Steadily decline of HBV DNA load under NAs in lymphoma patients and higher level of qAnti-HBc predict HBV reactivation

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Abstract

Background

Patients with lymphoma and hepatitis B virus infection need to be treated with both chemotherapy and nucleotide analogues (NAs) therapy. However, the dynamic change of HBV DNA with the increase of chemotherapy cycles is lacking. It is unknown that whether HBV replication markers: quantitative hepatitis B core antibody (qAnti-HBc), HBV RNA, and hepatitis B virus core-related antigen (HBcrAg) are also sensitive to predict HBV reactivation (HBVr).

Methods

From 29th June 2010 to 6th December 2021, clinical data and serial serum samples were collected from patients with diffuse large B lymphoma and HBV infection. Serum HBV DNA load (real time fluorescent quantitative PCR), qAnti-HBc (developed chemiluminescent particle immunoassay), HBV RNA (simultaneous amplification testing method based on real-time fluorescence detection), and HBcrAg (Lumipulse G HBcrAg assay) were tested and actors related to HBV DNA reactivation were analyzed.

Results

Under the NAs, load of HBV DNA in 69 HBsAg + lymphoma patients declined from 3.15 (2.13–4.73) lg IU/ml at baseline to 1.00 (1.00-1.75) lg IU/ml at the end of chemotherapy, and further declined to 1.00 (1.00-1.04) lg IU/ml at the end of 24-month follow-up. Serum qAnti-HBc level decreased gradually during chemotherapy in HBsAg + lymphoma patients ($F = 7.090$, $p = 0.009$). Serum HBV RNA and HBcrAg levels stayed stabled. Multivariate analysis revealed that a higher level of qAnti-HBc (1.97 ± 1.20 vs. 1.12 ± 0.84 lg IU/ml, OR = 8.367, [95% CI:1.439–48.645], $p = 0.018$) and a higher level of HBV RNA (1.00 ± 1.13 vs. 0.37 ± 0.80 lg copies/ml, OR = 3.654, [95% CI:1.208–11.048], $p = 0.022$) were related to HBVr in HBsAg-/anti-HBc + lymphoma patients.

Conclusions

The HBV DNA load declined by NAs under chemotherapy in lymphoma patients. In HBsAg-/anti-HBc + lymphoma patients, higher level of baseline serum qAnti-HBc and HBV RNA predict the HBVr during chemotherapy.

1. Introduction

Hepatitis B virus (HBV) infection is one of the most serious and prevalent health conditions, with an all-age prevalence of chronic HBV infection rate of 4.1%, affecting around 3.16 million people all over the

world[1]. HBV invading human body may cause liver damage, the virus optimizes its life cycle to allow for long-term persistence in liver tissue by establishing a plasmid-like covalently closed circular DNA (cccDNA) form[2]. Chronic active HBV infection leads to chronic hepatitis B (CHB), which accounts for 30% of all liver cirrhosis death and 40% of hepatocellular carcinoma death[3]. On the other hand, lymphoma is one of the most common malignant tumors in China. World Health Organization (WHO) GLOBOCAN 2020 shows 6,829 cases of new Hodgkin's lymphoma (HL) and 92,834 cases of new non-Hodgkin lymphoma (NHL) in China in 2020 [4]. Interestingly, people infected with HBV have a 2–3 fold greater risk of developing NHL compared to uninfected ones[5], the mechanism was not so clear but likely to be due to the hepatotropic and lymphotropic nature of HBV, which can assure its replication in lymphoid tissue[6]. But in HL, studies found HBV infection was not correlated with it[7].

Since immunosuppression is presently the mainstay of lymphoma treatment, many lymphoma patients coinfected with HBV may experience fluctuating serum HBV DNA loads or even reactivation (HBVr). Furthermore, patients with HBVr may postpone scheduled chemotherapy or present with abnormal liver function, leading to adverse effects on treatment outcome for the primary disease. According to the American Association for the Study of Liver Diseases (AASLD), HBVr from anti-cancer therapies occurred in 41–53% of HBsAg-positive, anti-HBc–positive patients and 8–18% of HBsAg-negative, anti-HBc–positive patients[8]. As a result, patients' HBV DNA levels must be monitored concurrently with chemotherapy, and an alanine aminotransferase (ALT) elevation may indicate a subsequent hepatitis flare.

Therefore, we detected HBV DNA in both HBsAg positive, anti-HBc positive (labeled as HBsAg+) lymphoma patients and HBsAg negative, anti-HBc positive (labeled as HBcAb+) lymphoma patients during the whole chemotherapy cycle and follow up to 24 months, in order to find out the changes and characteristics of HBV DNA in lymphoma patients during chemotherapy and follow-up. New factors related to HBV such as quantitative hepatitis B core antibody (qAnti-HBc), HBV RNA and hepatitis B virus core-related antigen (HBcrAg) were tested in these patients every two chemotherapy cycles. The dynamic changes were observed along with HBV DNA, since there were several patients with HBVr, the related factors in these patients were also examined.

2. Methods

2.1 Study design and patients

Eligible patients consented to participate in the study between 29th June 2010 to 6th December 2021. The inclusion criteria were: 1) HBsAg-positive, HBsAg-negative but anti-HBc-positive; 2) Confirmed diagnosis of diffuse large B-cell lymphoma (DLBCL) by biopsy; 3) Received at least four cycles of immunochemotherapy. Exclusion criteria were: 1) Involvement of the central nervous system; and 2) Human immunodeficiency virus or other hepatitis virus coinfection.

Procedures followed were in accordance with the Helsinki Declaration, approved by The Ethical Committees of Peking University First Hospital (2022 – 205). Informed consent was waived informed consent because data were de-identified.

2.2 Definition of HBV reactivation (HBVr)

The definition of HBVr was according to the AASLD guidelines[8]: in HBsAg-positive, anti-HBc–positive patients is reasonably defined as one of the following: 1) a more than (≥) 2 $\log_{10}$ (100-fold) increase in HBV DNA compared to the baseline level; 2) HBV DNA≥ 3 $\log_{10}$ (1,000) IU/mL in a patient with previously undetectable level (since HBV DNA levels fluctuate); 3) HBV DNA≥ 4 $\log_{10}$ (10,000) IU/mL, if the baseline level is not available. For HBsAg-negative but anti-HBc-positive patients, the following criteria are reasonable for HBVr: 1) HBV DNA is detectable or 2) reverse HBsAg seroconversion occurs (reappearance of HBsAg). A hepatitis flare is reasonably defined as an ALT increase to ≥ 3 times the baseline level and > 100 U/L.

2.3 Data Collection

Blood routine test, blood biochemistry test and HBV DNA were tested in every chemotherapy cycle and every three months after the cessation of chemotherapy. HBV DNA was assayed in Peking University Cancer Hospital by real time fluorescent quantitative PCR with a detection range of 10 to 10^8 IU/ml. (Northeast Pharm Co., Shenyang, China). Serum qAnti-HBc was measured by a newly developed chemiluminescent particle immunoassay with an upper limit of 100, 000 IU/ml. (Wantai Co., Xiamen, China). Serum HBV RNA was detected by RNA simultaneous amplification testing method (HBV-SAT) based on real-time fluorescence detection with an upper limit of 10^8 copies/ml. (Rendu Biotech Inc., Shanghai, China). Serum HBcrAg was quantified using the Lumipulse G HBcrAg assay and Lumipulse G1200 Analyzer with an upper limit of 10,000 KU/ml. (Fujirebio, Tokyo, Japan).

