group and AFP > 20 (ng/mL) during follow-up; 6- TDF group and AFP ≤ 20 (ng/mL) during follow-up

ETV entecavir, *TDF* tenofovir disoproxil fumarate, *AFP* alpha fetoprotein, *HCC* hepatic cell carcinoma, *BMI* body mass index, *HBV* hepatitis B virus, *NAFLD* non-alcoholic fatty liver disease, *T2DM* type 2 diabetes mellitus, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma-glutamyl transferase, *ALB* albumin, *GLB* globulin, *TB* total bilirubin, *DB* direct bilirubin, *PLT* platelet, *INR* international normalized ratio, *SWE* shear wave elastography

7 ng/ml, which could predict a higher risk of HCC within one year and had good specificity [33]. A meta-analysis by Choi WM showed that compared with entecavir, tenofovir treatment significantly reduced the risk of hepatocarcinogenesis in patients with chronic HBV infection (hazard ratio = 0.80; $P = 0.003$) [33]. Through our comparative study of entecavir and tenofovir, our results further showed that compared with entecavir, tenofovir is more effective in reducing the AFP level and the incidence of HCC.

A meta-analysis conducted by Choi WM included 42,939 patients who received TDF or ETV monotherapy, and the results showed that patients receiving TDF had significantly lower HCC risk. Lower HCC risk with TDF

