Appendix 1 Supplementary methods

Treatment for HBV infection and liver function

Before receiving anti-cancer treatment, patients with active hepatitis were instructed to receive routine hepatitis and antiviral treatments. Only when HBV DNA is reduced to 1,000 copies/mL and approved by the infection specialist, can the patients commence anti-cancer treatment. NPC patients with HBsAg(+) received antiviral treatment at the same time or 1 week before chemotherapy, and until 6–12 months after chemotherapy, if necessary. In the interim, liver function was closely monitored.

In this study, the liver function data were relatively complete in one of hospitals, as shown in Table S5. Furthermore, in further univariable analysis (not supplied), during chemotherapy, all of the variables (ALT, AST, Antiviral treatment, and HBV DNA) showed no statistical significance (all P>0.05) for all endpoints (OS, DMFS, LRFS and PFS), suggesting that they may not be confounding variables. Even in the stratified analysis of patients with HBsAg(+), there was no statistical significance in those indicators of survival in NPC.

Treatment for NPC

Per the treatment principle for nasopharyngeal carcinoma (NPC) at the Sun Yat-sen University Cancer Center and First People's Hospital of Foshan, all included patients received intensity-modulated radiation therapy during the radiotherapy course. Target volumes were delineated slice-by-slice on treatment planning computed tomography (CT) scans using an individualized delineation protocol that complies with the International Commission on Radiation Units and Measurements Reports 50 and 62. The prescribed doses were 66–72 Gy at 2.12–2.43 Gy/fraction to the planning target volume (PTV) of the primary GTV (GTVnx), 64–70 Gy per 28–33 fractions to the PTV of the GTV of involved lymph nodes (GTVnd), 60–63 Gy per 28–33 fractions to the PTV of the high-risk clinical target volume (CTV1), and 54–56 Gy per 28–33 fractions to the PTV of the low-risk clinical target volume (CTV2). The median dose delivered was 72.77 ± 1.26 Gy to the PTV of the GTVnx. Neoadjuvant, concurrent, or adjuvant chemotherapy based on platinum was administered to patients with stage II–IV NPC. Patients who developed local recurrence or had persistent disease received salvage therapy, such as secondary radiation, surgery, and chemotherapy. Induction chemotherapy (IC) included docetaxel (60 mg/m^2 on day 1), cisplatin (60 mg/m^2 on day 1), fluorouracil (600 mg/m^2 per day for the first 5 days), or gemcitabine (1,000 mg/m^2 on days 1 and 8) and cisplatin (80 mg/m^2 on day 1), repeated every 3 weeks for two to four cycles. Concurrent chemotherapy included 30–40 mg/m^2 cisplatin per week, 80–100 mg/m^2 cisplatin on day 1, and 28–37 times of IMRT for radiotherapy.