2.4 Statistical analysis

Data were reported as mean ± standard deviation (SD, for Gaussian distribution) or median (interquartile range; IQR, Q1-Q3, for skewed distribution) for continuous variables and as numbers (percentages) for categorical variables. Chi-square or Fisher's exact tests (categorical variables), student t-test (normal distribution) or Man-Whitney U test (skewed distribution) were used to detect the differences between binary variables. One-way ANOVA and Post-Hoc analysis (Bonferroni) were used to compare the differences of qAnti-HBc/HBV RNA/HBcrAg in different DNA levels. The HBVr related factors were explored using univariate ($p < 0.1$) and multivariate logistic regression. The diagnostic accuracy of markers about HBVr were analyzed with receiver operating characteristic curves (ROC) and expressed as the area under the ROC curves (AUROC) and 95% confidence interval (CI). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The optimal cut-off values of markers were obtained when Youden's index was fixed at the maximum value. Spearman's rank tests were used to analyze the associations between HBV DNA and HBV RNA/HBcrAg. All statistical

analyses were performed using SPSS version 26.0 software (SPSS, Inc., Chicago, IL, USA). P values less than 0.05 (two-sided) were considered statistically significant.

3. Results

3.1 Study population

A total of 1029 patients were screened and 181 patients were enrolled in this study, including 114 HBsAg + patients and 67 HBsAg -/anti-HBc + patients. In HBsAg + patients, 69 patients' HBV DNA load were higher than 10 IU/mL (Group A). Among them, 53 patients retained paired serum samples before and during chemotherapy (Group B). All 67 HBsAg -/anti-HBc + patients (Group C) had paired serum samples before and during chemotherapy. The patient's enrollment flow chart was shown in Fig. 1. Among 114 HBsAg + patients, 111 received nucleoside analogue drugs (NAs) before chemotherapy, all used Entecavir (ETV) except two patients received ETV combined with Adefovir dipivoxil and one patient received Lamivudine. Ninety-two out of 111 (82.88%) received NAs therapy less than one month before chemotherapy. Only one HBsAg + patients started NAs treatment after chemotherapy whose HBV DNA was negative at baseline and added ETV when HBV DNA raised to 2.78 lg IU/ml after three months.

Patients were mainly male, no matter in HBsAg + or HBsAg-/anti-HBc + patients. The average age was 56.62 years old. Subgroup of HBV DNA positive (group A, 51.86 years old) were youngest compared with HBV DNA negative subgroup (55.02 years old) and HBsAg-/anti-HBc + patients (group C, 62.51 years old), $p < 0.05$. There were only eight cirrhotic patients, all were HBsAg + and half were HBV DNA positive. Liver function, platelet count, and prothrombin time (PT) activity were all comparable between HBsAg + vs. HBsAg-/anti-HBc + patients and subgroups HBV DNA positive vs. HBV DNA negative. The International Prognostic Index (IPI) score in lymphoma was comparable in HBsAg + patients and HBsAg-/anti-HBc + patients, also comparable in HBsAg + subgroups (HBV DNA positive and HBV DNA negative). The baseline dosages of Vincristine, Anthracycline, Cyclophosphamide (CTX), and Glucocorticoids (GCs) were all comparable. HBsAg-/anti-HBc + patients received a much larger dose of Rituximab (R) (606.06 ± 88.45 mg vs 485.49 ± 262.38 mg, $p < 0.001$) and a much higher percentage of using R at baseline (98.5% vs.74.6%, $p < 0.001$) than HBsAg + patients. Detailed baseline characteristics were shown in Table 1.

Table 1
Baseline characteristics of all patients.

	Total	HBsAg+				HBsAg-/anti-HBc+	p^2
		all	Baseline HBV DNA+	Baseline HBV DNA-	p^1		
N*	181 (120)	114 (53)	69 (38)	45 (15)		67 (67)	
Male, n (%)	103 (56.9%)	65 (57.0%)	39 (56.5%)	26 (57.8%)	0.895	38 (56.8%)	0.968
Age, year	56.62 ± 12.62	53.16 ± 11.97	51.86 ± 12.49	55.02 ± 11.35	0.151	62.51 ± 11.53	0.000
BMI, kg/m^2	23.92 ± 3.93	23.93 ± 4.30	23.9 ± 3.87	24.02 ± 5.10	0.921	23.91 ± 3.24	0.975
Cirrhosis, n (%)	8 (4.4%)	8 (7.0%)	4 (5.8%)	4 (8.9%)	0.528	0	0.027
History of HBsAg+, year	2.00 (0.00–20.0)	19.5 (5.00–30.0)	20.0 (10.0–30.0)	15.0 (4.50–20.0)	0.167	0.00 (0.00–0.00)	0.000
HBV DNA, lg IU/ml	1.00 (1.00-1.86)	2.01 (1.00-4.20)	3.30 (1.88–5.74)	1.00 (1.00–1.00)	0.000	1.00 (1.00–1.00)	0.000
PLT, ·10^9/L	216.51 ± 84.64	224.16 ± 87.69	215.64 ± 84.63	231.19 ± 90.47	0.200	203.49 ± 78.11	0.113
PTA, %	92.75 ± 16.32	91.28 ± 16.40	90.04 ± 15.73	93.23 ± 17.72	0.328	95.19 ± 16.03	0.131
ALB, g/L	42.22 ± 5.19	42.22 ± 5.60	42.40 ± 5.79	42.17 ± 5.33	0.663	42.23 ± 4.46	0.985
ALT, U/L	16.0 (12.0-23.5)	16.0 (13.0–23.0)	20.0 (14.0–26.0)	13.0 (10.5–17.0)	0.090	15.0 (11.0-22.7)	0.482

All values shown are based on available data. Numeric data are represented as (mean ± SD) or median (upper quartile, lower quartile); *, the number in brackets was patients with serum sample and tested qAnti-HBc, HBV RNA and HBcrAg; p^1: p value between baseline HBV DNA positive and baseline HBV DNA negative; p^2: p value between HBsAg + group and HBsAg-/anti-HBc + group.

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CTX: Cyclophosphamide; DBil, direct bilirubin; GCs, glucocorticoid; GGT, glutamyl transferase; HBcAb, Hepatitis B core antibody; HBcrAg, hepatitis B virus core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IPI score, International Prognostic Index score; PLT, platelet; PTA, prothrombin time activity; qAnti-HBc, quantitative anti-hepatitis B core antigen; TBil, total bilirubin.

	Total	HBsAg+				HBsAg-/anti-HBc+	p^2
		all	Baseline HBV DNA+	Baseline HBV DNA-	p^1		
AST, U/L	23.0 (18.0–27.0)	23.0 (18.0–27.0)	25.0 (21.0–29.0)	20.0 (16.0–24.0)	0.013	23.0 (19.0–27.0)	0.503
GGT, U/L	23.0 (17.0–32.0)	23.0 (17.0–33.0)	23.0 (17.0–37.0)	23.0 (16.5–33.0)	0.621	22.0 (16.0–30.0)	0.307
ALP, U/L	73.0 (59.0–85.0)	74.5 (58.2–85.5)	75.0 (60.0–86.0)	73.0 (57.0–89.0)	0.614	70.5 (59.0-86.7)	0.964
TBiL, μmol/L	11.7 (9.07–16.6)	12.6 (9.20–17.7)	12.3 (9.05–17.7)	12.7 (9.45–17.6)	0.686	11.3 (9.00-14.9)	0.111
DBiL, μmol/L	3.70 (2.80–4.80)	3.70 (3.10–4.95)	3.90 (3.10–5.90)	3.40 (3.05–4.51)	0.312	3.20 (2.32–4.50)	0.177
HBeAg+, n (%)	22 (12.2%)	21 (18.4)	16 (23.2%)	5 (11.1%)	0.139	1 (1.5%)	0.000
qAnti-HBc, lg IU/ml	2.20 ± 1.43	3.48 ± 0.84	3.69 ± 0.84	2.93 ± 0.55	0.000	1.19 ± 0.90	0.000
HBV RNA, lg copies/ml	0.00 (0.00-2.33)	2.34 (0.00-3.96)	2.39 (1.56–4.95)	1.40 (0.00-2.70)	0.004	0.00 (0.00–0.00)	0.000
HBcrAg, lg KU/ml	3.38 ± 1.59	4.27 ± 1.99	4.57 ± 2.13	3.50 ± 1.04	0.021	2.67 ± 0.54	0.000
IPI score	1.00 (1.00–3.00)	1.00 (1.00-2.50)	1.00 (0.00–3.00)	1.00 (1.00–3.00)	0.051	1.00 (1.00–3.00)	0.984