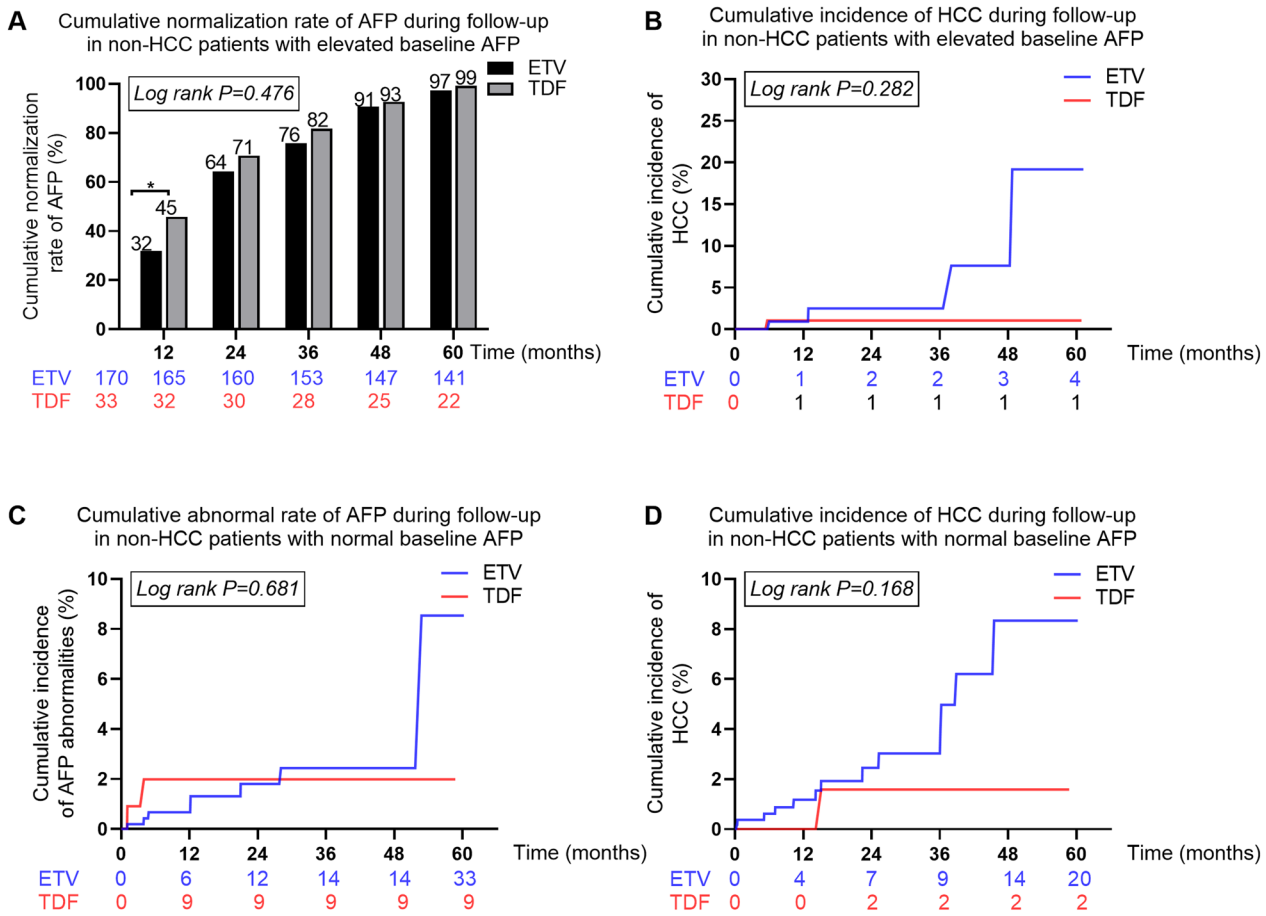

Fig. 2 Survival analysis chart of baseline non-HCC patients during follow-up. **A** Cumulative normalization rate of AFP during follow-up in non-HCC patients with elevated baseline AFP; **B** Cumulative incidence of HCC during follow-up in non-HCC patients with elevated baseline AFP; **C** Cumulative abnormal rate of AFP during follow-up in non-HCC patients with normal baseline AFP; **D** Cumulative incidence of HCC during follow-up in non-HCC patients with normal baseline AFP. * $P < 0.05$

was consistently observed in PSM analyses and in all subgroups, with statistical significance in the ≥ 50 years of age, male, HBeAg-positive and non-diabetic subgroups [34]. While our analysis demonstrated a clear difference between TDF and ETV, building on previous findings, the mechanisms behind this difference are not fully understood. Studies have suggested that TDF may provide faster and more complete suppression of HBV DNA levels than ETV, particularly in patients with high baseline HBV DNA levels [35]. Multiple clinical studies also suggested that the reduction in HBsAg level was more profound with TDF treatment than ETV treatment. These superior virologic and serologic responses by TDF compared to ETV may result in different levels of effectiveness in HCC prevention [36, 37].

In addition, a recent study has shown that patients with chronic hepatitis B treated with TDF have higher levels of interferon λ 3 than those treated with ETV [13]. Previous cancer mouse model experiments have demonstrated the effectiveness and anti-tumor activity of interferon mediated antiviral therapy, which may be related to TDF's better inhibitory effect on the virus [38]. In clinical patients, chronic liver inflammation leads to fibrosis and cirrhosis, and stopping or reversing these are key targets for treating chronic hepatitis B. Although both TDF and ETV have been shown to reverse cirrhosis, a large real-world cohort study report showed that TDF treatment had a higher rate of cirrhosis reversal after 5 years (73.8% vs. 61.5%, $P = 0.038$) [39]. A study by Pengpeng Li involving 4451 patients showed that, in patients undergoing curative liver resection for HBV-related HCC, tenofovir disoproxil was associated with better long-term overall survival and recurrence-free survival rates compared with entecavir [40]. These studies all suggest that, especially those at high risk for HCC, choosing TDF as a potentially preferable choice for such patients may have better clinical outcomes.