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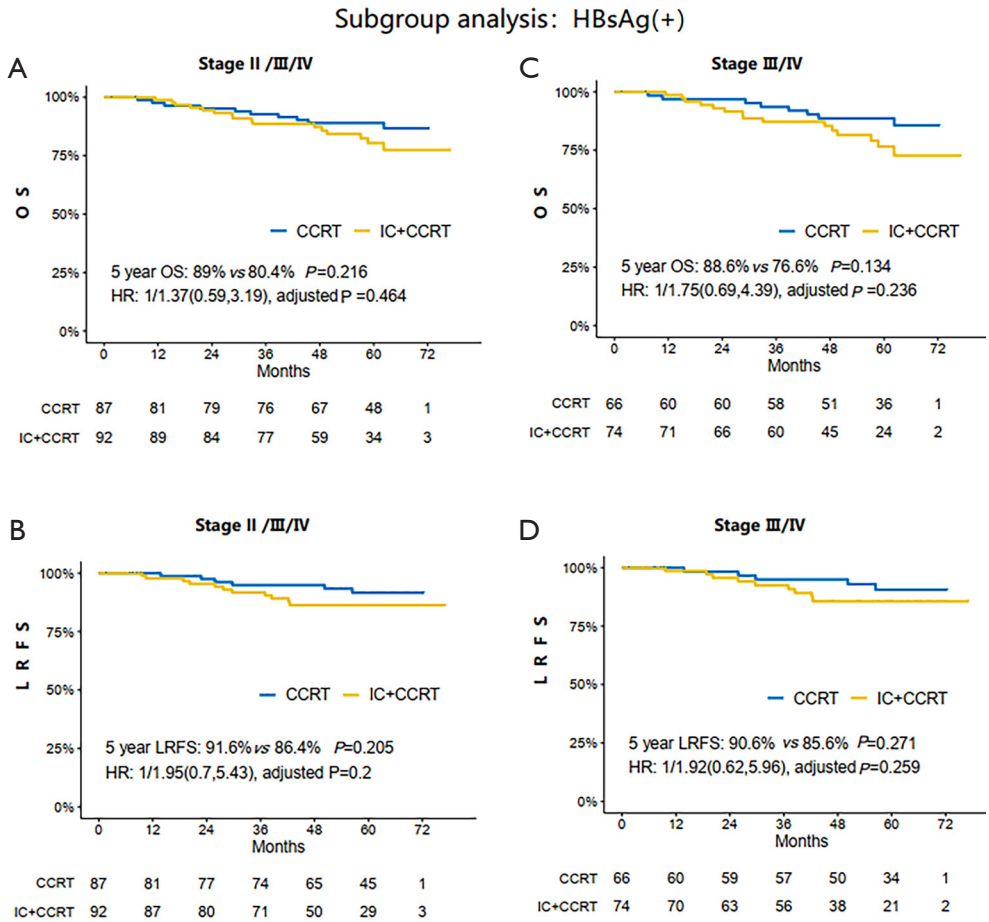

Figure S1 Subgroup analysis for OS and LRFS in NPC patients with HBsAg(+). In stage II/III/IV and stage III/IV subgroups, NPC patients with HBsAg(+) showed no significant differences in OS (A,C) or LRFS (B,D) between those treated with IC + CCRT or CCRT. Kaplan–Meier survival analysis with the log-rank test was used to calculate the 5-year survival difference between the CCRT and IC + CCRT groups. The Y-axis represented survival probability. HRs and P values were calculated using multivariate Cox regression analysis. Detailed results are shown in Table S3. OS, overall survival; LRFS, local recurrence-free survival; NPC, Nasopharyngeal carcinoma; HBsAg, hepatitis B surface antigen; +, positive; CCRT, concurrent chemotherapy; IC, induction chemotherapy; HR, hazard ratio.

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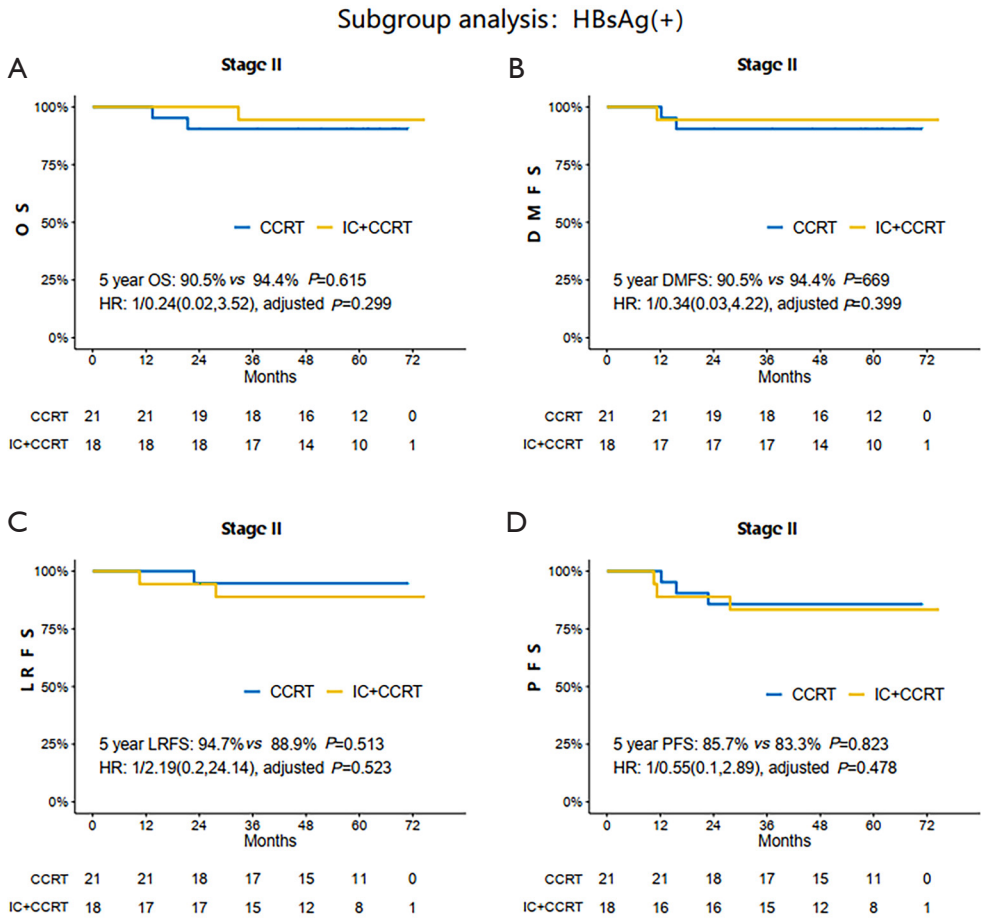