All values shown are based on available data. Numeric data are represented as (mean ± SD) or median (upper quartile, lower quartile); *, the number in brackets was patients with serum sample and tested qAnti-HBc, HBV RNA and HBcrAg; p^1: p value between baseline HBV DNA positive and baseline HBV DNA negative; p^2: p value between HBsAg + group and HBsAg-/anti-HBc + group.

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CTX: Cyclophosphamide; DBil, direct bilirubin; GCs, glucocorticoid; GGT, glutamyl transferase; HBcAb, Hepatitis B core antibody; HBcrAg, hepatitis B virus core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IPI score, International Prognostic Index score; PLT, platelet; PTA, prothrombin time activity; qAnti-HBc, quantitative anti-hepatitis B core antigen; TBil, total bilirubin.

	Total	HBsAg+				HBsAg-/anti-HBc+	p^2
		all	Baseline HBV DNA+	Baseline HBV DNA-	p^1		
First-line chemotherapy cycles	6.26 ± 1.36	6.29 ± 1.37	6.45 ± 1.33	6.04 ± 1.41	0.125	6.21 ± 1.34	0.702
Using Rituximab at baseline, n (%)	151 (83.4%)	85 (74.6%)	42 (60.9%)	43 (95.6%)	0.000	66 (98.5%)	0.000
Dose of Rituximab, mg	530.12 ± 222.48	485.49 ± 262.38	411.59 ± 296.32	598.81 ± 139.32	0.000	606.06 ± 88.45	0.000
Dose of Vincristine, mg	2.76 ± 1.38	2.72 ± 1.39	2.79 ± 1.39	2.61 ± 1.39	0.505	2.82 ± 1.38	0.620
Dose of Anthracycline, mg	67.46 ± 28.80	70.12 ± 25.56	74.32 ± 24.61	63.67 ± 25.92	0.029	62.95 ± 33.33	0.132
Dose of CTX, mg	1182.6 ± 220.18	1203.18 ± 212.54	1207.97 ± 195.71	1195.83 ± 238.19	0.767	1147.59 ± 229.98	0.101
First dose of GCs, mg	50.0 (0.00-100)	50.0 (0.00-100)	30.0 (0.00-100)	60.0 (30.0-100)	0.066	60.0 (30.0-100)	0.114

All values shown are based on available data. Numeric data are represented as (mean ± SD) or median (upper quartile, lower quartile); *, the number in brackets was patients with serum sample and tested qAnti-HBc, HBV RNA and HBcrAg; p^1: p value between baseline HBV DNA positive and baseline HBV DNA negative; p^2: p value between HBsAg + group and HBsAg-/anti-HBc + group.

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CTX: Cyclophosphamide; DBil, direct bilirubin; GCs, glucocorticoid; GGT, glutamyl transferase; HBcAb, Hepatitis B core antibody; HBcrAg, hepatitis B virus core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IPI score, International Prognostic Index score; PLT, platelet; PTA, prothrombin time activity; qAnti-HBc, quantitative anti-hepatitis B core antigen; TBil, total bilirubin.

The serum levels of qAnti-HBc, HBV RNA, and HBcrAg were measured using paired serum samples from patients in group B and C collected before and during chemotherapy. At baseline, the levels of qAnti-HBc (3.48 ± 0.84 lg IU/ml), HBV RNA (3.34 (0.00-3.96) lg copies/ml) and HBcrAg (4.27 ± 1.99 lg KU/ml) were much higher in group B patients than that of group C patients, $p < 0.001$. Detailed baseline of group B and C patients's characteristics were shown in Table S1.

3.2 HBV DNA load declined steadily by NAs under the chemotherapy

Sixty-nine patients in group A were HBV DNA positive at baseline and were all given ETV, 64 of them were prescribed less than one month before chemotherapy. Serum HBV DNA load decreased steadily by using NAs (F = 13.748, $p < 0.001$), regardless the number of chemotherapy cycles increased. This decline trend persisted throughout the 24-month follow-up period (Fig. 2A and B). The load of HBV DNA reduced from 3.15 (2.13–4.73) lg IU/ml at baseline to 1.00 (1.00-1.75) lg IU/ml at the end of chemotherapy, and further declined to 1.00 (1.00-1.04) lg IU/ml at the end of 24-month follow-up.

A large amount of evidence shows that Rituximab can effectively improve the complete remission rate of lymphoma[9]. On the other hand, Rituximab is an evidence-based drug that can potentially inducing HBV reactivation[10]. Twenty-seven patients in group A using chemotherapy without Rituximab from the 1st cycle because of significantly higher HBV DNA load (5.60 (3.72–7.03) lg IU/ml) than the other 42 patients (2.46 (1.79–3.17) lg IU/m), $p < 0.001$. In patients treated with R from the 1st cycle chemotherapy, HBV DNA load still showed a downward trend under the effect of NAs, F = 9.549, $p = 0.002$. However, the decrease of HBV DNA load was less than that of patients not using R at baseline. (Fig. 2C).

At baseline, HBV DNA level of 16 HBeAg positive patients (6.77 (4.42–8.54) lg IU/ml) was significantly higher than that of 53 HBeAg negative patients (2.84 (2.04–3.55) lg IU/ml). The load of HBV DNA in HBeAg positive patients decreased to 3.82 (2.70–4.27) lg IU/ml and 3.36 (2.01–3.76) lg IU/ml after four and eight cycle chemotherapy. The load of HBV DNA level in HBeAg negative patients decreased to 1.00 (1.00-1.99) lg IU/ml after the first cycle chemotherapy and was stable with a median load of 1.00 lg IU/ml throughout chemotherapy. Figure 2D showed the decreased value of HBV DNA from baseline.

We further explored whether chemotherapy drugs and lymphoma could affect the antiviral effect of NAs. Since we had 64 naïve patients who were prescribed ETV just before chemotherapy, we compared the antiviral efficacy of ETV of our patients with patients in ETV pre-marketing registration trial[11,12]. Unsurprisingly, in the pre-marketing registration trial set of ETV in patients without lymphoma and chemotherapy drug pressure, the decrease load of HBV DNA after 48-week treatment was significantly higher (6.9 ± 2.0 lg IU/ml and 5.0 ± 1.7 lg IU/ml in HBeAg positive and negative patients, respectively) than patients with lymphoma in our study (3.97 ± 1.94 lg IU/ml and 2.73 ± 1.57 lg IU/ml in HBeAg positive and negative patients, respectively), $p < 0.001$.