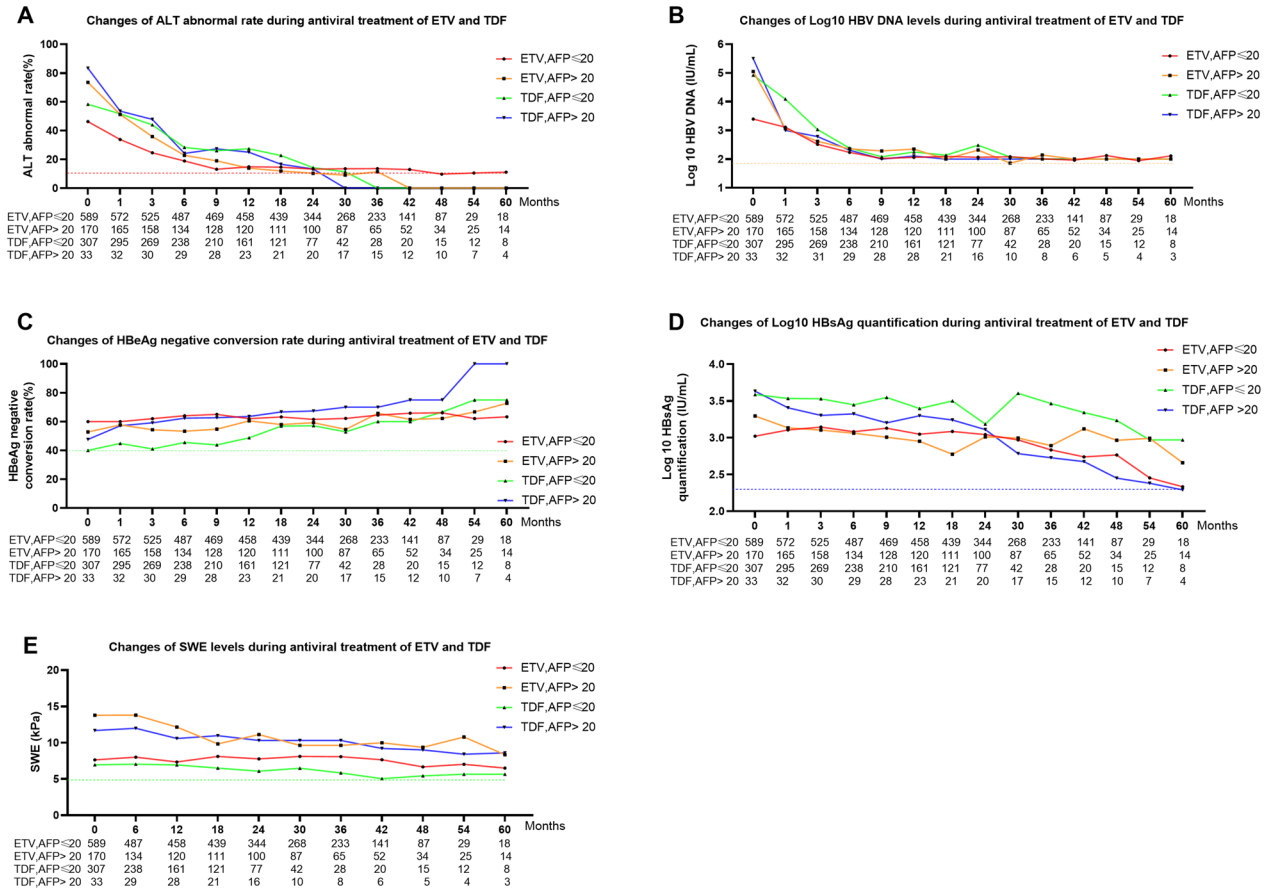

Fig. 3 Changes in the abnormal ALT rate, virological indices and SWE levels during the follow-up of ETV and TDF treatment. **A** Changes in the rate of abnormal ALT levels during antiviral treatment with ETV and TDF; **B** Changes of Log10 HBV DNA levels during antiviral treatment with ETV and TDF; **C** Changes of HBeAg negative conversion rate during antiviral treatment with ETV and TDF; **D** Changes of Log10 HBsAg quantification during antiviral treatment with ETV and TDF; **E** Changes of SWE levels during antiviral treatment with ETV and TDF

This prospective cohort study with a follow-up period of approximately 60 months. The findings indicate that the age of CHB patients, elevation SWE, REACH-B score, Page-B score, mPage-B score, aMAP score and T2DM are independent predictors of hepatocellular carcinoma occurrence.

Several limitations were identified in this study. Firstly, the selection of antiviral treatment for CHB patients were based on patients consent, taking into account their age, sex, underlying conditions, and other factors. This method did not involve random allocation of antiviral drugs, nor did it match patients between different antiviral drug groups, leading to potential selection bias in the study. Without randomization, patients may have been assigned to treatments based on clinical characteristics, preferences, or availability, which could systematically favor one group over the other. Patients with more severe liver disease or higher HCC risk might have been more likely to receive TDF over ETV due to perceived advantages or clinician preference. Alternatively, younger or healthier patients might have been more inclined toward one treatment due to fewer contraindications. This imbalance could affect key outcomes, such as AFP levels and HCC occurrence, making it unclear whether the differences observed were due to the treatments themselves or the underlying differences in patient characteristics.