Figure S2 Survival analysis in stage II NPC patients with HBsAg(+). Stage II NPC patients with HBsAg(+) showed no significant differences in OS (A), DMFS (B), LRFS (C), and PFS (D) between those treated with IC + CCRT or CCRT. Kaplan–Meier survival analysis with the log-rank test was used to calculate the 5-year survival difference between the CCRT and IC + CCRT groups. The Y-axis represented survival probability. HRs and P values were calculated using multivariate Cox regression analysis. A table of detailed results will be provided if necessary. NPC, Nasopharyngeal carcinoma; HBsAg, hepatitis B surface antigen; +, positive; CCRT, concurrent chemotherapy; IC, induction chemotherapy; OS, overall survival; DMFS, distant metastasis-free survival; LRFS, local recurrence-free survival; PFS, progression-free survival; HR, hazard ratio.

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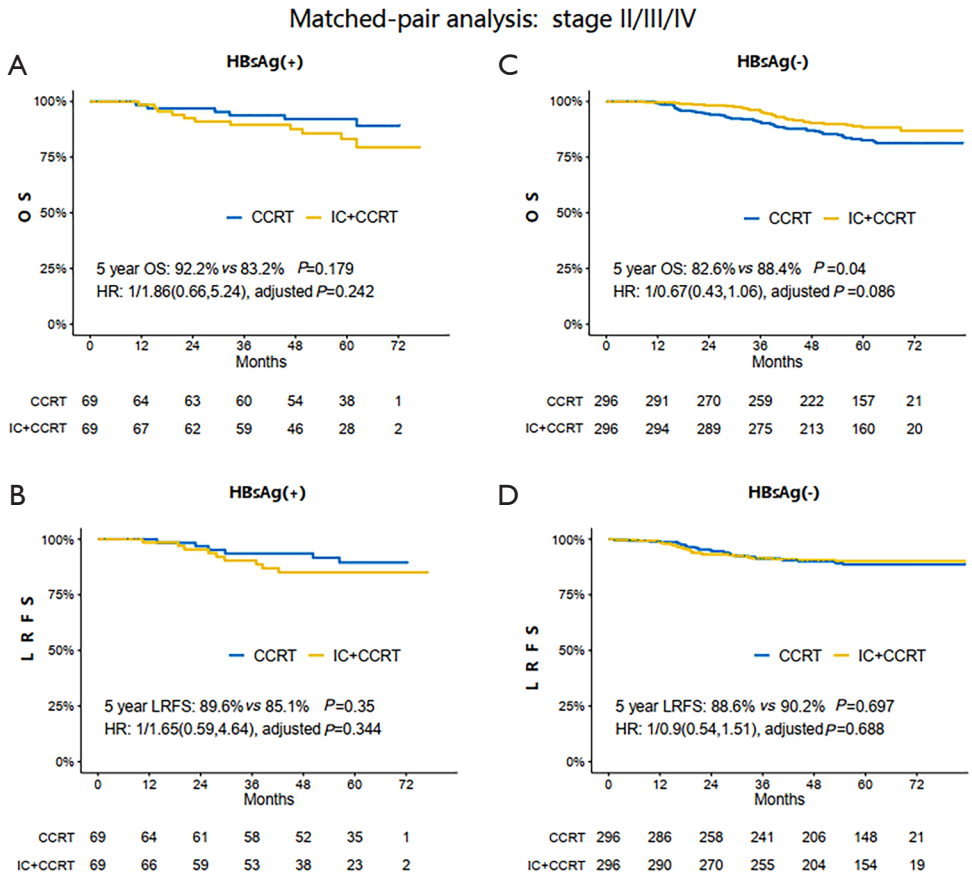