3.3 Serum qAnti-HBc level decreased gradually during chemotherapy in HBsAg positive lymphoma patients

At baseline, HBsAg positive lymphoma patients (group B) had a remarkable higher qAnti-HBc level (3.48 ± 0.84 lg IU/ml) than that of HBsAg-/anti-HBc + patients (group C) (1.19 ± 0.90 lg IU/ml), $p < 0.001$.

Patients in group C, the baseline serum qAnti-HBc level was 1.19 ± 0.90 lg IU/ml and the median ALT/AST level was 15/23 IU/L. The serum qAnti-HBc level increased slightly after receiving two cycle chemotherapy at 1.69 ± 0.40 lg IU/ml and stable at this level throughout the chemotherapy (Fig. 3B).

In group B, patients with HBV DNA positive had a much higher qAnti-HBc level than those with undetectable HBV DNA (3.69 ± 0.84 lg IU/ml vs. 2.93 ± 0.55 lg IU/ml, $p < 0.001$). During chemotherapy, serum qAnti-HBc level decreased gradually (F = 7.090, $p = 0.009$) (Fig. 3A), no matter whether baseline HBV DNA was detectable or not (Fig. 3C). At the end of chemotherapy, 13 patients with HBV DNA positive turned to negative, and their qAnti-HBc decreased simultaneously from 3.96 ± 0.77 lg IU/ml (baseline) to 3.33 ± 0.71 lg IU/ml (at the end of chemotherapy), $p < 0.001$.

There were 32 patients in group B with ALT level lower than 20 U/L (0.5×ULN) at baseline. Serum qAnti-HBc of these patients (3.29 ± 0.85 lg IU/ml) was significantly lower than patients with ALT≥0.5×ULN (3.76 ± 0.76 lg IU/ml), $p = 0.046$. While serum qAnti-HBc level remained at about 3.2 lg IU/ml in patients with baseline ALT < 20 IU/L, the level of qAnti-HBc in patients with baseline ALT > 20 IU/L decreased gradually during chemotherapy (Fig. 3D). We further divided the ALT level into four grades, and found that the synchronous rising trend between qAnti-HBc and ALT levels was much more clearly presented (F = 13.723, $p = 0.001$) (Fig. 3E). During chemotherapy, there were 135 paired ALT levels and qAnti-HBc levels results. Stratified analysis showed that serum qAnti-HBc of different ALT levels was maintained at about 1.5 lg IU/ml during chemotherapy (Fig. 3F).

3.4 Serum HBV RNA and HBcrAg stabled under the chemotherapy

The serum HBV RNA level showed no obvious change throughout the chemotherapy. The median HBV RNA level in HBsAg + patients (group B) was stable around at 2.20 lg copies/ml (Fig. 4A). The baseline HBV RNA level in HBsAg-/anti-HBc + patients (group C) was significantly lower (0.00 (0.00, 0.00) lg copies/ml) than that of group B (2.34 (0.00-3.96) lg copies/ml), $p < 0.001$) and stayed stable during chemotherapy (Fig. 4B). The level of HBV RNA and HBV DNA in group B patients showed a positive correlation, no matter before (r = 0.583, $p < 0.001$, Fig. 4C) or during chemotherapy (r = 0.713, $p < 0.001$, Fig. 4D). Further analysis showed that the higher the HBV DNA level, the higher HBV RNA level, regardless with or without chemotherapy (Fig. 4E, F).

Although 13 patients in group B whose HBV DNA turned undetectable at the end of chemotherapy, their HBV RNA showed no significant change: 1.61 (0.00-2.26) lg copies/ml at baseline and 2.02 (0.00-2.16) lg copies/ml at the end of chemotherapy ($p = 0.821$).

The serum HBcrAg level stabled throughout the study, either in HBsAg + patients (group B) (Fig. 5A) or group C (Fig. 5B). The level of HBcrAg in group B positively correlated with HBV DNA, regardless of before chemotherapy (r = 0.402, $p < 0.001$, Fig. 5C) or during chemotherapy (r = 0.741, $p < 0.001$, Fig. 5D). The higher the HBV DNA level, the higher HBcrAg level, regardless of chemotherapy (Fig. 5E, F). In the 13 people whose HBV DNA turned undetectable at the end of chemotherapy, their HBcrAg showed no significant change too: 2.85 ± 0.49 lg KU/ml, at baseline and 2.78 ± 0.42 lg KU/ml at the end of chemotherapy ($p = 0.866$).

3.5 Higher baseline level of qAnti-HBc and HBV RNA predicted HBVr in HBsAg-/anti-HBc + lymphoma patients

There were ten patients occurred HBVr: four in HBsAg + patients (group B) and six in HBsAg-/anti-HBc + patients (group C). Multivariate analysis revealed that a higher qAnti-HBc (1.97 ± 1.20 vs. 1.12 ± 0.84 lg IU/ml, OR = 8.367, [95% CI:1.439–48.645], $p = 0.018$) and a higher HBV RNA (1.00 ± 1.13 vs. 0.37 ± 0.80 lg copies/ml, OR = 3.654, [95% CI:1.208–11.048], $p = 0.022$) were related to HBVr in HBsAg-/anti-HBc + lymphoma patients (Table 2). In HBsAg + patients, a higher dose of R at baseline (600.00 ± 81.65 mg vs. 459.18 ± 282.05 mg, $p = 0.032$) was related to HBVr in univariate analysis (Table S2).

Table 2
Univariate and multivariate analysis of HBV reactivation in HBsAg-/anti-HBc + lymphoma patients.

	Reactivation	Without Reactivation	*p'*-value	OR (95%CI)	*p*-value
N	6	61			
Age, year	61.50 ± 13.40	62.61 ± 11.45	0.824		
HBV DNA, lg IU/ml	1.00 ± 0.00	1.02 ± 0.09	0.682		
ALT, U/L	15.5 (14.3–19.8)	15.0 (10.0-23.5)	0.701		
qAnti-HBc, lg IU/ml	1.97 ± 1.20, 1.89	1.12 ± 0.84, 1.38	0.025	8.367 (1.439–48.645)	0.018
HBV RNA, lg copies/ml	0.86 (0.00-1.94)	0.00 (0.00–0.00)	0.082	3.654 (1.208–11.048)	0.022
HBcrAg, lg KU/ml	2.52 ± 0.60, 2.39	2.68 ± 0.54, 2.70	0.492		
IPI score	1.00 (0.50–2.05)	1.00 (0.50-3.00)	0.566		
Using Rituximab at baseline, n (%)	6 (100)	60 (98.4)	1.000		
Dose of Rituximab, mg	617.67 ± 73.43	604.92 ± 90.23	0.739		
Total dose of GCs, mg	215 (45.0-800)	360 (163.5–600)	0.926		
ARDI	0.43 ± 0.33	0.73 ± 0.23	0.005		

All values shown are based on available data. Numeric data are represented as (mean ± SD) or median (upper quartile, lower quartile); *p'*-value: *p* value of single factor analysis; *p*-value: *p* value of multi factor analysis.

Abbreviations: ALT, alanine aminotransferase; GCs, glucocorticoid; HBV, hepatitis B virus; HBcrAg, hepatitis B virus core-related antigen; IPI score, International Prognostic Index score; qAnti-HBc, quantitative anti-hepatitis B core antigen.

The AUROC of qAnti-HBc, HBV RNA and HBcrAg in group C patients predict HBVr were 0.743, 0.649 and 0.605, respectively (Figure S). The cut-off value of qAnti-HBc was 1.60 [95%CI: 0.487-1.000] lg IU/ml. Sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) were 83.3%, 67.2%, 20.0% and 97.6%, respectively. Other details and details of the three factors in group B patients were shown in Table 3.