Secondly, this study did not fully consider the impact of confounding factors on the occurrence of HCC, such as family history of HCC, alcohol consumption, or HBV genotype. Previous studies have shown that changes in HBV genotype are associated with differences in disease progression and HCC risk [41], and family history and genetic susceptibility of HCC play important roles in HCC risk [42]. In addition, drinking alcohol can accelerate the progression of liver fibrosis and increase the risk of HCC [43]. This study excludes people with alcohol abuse, but a small amount of alcohol may still affect the risk of HCC in patients with hepatitis B, which needs further verification in future studies.

Table 4 Predictors associated with elevated of AFP after treatment by Cox regression model in patients with HBV infection

Factors	Group	Univariate analysis			Multivariable analysis		
		Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, years	≥ 60 VS < 60	1.66	0.92–3.01	0.09			
Sex	Male VS Female	1.16	0.70–1.91	0.57			
SWE, kPa		1.08	1.06–1.10	< 0.001	1.05	1.03–1.08	< 0.001
AFP in Baseline		1.17	1.09–1.25	< 0.001	1.00	1.00–1.00	< 0.001
PLT, 10^9/L	< 100 VS ≥ 100	1.06	0.65–1.72	0.83			
Log10 HBVDNA		1.19	1.07–1.31	0.001	1.09	0.91–1.29	0.36
HBeAg(+)	Positive VS Negative	0.99	0.64–1.51	0.95			
Log10 HBsAg, IU/mL		0.87	0.71–1.08	0.21			
REACH-B score		1.21	1.14–1.30	< 0.001	1.08	0.95–1.23	0.26
Page-B score		1.10	1.05–1.14	< 0.001	0.99	0.89–1.11	0.94
mPage-B score		1.13	1.07–1.19	< 0.001	0.87	0.72–1.07	0.19
aMAP score		1.06	1.04–1.09	< 0.001	1.08	0.98–1.17	0.12
NAFLD	Yes VS No	0.72	0.37–1.39	0.33			
T2DM	Yes VS No	2.04	0.89–4.68	0.09			
Nucleoside analogues	ETV VS TDF	1.65	0.93–2.90	0.08			

AFP alpha fetoprotein, *HBV* hepatitis B virus, *SWE* shear wave elastography, *PLT* platelet, *NAFLD* non-alcoholic fatty liver disease, *T2DM* type 2 diabetes mellitus, *ETV* entecavir, *TDF* tenofovir disoproxil fumarate

Table 5 Predictors associated with occurrence of HCC after treatment in patients with HBV infection

Factors	Group	Univariate analysis			Multivariable analysis		
		Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age, years	≥ 60 VS < 60	4.41	1.88–10.32	0.001	1.13	0.29–4.43	0.87
Sex	Male VS Female	1.30	0.49–3.50	0.60			
SWE, kPa		1.09	1.06–1.12	< 0.001	1.07	1.02–1.12	0.01
AFP in Baseline		1.02	0.98–1.04	0.45			
PLT, 10^9/L	< 100 VS ≥ 100	2.15	0.95–4.84	0.07			
Log10 HBVDNA		1.10	0.92–1.32	0.30			
HBeAg(+)	Positive VS Negative	0.96	0.43–2.14	0.92			
Log10 HBsAg, IU/mL		0.69	0.55–1.03	0.11			
REACH-B score		1.32	1.17–1.50	< 0.001	1.12	0.95–1.32	0.18
Page-B score		1.18	1.08–1.29	< 0.001	0.95	0.77–1.18	0.64
mPage-B score		1.29	1.16–1.43	< 0.001	1.19	0.78–1.79	0.42
aMAP score		1.12	1.06–1.17	< 0.001	1.01	0.86–1.18	0.94
NAFLD	Yes VS No	0.48	0.11–2.06	0.33			
T2DM	Yes VS No	4.21	1.25–14.17	0.02	0.63	0.11–3.69	0.61
Nucleoside analogues	ETV VS TDF	6.90	0.92–51.68	0.06			

HCC hepatic cell carcinoma, *HBV* hepatitis B virus, *SWE* shear wave elastography, *AFP* alpha fetoprotein, *PLT* platelet, *NAFLD* non-alcoholic fatty liver disease, *T2DM* type 2 diabetes mellitus, *ETV* entecavir, *TDF* tenofovir disoproxil fumarate

Conclusions

Over a period of 60 months, we conducted a study to monitor AFP levels and the incidence of HCC in CHB patients, and assessed the risk factors associated with abnormal AFP elevation and HCC development. Our findings indicated that CHB patients receiving ETV or TDF treatment had similar rates of AFP elevation and HCC development. Elevated SWE and abnormal AFP levels at baseline were identified as independent risk factors for abnormal AFP elevation in CHB patients. Additionally, elevated SWE weas identified as an independent risk factor for HCC development in CHB patients.