Figure S3 Matched-pair analysis for OS and LRFS between IC + CCRT and CCRT in stage II/III/IV NPC patients with different HBsAg status. For 69 pairs of NPC patients with HBsAg(+), no significant differences were observed in OS (A) and LRFS (B) between those treated with IC + CCRT or CCRT. For 296 pairs of NPC patients with HBsAg(–), patients treated with IC + CCRT had a significantly higher OS (C) than those treated with CCRT; however, this was no longer significant after adjusting for confounding factors. The LRFS survival curves of HBsAg(–) patients overlapped (D). Kaplan–Meier survival analysis with the log-rank test was used to calculate the 5-year survival difference between the CCRT and IC + CCRT groups. The Y-axis represented survival probability. HRs and P values were calculated using multivariate Cox regression analysis. A table of detailed results will be provided if necessary. OS, overall survival; LRFS, local recurrence-free survival; IC, induction chemotherapy; NPC, nasopharyngeal carcinoma; HBsAg, hepatitis B surface antigen; +, positive; –, negative; CCRT, concurrent chemotherapy.

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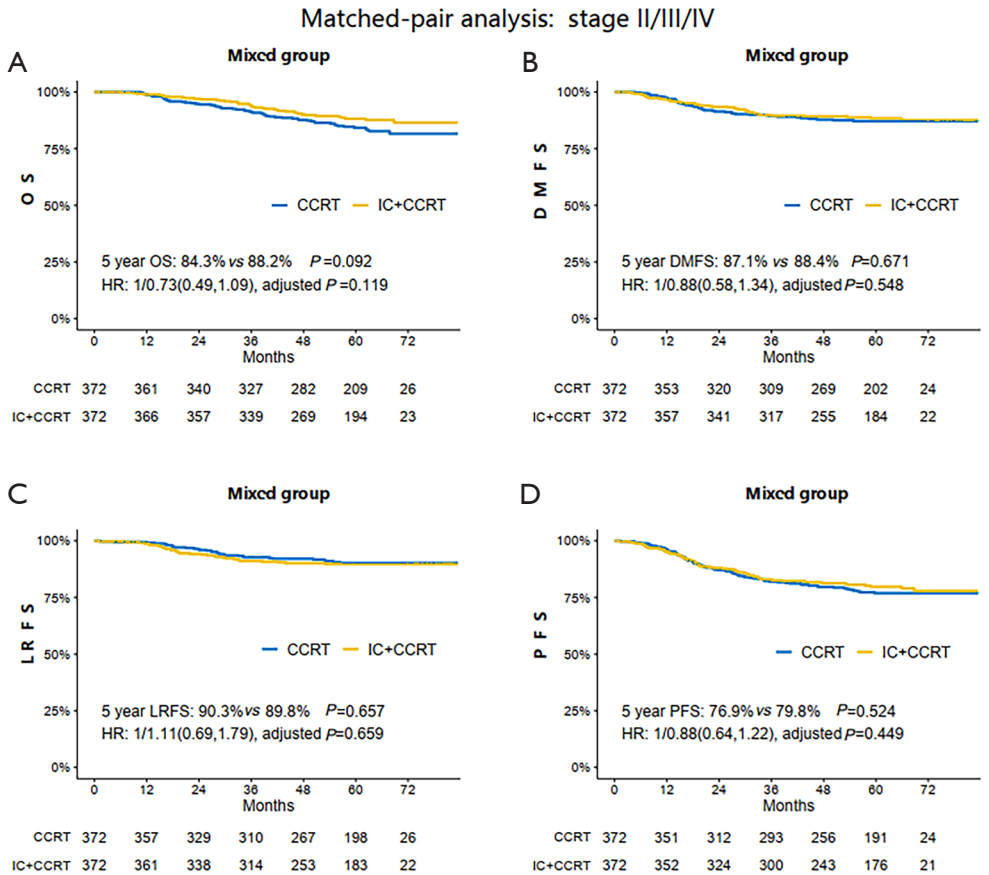