Table 3
Parameters of ROC curve analysis.

	AUROC	95% CI	Cut-off	SE	SP	PPV	NPV
HBsAg + patients							
qAnti-HBc	0.633	0.190-1.000	2.679	0.500	0.898	0.286	0.957
HBV RNA	0.704	0.471–0.937	3.548	0.750	0.755	0.200	0.074
HBcrAg	0.689	0.469–0.909	4.668	0.750	0.694	0.167	0.971
HBsAg-/anti-HBc + patients							
qAnti-HBc	0.743	0.487-1.000	1.604	0.833	0.672	0.200	0.976
HBV RNA	0.649	0.422–0.876	1.477	0.500	0.852	0.250	0.945
HBcrAg	0.605	0.334–0.877	2.540	0.667	0.639	0.154	0.951

Abbreviations: AUROC, area under the receiver operating characteristic curve; HBV, hepatitis B virus; HBcrAg, hepatitis B virus core-related antigen; qAnti-HBc, quantitative anti-hepatitis B core antigen; SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

4. Discussion

Our study highlights serum HBV DNA load decreased steadily by using NAs regardless the number of chemotherapy cycles increased, and the decline trend persisted throughout the 24-month follow-up period. HBV DNA load still showed a downward trend under the effect of NAs in patients treated with R from the 1st cycle chemotherapy. Serum qAnti-HBc level decreased gradually during chemotherapy in HBsAg positive lymphoma patient**s.** In HBsAg-/anti-HBc + patients, the serum qAnti-HBc level increased slightly up to 1.69 ± 0.40 lg IU/ml after receiving two cycle chemotherapy and stayed stable near this level throughout the chemotherapy. Serum HBV RNA and HBcrAg stabled under the chemotherapy. Higher baseline level of qAnti-HBc and HBV RNA predicted HBVr in HBsAg-/anti-HBc + lymphoma patients.

Studies have shown that qAnti-HBc was related to serum ALT and AST in CHB patients, especially, when ALT is within normal value, qAnti-HBc can better reflect the histological inflammation in CHB patients[13,14]. Our research showed a positive correlation between qAnti-HBc and ALT in these CHB patients with lymphoma: qAnti-HBc in ALT≥ 0.5·ULN group was higher than ALT < 0.5·ULN group ($p = 0.046$), but we did not obtain liver tissue in them. Since some patients occurred HBV reactivation according to AASLD as mentioned above[8], we also compared patients with or without HBV reactivation in demographic, biochemical and virological indicators. And found that a higher qAnti-HBc at baseline was related to HBV reactivation in HBsAg-/anti-HBc + patients (OR = 8.367, [95% CI:1.439–48.645], $p = 0.018$), with an AUROC of 0.743 (95% CI: 0.487-1.000). This result was consistent with an early study of Yang HC et al[15] in 2018, who found high anti-HBc more than 6.41 IU/ml at baseline was significantly associated with HBV reactivation (HR = 4.52, [95% CI:1.75–11.65], $p = 0.002$)

For the other two HBV virological biomarkers: HBV RNA and HBcrAg, our study found a positive correlation between them and HBV DNA. The correlation index of HBV RNA and HBV DNA at baseline was 0.583, while during chemotherapy, the index was 0.713 (all $p < 0.001$). The correlation index of HBcrAg and HBV DNA were 0.402 and 0.741, respectively (all $p < 0.001$). It is noteworthy that the correlation between the two indicators and HBV DNA has increased during chemotherapy. And we may need more samples to explain this.

Also, HBV RNA and HBcrAg were both reported related to covalently closed circular DNA (cccDNA), the transcriptional and replicative template of HBV. A newly published online literature reviewed the literatures of the past ten years and confirmed that HBcrAg and HBV RNA may accurately reflect cccDNA transcriptional activity[16]. But the specific methods and technical details of serum RNA detection vary widely between different studies[17]. Chen EQ et al[18] reported a positive correlation between HBcrAg levels and liver cccDNA too. In situations where serum HBV DNA levels become undetectable or HBsAg loss is achieved, HBcrAg can still be detectable[19]. Testoni, B et al[20] proved that HBcrAg is strongly correlated with HBV DNA and cccDNA both in HBeAg + and HBeAg − patients. Its profile differs drastically in patients in different disease phases, and the level declines with antiviral therapies. Further more, one study showed in anti-HBe positive patients with HBV reactivation who underwent long-term NAs treatment and achieved HBsAg loss, had detectable HBV RNA at treatment withdrawal, but HBcrAg and HBV DNA were not detected[21].

The predominant component of serum HBV RNA is full-length pregenomic RNA (pgRNA), which is encapsidated by HBc protein[22]. This component may serve as a possible predictive biomarker to track the safe cessation of antiviral medication. Since we found that the HBV DNA decreased to negative at the end of chemotherapy, the HBV RNA and HBcrAg were still positive, and had no significant change from baseline, it may suggest that these patients still need to continue antiviral treatment after chemotherapy.

There are several limitations in our study: Firstly, we have not obtained the liver tissues of these patients, so we cannot directly detect the activity of cccDNA in the liver; Secondly, number of patients is limited, especially those with HBV reactivation. Third, the ability of qAnti-HBc prediction HBV reactivation is not verified in the external cohort. increasing sample size and validation were guaranteed.

5. Conclusion

Despite the cancer and chemotherapy pressure, serum HBV DNA load in all lymphoma patients decreased gradually by NAs during chemotherapy and follow-up. Higher qAnti-HBc level and HBV RNA before chemotherapy predict HBV reactivation in HBsAg-/anti-HBc + lymphoma patients. HBV RNA and HBcrAg were closely correlated with HBV DNA in lymphoma patients receiving chemotherapy. NAs should be continued even if HBV DNA converted to undetectable during chemotherapy.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