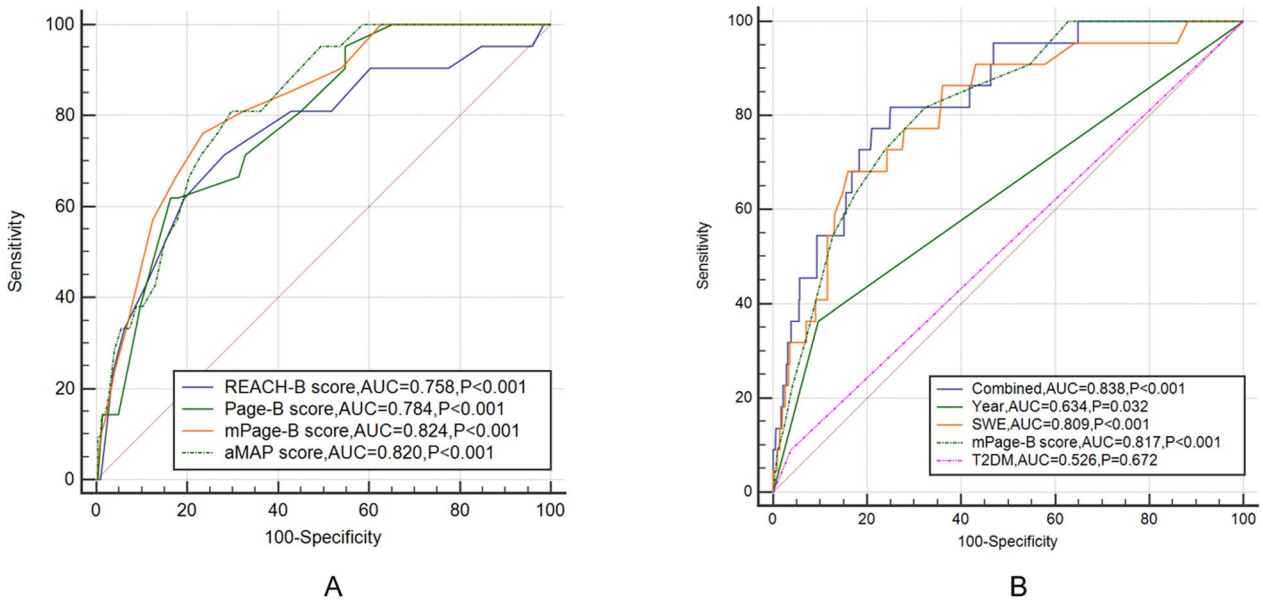

Fig. 4 ROC curve predicts the risk of hepatocellular carcinoma

Abbreviations

ADV	Adefovir dipivoxi
AFP	Alpha fetoprotein
ALB	Albumin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
DB	Direct bilirubin
ETV	Entecavir
GGT	Gamma-glutamyl transferase
GLB	Globulin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
INR	International normalized ratio
LAM	Lamivudine
LdT	Telbivudine
PLT	Platelet
PSM	Propensity score matching
SWE	Shear wave elastography
TAF	Tenofovir alafenamide fumarate
TB	Total bilirubin
TDF	Tenofovir disoproxil fumarate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13027-025-00639-1.

Additional file 1

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Author contributions
Bihui Zhong: Conceptualization, Project administration, Supervision; Qianqian Ma and Junzhao Ye: Data curation, Software, Supervision, Writing, Writing – review & editing; Ling Luo: Data curation, Validation; Yanhong Sun, Wei Wang, Shiting Feng and Bing Liao: Supervision.All authors reviewed the manuscript.

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Data Availability
Data is provided within the manuscript or supplementary information files.

Declarations

Competing interest
The authors declare no competing interests.

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