Figure S4 Survival in NPC with mixed HBsAg status using matched-pair analysis. For 372 pairs of NPC patients in stage II/III/IV, the OS (A) in patients treated with IC + CCRT was not significantly different than that in patients treated with CCRT. The survival curves between patients treated with IC + CCRT and CCRT overlapped for DMFS (B), LRFS (C), and PFS (D). Kaplan–Meier survival analysis with the log-rank test was used to calculate the 5-year survival difference between the CCRT and IC + CCRT groups. The Y-axis represented survival probability. HRs and P values were calculated using multivariate Cox regression analysis. Mixed group refers to patients without considering the HBsAg status when pairing. A table of detailed results will be provided if necessary. NPC, nasopharyngeal carcinoma; HBsAg, hepatitis B surface antigen; CCRT, concurrent chemotherapy; IC, induction chemotherapy; OS, overall survival; DMFS, distant metastasis-free survival; LRFS, local recurrence-free survival; PFS, progression-free survival; HR, hazard ratio.

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Table S1 Clinical characteristics of nasopharyngeal carcinoma patients in the two hospitals

Variables	Total (N=1,076)	Hospital 1 (N=719)	Hospital 2 (N=357)	P value[†]
Age (years)				<0.001*
Median (IQR)	46.0 (38.0–55.0)	45 (37–53)	47 (40–58)	
Sex				0.408
Male	799 (74.3%)	528 (73.4%)	271 (75.9%)	
Female	277 (25.7%)	191 (26.6%)	86 (24.1%)	
Histologic type				<0.001*
WHO type 1/2	41 (3.8%)	41 (5.7%)	0 (0%)	
WHO type 3	1035 (96.2%)	678 (94.3%)	357 (100%)	
Plasma EBV DNA level (1000 copy/mL)				<0.001*
<1	482 (44.8%)	287 (39.9%)	195 (54.6%)	
<10	337 (31.3%)	184 (25.6%)	153 (42.9%)	
≥10	257 (23.9%)	248 (34.5%)	9 (2.5%)	
T classification[‡]				0.567
T1	205 (19.1%)	131 (18.2%)	74 (20.7%)	
T2	150 (13.9%)	97 (13.5%)	53 (14.8%)	
T3	429 (39.9%)	296 (41.2%)	133 (37.3%)	
T4	292 (27.1%)	195 (27.1%)	97 (27.2%)	
N classification[‡]				0.019*
N0	143 (13.3%)	109 (15.2%)	34 (9.5%)	
N1	655 (60.9%)	438 (60.9%)	217 (60.8%)	
N2	189 (17.6%)	113 (15.7%)	76 (21.3%)	
N3	89 (8.3%)	59 (8.2%)	30 (8.4%)	
Stage[‡]				0.844
II	264 (24.5%)	175 (24.3%)	89 (24.9%)	
III	447 (41.5%)	303 (42.1%)	144 (40.3%)	
IV	365 (33.9%)	241 (33.5%)	124 (34.7%)	
Chemotherapy				0.084
CCRT	480 (44.6%)	334 (46.5%)	146 (40.9%)	
IC + CCRT	596 (55.4%)	385 (53.5%)	211 (59.1%)	
HBsAg				0.056
(–)	897 (83.4%)	588 (81.8%)	309 (86.6%)	
(+)	179 (16.6%)	131 (18.2%)	48 (13.4%)	

Hospital 1, Sun Yat-sen University Cancer Center; Hospital 2, First People's Hospital of Foshan. No significant difference was found in terms of stage, treatment mode, and HBsAg(+/–) between the two hospitals. [†], P values were calculated using Fisher's exact test or the chi-square test for categorical variables, and Student's t-test for continuous variables. [‡], according to the eighth edition of the AJCC/UICC staging system. *, P value <0.05. IQR, interquartile range; WHO, World Health Organization; plasma EBV DNA level, plasma Epstein-Barr virus DNA level; CCRT, concurrent chemotherapy; IC, induction chemotherapy; HBsAg, hepatitis B surface antigen; +, positive; –, negative.