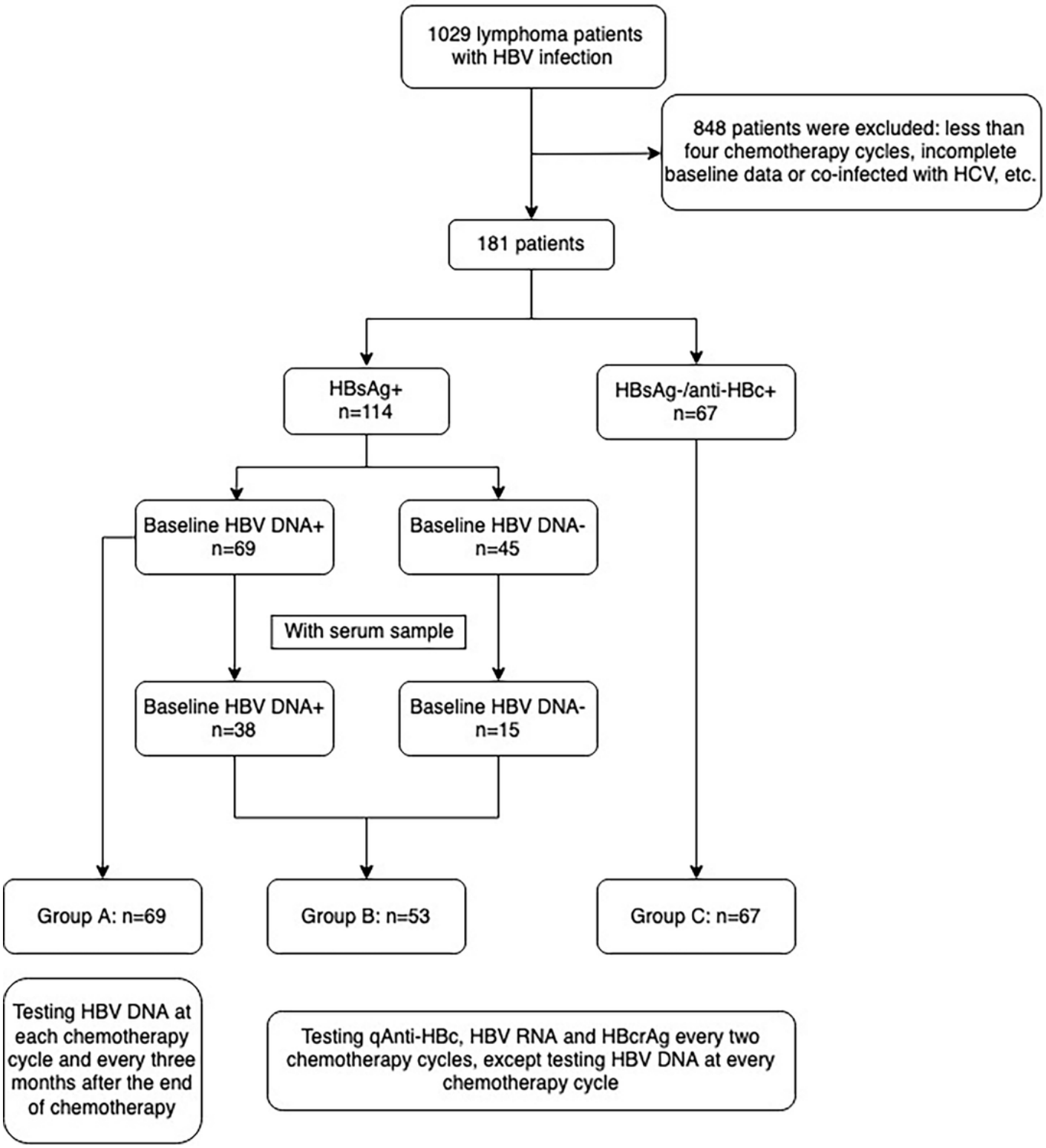

Figure 1

Flow chart of patients enrollment and grouping.

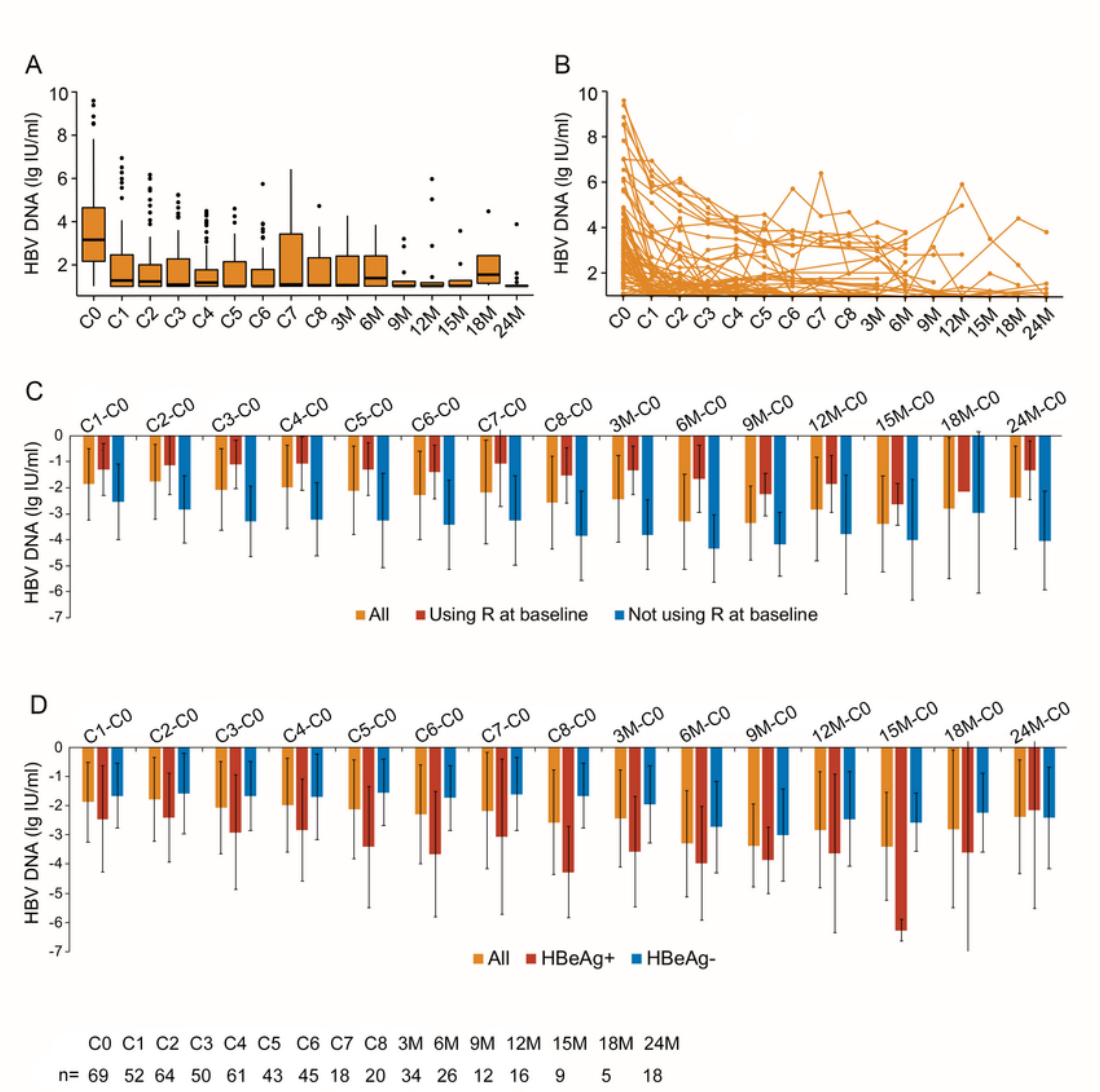

Figure 2

Dynamic decline of HBV DNA load in HBsAg+ lymphoma patients with detectable baseline HBV DNA (group A).

A: The median load of HBV DNA in group A patients before each chemotherapy cycle and every three months after chemotherapy; B: The HBV DNA load of each patient in group A before each chemotherapy

cycle and follow-up period; C: The decrease load of HBV DNA from baseline (mean) in each cycle of chemotherapy and follow-up period, according to whether the patients use Rituximab (R) at baseline (red color) or not (blue color); D: The decrease load of HBV DNA from baseline (mean) in each cycle of chemotherapy and every three months after chemotherapy, according to patients with HBeAg positive (red color) or negative (blue color). The number of patients at each time point was shown at the bottom.