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Table S2 Univariate analysis for elucidating confounding variables associated with prognosis

Variables	OS		DMFS		LRFS		PFS	
	5-years	P value†	5-years	P value†	5-years	P value†	5-years	P value†
Age (years)	–	<0.001*	–	0.394	–	0.453	–	0.008*
Sex		0.061		0.736		0.972		0.391
Male	83.57		85.76		88.92		75.25	
Female	88.30		86.77		89.11		78.21	
Histologic type		0.405		0.250		0.578		0.313
WHO type 1/2	79.10		79.45		86.83		69.65	
WHO type 3	85.02		86.30		89.06		76.29	
Plasma EBV DNA level (10^3 copy/mL)		0.001*		<0.001*		0.131		<0.001*
<1	88.60		91.02		91.04		82.32	
<10	85.49		82.82		87.21		73.19	
≥10	78.73		81.15		87.20		68.88	
T classification‡		<0.001*		<0.001*		0.133		<0.001*
T1	93.08		92.38		92.81		84.94	
T2	84.67		87.43		90.72		78.61	
T3	88.03		87.54		88.54		77.93	
T4	74.16		78.36		85.75		65.45	
N classification‡		<0.001*		<0.001*		0.035*		<0.001*
N0	88.93		92.84		95.60		85.55	
N1	88.16		88.67		88.84		78.56	
N2	77.12		78.50		86.56		68.14	
N3	68.00		70.19		83.63		58.00	
Stage‡		<0.001*		<0.001*		0.015*		<0.001*
II	93.40		93.77		93.34		86.63	
III	88.51		88.06		89.07		78.90	
IV	73.67		77.55		85.35		64.61	
Chemotherapy		0.123		0.014*		0.068		0.026*
CCRT	86.91		88.99		90.91		79.13	
IC + CCRT	83.07		83.58		87.43		73.52	
HBsAg		0.873		0.500		0.927		0.876
–	84.80		86.34		89.00		75.90	
+	84.89		84.44		88.82		76.78	
ALT		0.088		0.425		0.741		0.300
<50 U/L	84.26		85.750		88.95		75.68	
≥50 U/L	91.99		89.380		89.22		80.43	
AST		0.309		0.394		0.467		0.262
<40 U/L	84.55		85.810		88.85		75.72	
≥40 U/L	90.91		90.800		91.52		82.81	

The above significant factors (P<0.05) in the univariate analysis were entered in the multivariate analysis to further test and determine the confounding variables; this table will be provided if necessary. †, P values were calculated using the log-rank test. ‡, According to the eighth edition of the AJCC/UICC staging system. *, P value <0.05. OS, overall survival; DMFS, distant metastasis-free survival; LRFS, locoregional recurrence-free survival; PFS, progression-free survival; WHO, World Health Organization; plasma EBV DNA level, plasma Epstein-Barr virus DNA level; CCRT, concurrent chemotherapy; IC, induction chemotherapy; HBsAg, hepatitis B surface antigen; +, positive; –, negative; ALT, alanine aminotransferase; AST, aspartate transaminase.

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Table S3 Stage-based subgroup survival analysis in NPC patients with HBsAg(+)

Subgroups	Variables	OS		DMFS		LRFS		PFS	
		HR (95% CI)[†]	P value[‡]	HR (95% CI)[†]	P value[‡]	HR (95% CI)[†]	P value[‡]	HR (95% CI)[†]	P value[‡]
Stage II/III/IV	Age (years)	1.05 (1.01–1.08)	0.007*	Na		Na		1.03 (1–1.05)	0.069
	Plasma EBV DNA level (1000 copy/mL)					Na			
	<1	1 (reference)		1 (reference)		1 (reference)			
	<10	1.12 (0.42–3.01)	0.817	0.49 (0.14–1.68)	0.259	1.27 (0.56–2.91)		0.564	
	≥10	1.05 (0.38–2.89)	0.926	2.81 (1.15–6.88)	0.023*	1.91 (0.86–4.25)		0.112	
	Stage[§]								
	II	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
	III	1.37 (0.37–5.11)	0.642	1.59 (0.44–5.75)	0.48	1.45 (0.39–5.36)	0.579	1.27 (0.49–3.26)	0.621
	IV	3.01 (0.82–11.1)	0.097	2 (0.54–7.41)	0.298	1.19 (0.28–5.05)	0.811	1.56 (0.59–4.13)	0.369
	Chemotherapy								
	CCRT	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
	IC + CCRT	1.37 (0.59–3.19)	0.464	2.47 (1.04–5.88)	0.041*	1.95 (0.7–5.43)	0.2	1.97 (0.98–3.99)	0.059
Stage III/IV	Age (years)	1.04 (1.01–1.08)	0.016*	Na		Na		1.03 (1–1.06)	0.04*
	Plasma EBV DNA level (1,000 copy/mL)					Na			
	<1	1 (reference)		1 (reference)		1 (reference)			
	<10	0.83 (0.28–2.41)	0.728	0.24 (0.05–1.19)	0.081	0.69 (0.27–1.77)		0.438	
	≥10	1.01 (0.36–2.82)	0.989	2.7 (1.08–6.75)	0.033*	1.63 (0.73–3.66)		0.233	
	Stage[§]								
	III	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
	IV	2.23 (0.91–5.49)	0.081	1.21 (0.52–2.79)	0.659	0.83 (0.27–2.59)	0.754	1.21 (0.59–2.46)	0.603
	Chemotherapy								
	CCRT	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
	IC + CCRT	1.75 (0.69–4.39)	0.236	3.42 (1.3–8.97)	0.013*	1.92 (0.62–5.96)	0.259	2.69 (1.23–5.88)	0.014*

Variables that were statistically significant in the univariate analysis (as shown in Table S2) were selected. Survival curves are shown in *Figure 1* and Figure S1. [†,‡], Hazard ratios (HRs) and P values were calculated using multivariate Cox regression analysis. [§], According to the eighth edition of the AJCC/UICC staging system. *, P value <0.05. NPC, Nasopharyngeal carcinoma; HBsAg, hepatitis B surface antigen; +, positive; CCRT, concurrent chemotherapy; IC, induction chemotherapy; plasma EBV DNA level, plasma Epstein-Barr virus DNA level; OS, overall survival; DMFS, distant metastasis-free survival; LRFS, local recurrence-free survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval.

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Table S4 Clinical characteristics of nasopharyngeal carcinoma patients in the matched-pair analysis