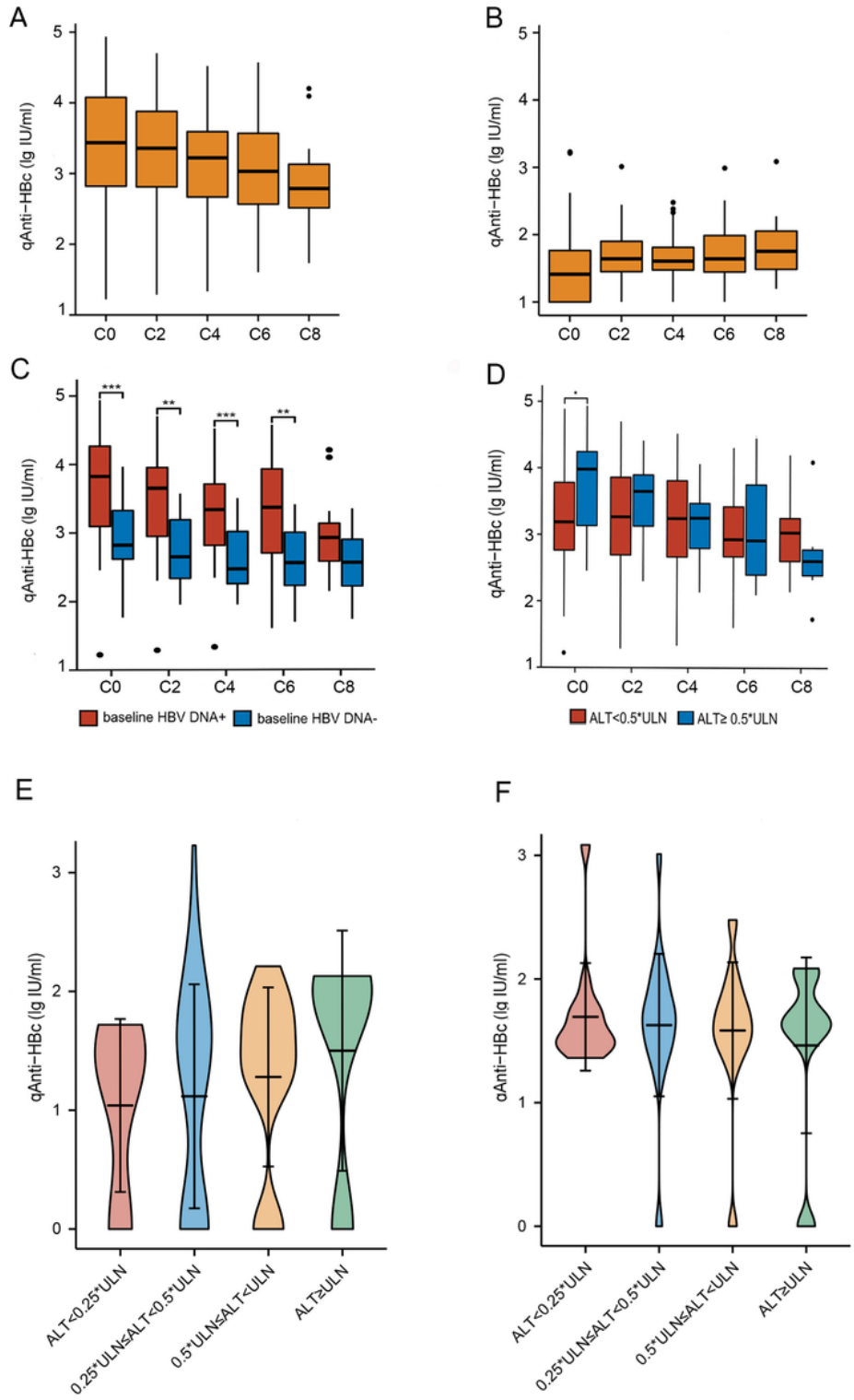

Figure 3

Dynamic changes of serum qAnti-HBc level in lymphoma patients and relationship between ALT level in HBsAg+ patients.

A: In HBsAg+ patients, serum qAnti-HBc level decreased gradually,no matter whether baseline HBV DNA was detectable (C, red color) or not (C, blue color); B: In HBsAg-/anti-HBc+ patients, serum qAnti-HBc level increased slightly after receiving two cycle chemotherapy and then stabled throughout the chemotherapy; D: In HBsAg+ patients, serum qAnti-HBc level remained stabled in patients with baseline ALT<20 IU/L(red color) but decreased gradually in patients with baseline ALT≥20 IU/L(blue color) during chemotherapy; E: In HBsAg+ patients, a synchronous rising trend between qAnti-HBc and ALT was presented (F=13.723, p=0.001) before chemorheapy when baseline ALT were further divided into four grades; F: In HBsAg+ patients, serum qAnti-HBc level was basically stabled during chemotherapy (the 2^{nd}, 4^{th}, 6^{th}, and 8^{th} cycle) regardless different grades of ALT.

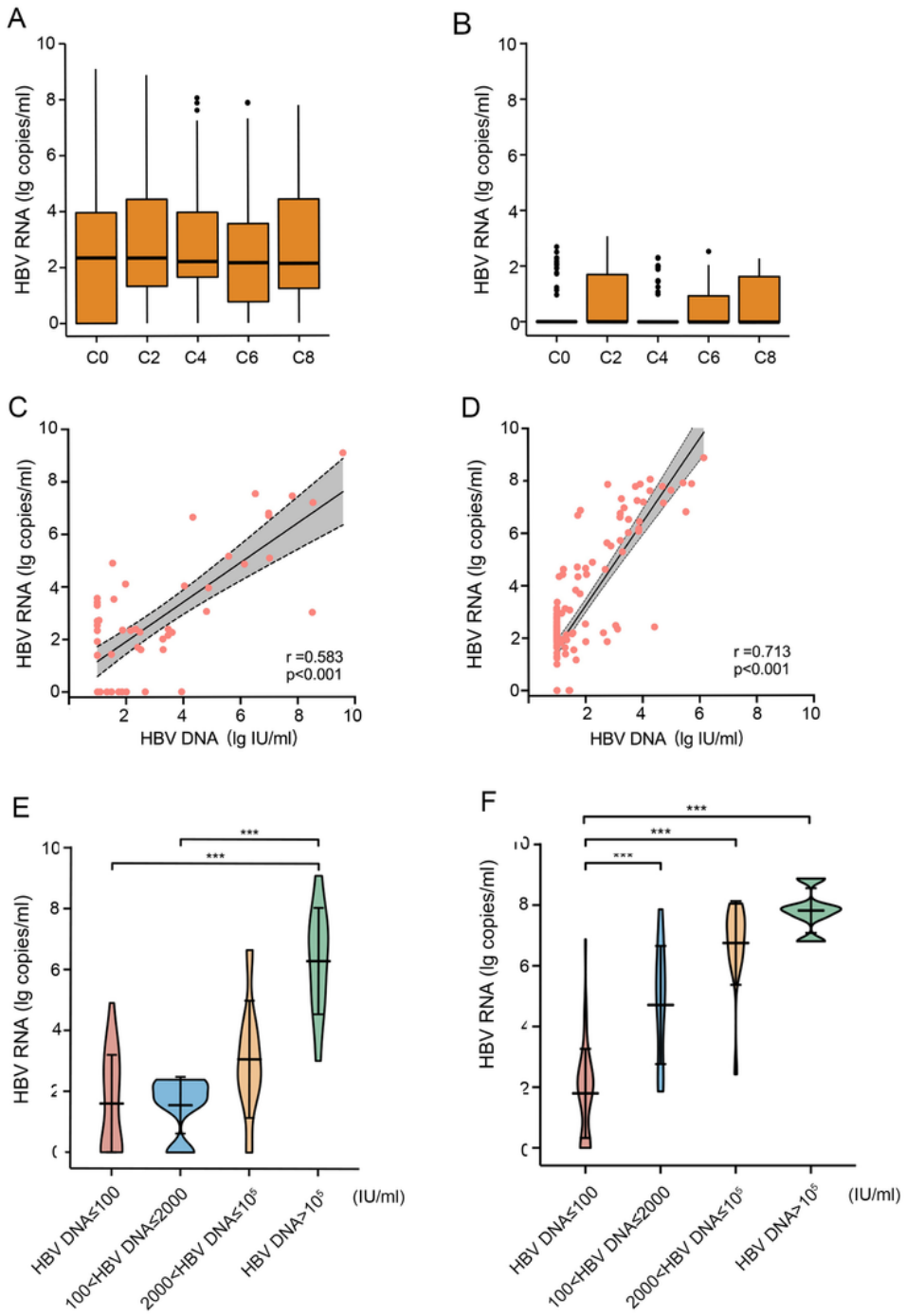

Figure 4

Serum HBV RNA stabled under the chemotherapy and positive correlated with HBV DNA load.

A: The median HBV RNA level was stable around at 2.20 lg copies/ml in HBsAg+ patients; B: The median HBV RNA level was stable around at 0.00 lg copies/ml in HBsAg-/anti-HBc+ patients; C: In HBsAg+ patients, a positive correlation was found between the level of HBV RNA and HBV DNA before

chemotherapy; D: In HBsAg+ patients, a stronger positive correlation was found between the level of HBV RNA and HBV DNA during chemotherapy; E: In HBsAg+ patients, HBV RNA level in the four subgroups before chemotherapy was higher when the grade of HBV DNA was higher: 1.41 (0.00-2.72) lg copies/ml in HBV DNA≤ 100 IU/ml group, 1.70 (1.61-2.28) lg copies/ml in 100< HBV DNA≤2000 IU/ml group, 2.72 (2.23-3.98) lg copies/ml in 2000< HBV DNA≤10^5 IU/ml group and 6.76 (2.27-5.11) lg copies/ml in HBV DNA>10^5 IU/ml group. But only in HBV DNA≤ 100 IU/ml group compared with HBV DNA>10^5 IU/ml group and 100< HBV DNA≤2000 IU/ml group compared with HBV DNA>10^5 IU/ml group had statistical differences (p<0.001, labled as***). F: In HBsAg+ patients, during chemotherapy (the 2^{nd}, 4^{th}, 6^{th}, and 8^{th} cycle), the trend was likely to before chemotherapy, HBV RNA levels were: 1.96 (0.00-4.67) lg copies/ml, 4.89 (2.48-5.72) lg copies/ml, 7.15 (6.30-7.69) lg copies/ml and 7.88 (7.63-7.91) lg copies/ml, respectively. Differences between three groups were statistically significant (p<0.001, labled as ***).

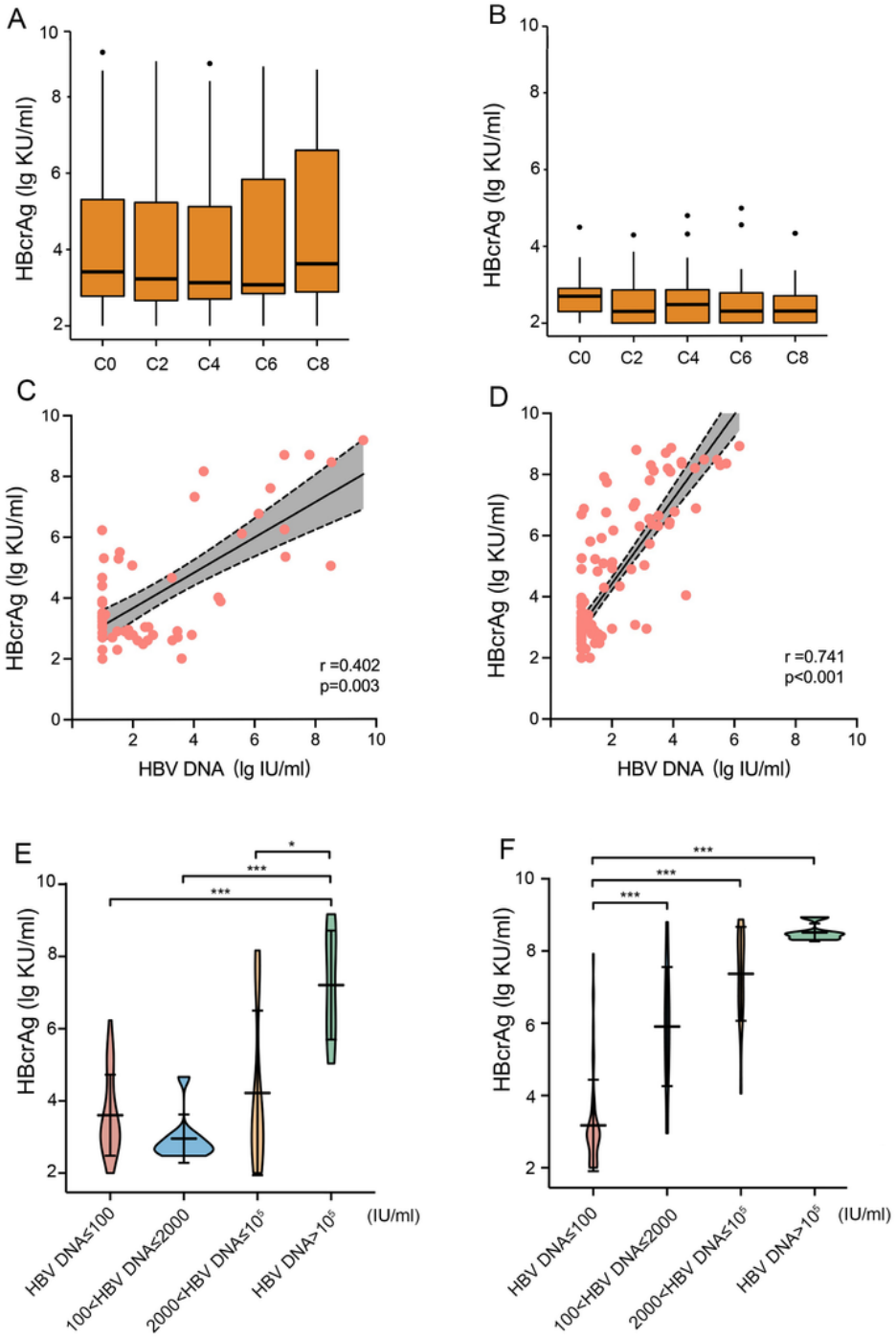

Figure 5

Serum HBcrAg stabled under the chemotherapy and positive correlated with HBV DNA load.

A: The HBcrAg level in HBsAg+ patients seems to decline along with the chemotherapy cycle but increased at C8; B: The HBcrAg level in HBsAg-/anti-HBc+ patients stayed stable during chemotherapy; C: In HBsAg+ patients, a positive correlation between HBcrAg and HBV DNA was found before

chemotherapy; D: In HBsAg+ patients, a stronger positive correlation between HBcrAg and HBV DNA was found during chemotherapy; E: In HBsAg+ patients, HBV DNA>10^5 IU/ml group had the highest HBcrAg level (7.21±1.51 lg KU/ml) before chemotherapy compared to HBV DNA≤ 100 IU/ml group (3.74±1.26 lg KU/ml), 100< HBV DNA≤2000 IU/ml group (2.95±0.67 lg KU/ml) and 2000< HBV DNA≤10^5 IU/ml group (4.22±2.28 lg KU/ml), all p<0.001 (labled as***); F: In HBsAg+ patients, HBcrAg level in the four subgroups was higher when the grade of HBV DNA was higher during chemotherapy: 3.21±1.32 lg KU/ml, 5.91±1.65 lg KU/ml, 7.37±1.30 lg KU/ml and 8.51±0.25 lg KU/ml, respectively. But significantly differences were found only between in three groups (p<0.001, labled as ***).

Supplementary Files

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