Variables	HBsAg(+)			HBsAg(–)			Mixed		
	CCRT (N=69)	IC + CCRT (N=69)	P value†	CCRT (N=296)	IC + CCRT (N=296)	P value†	CCRT (N=372)	IC + CCRT (N=372)	P value†
Age (years)			1.000			0.004*			0.006*
Median (IQR)	44 (37–53)	45 (37–49)		47 (40–58)	45 (39–52)		47 (39–56)	45 (38–52)	
Sex			0.532			0.399			0.675
Male	56 (81.2%)	52 (75.4%)		211 (71.3%)	221 (74.7%)		272 (73.1%)	278 (74.7%)	
Female	13 (18.8%)	17 (24.6%)		85 (28.7%)	75 (25.3%)		100 (26.9%)	94 (25.3%)	
Histologic type			1.000			0.680			1.000
WHO type 1/2	2 (2.9%)	2 (2.9%)		14 (4.7%)	11 (3.7%)		12 (3.2%)	12 (3.2%)	
WHO type 3	67 (97.1%)	67 (97.1%)		282 (95.3%)	285 (96.3%)		360 (96.8%)	360 (96.8%)	
Plasma EBV DNA level (1000 copy/mL)			0.000*			0.034*			0.003*
<1	44 (63.8%)	20 (29%)		155 (52.4%)	124 (41.9%)		194 (52.2%)	149 (40.1%)	
<10	11 (15.9%)	28 (40.6%)		80 (27%)	103 (34.8%)		99 (26.6%)	136 (36.6%)	
>10	14 (20.3%)	21 (30.4%)		61 (20.6%)	69 (23.3%)		79 (21.2%)	87 (23.4%)	
T classification‡			1.000			1.000			1.000
T1	14 (20.3%)	14 (20.3%)		58 (19.6%)	58 (19.6%)		73 (19.6%)	73 (19.6%)	
T2	7 (10.1%)	7 (10.1%)		40 (13.5%)	40 (13.5%)		53 (14.2%)	53 (14.2%)	
T3	38 (55.1%)	38 (55.1%)		129 (43.6%)	129 (43.6%)		167 (44.9%)	167 (44.9%)	
T4	10 (14.5%)	10 (14.5%)		69 (23.3%)	69 (23.3%)		79 (21.2%)	79 (21.2%)	
N classification‡			1.000			1.000			1.000
N0	12 (17.4%)	12 (17.4%)		28 (9.5%)	28 (9.5%)		40 (10.8%)	40 (10.8%)	
N1	42 (60.9%)	42 (60.9%)		215 (72.6%)	215 (72.6%)		257 (69.1%)	257 (69.1%)	
N2	11 (15.9%)	11 (15.9%)		48 (16.2%)	48 (16.2%)		64 (17.2%)	64 (17.2%)	
N3	4 (5.8%)	4 (5.8%)		5 (1.7%)	5 (1.7%)		11 (3%)	11 (3%)	
Stage‡			1.000			1.000			1.000
II	18 (26.1%)	18 (26.1%)		87 (29.4%)	87 (29.4%)		105 (28.2%)	105 (28.2%)	
III	37 (53.6%)	37 (53.6%)		135 (45.6%)	135 (45.6%)		177 (47.6%)	177 (47.6%)	
IV	14 (20.3%)	14 (20.3%)		74 (25%)	74 (25%)		90 (24.2%)	90 (24.2%)	
Volume (cm^3)			0.967			0.919			0.652
Median (IQR)	27.6 (18.1–47.5)	28.2 (16.8–49.7)		30 (17.7–50.6)	29 (17–48.8)		30 (17.8–49.9)	28.6 (16.8–47.5)	

T and N classifications were used for 1:1 random pairing matching, which can be used to eliminate some known confounding factors such as stage and may further eliminate some unknown confounding factors. The mixed pairs were set as reference pairs, which do not consider the status of HBsAg when pairing. †, P values were calculated using Fisher's exact test or the Chi-square test for categorical variables, and Student's *t*-test for continuous variables. ‡, According to the eighth edition of the AJCC/UICC staging system. *, P value <0.05. HBsAg, hepatitis B surface antigen; +, positive; –, negative; CCRT, concurrent chemotherapy; IC, induction chemotherapy; IQR, interquartile range; WHO, World Health Organization; plasma EBV DNA level, plasma Epstein-Barr virus DNA level.

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Table S5 HBV-related clinical characteristics of nasopharyngeal carcinoma patients during chemotherapy

Variables	Hospital 1 (N=719)	HBsAg(–) (N=897)	HBsAg(+) (N=179)	P value[a]
ALT				0.002
<50 U/L	584 (81.2%)	490 (83.3%)	94 (71.8%)	
≥50 U/L	135 (18.8%)	98 (16.7%)	37 (28.2%)	
AST				0.004
<40 U/L	640 (89%)	533 (90.6%)	107 (81.7%)	
≥40 U/L	79 (11%)	55 (9.4%)	24 (18.3%)	
ALT/AST				0.013
≤1	579 (80.5%)	484 (82.3%)	95 (72.5%)	
>1	140 (19.5%)	104 (17.7%)	36 (27.5%)	
Antiviral treatment				<0.001
None treatment/record	690 (96%)	588 (100%)	102 (77.9%)	
Yes	29 (4%)	0 (0%)	29 (22.1%)	
HBV DNA level				<0.001
None record/undetected	690 (96%)	588 (100%)	102 (77.9%)	
<1000 copies/mL	6 (0.8%)	0 (0%)	6 (4.6%)	
≥1000 copies/mL	23 (3.2%)	0 (0%)	23 (17.6%)	

[a], P values were calculated using Fisher's exact test or the Chi-square test for categorical variables. ALT, alanine aminotransferase; AST, aspartate transaminase.

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