Supplementary Methods

Pre-treatment evaluation

All patients were treated according to the principle of pretreatment for nasopharyngeal carcinoma (NPC) patients at SYSUCC. Detailed pretreatment evaluation included complete history; physical examination, and fiber-optic endoscopic examination of the nasopharynx, oropharynx, and larynx; magnetic resonance imaging (MRI) or computerized tomography (CT) of the head and neck; a conventional work-up including chest radiography, abdominal sonography, and whole-body bone scanning using single-photon emission computed tomography (SPECT) or 18F-fluorodeoxyglucose positron emission tomography and computed tomography (PET/CT).

Treatment information

In total of eligible 1357 NPC patients, 909 patients received IMRT in combination with platinum-based concurrent chemotherapy (CCRT), and 448 patients were treated with IMRT alone. The CCRT consisted of concurrent cisplatin (30-40 mg/m^2) weekly or every 3 weeks (80-100 mg/m^2) during IMRT. (1. Chen QY, Wen YF, Guo L, Liu H, Huang PY, Mo HY, et al. Concurrent chemoradiotherapy vs radiotherapy alone in stage II nasopharyngeal carcinoma: phase III randomized trial. J Natl Cancer Inst 2011;103(23):1761-70. 2. Chen L, Hu CS, Chen XZ, Hu GQ, Cheng ZB, Sun Y, et al. Concurrent chemoradiotherapy plus adjuvant chemotherapy versus

concurrent chemoradiotherapy alone in patients with locoregionally advanced nasopharyngeal carcinoma: a phase 3 multicentre randomised controlled trial. Lancet Oncol 2012;13(2):163-71.) Of patients receiving IMRT-plus-CCRT, a total of 315 patients (34.7%) were treated with platinum weekly, and 594 patients (65.3%) received 2 or 3 cycles of chemotherapy every 3 weeks.

All patients were treated with intensity-modulated radiotherapy (IMRT). The target volumes were delineated according to a previously described institutional treatment protocol, which is in accordance with the International Commission on Radiation Units and Measurements (ICRU) Reports No. 50 and No. 62. Specifically, the primary nasopharyngeal gross tumor volume (GTVnx) and corresponding cervical lymph nodes were determined based on MRI or CT imaging as well as clinical and endoscopic data. The enlarged retropharyngeal nodes together with primary gross tumor volume (GTV) were outlined as the GTVnx on the IMRT plans. The first clinical tumor volume (CTV1) was defined as the GTV within 0.5-1.0 cm margin (0.2 to 0.3 cm posterior margin) to encompass the high-risk sites of microscopic extension and the whole nasopharynx. Clinical target volume 2 (CTV2) was defined as the CTV1 plus a 0.5-1.0 cm margin (0.2 to 0.3 cm posterior margin) to encompass the low-risk sites of microscopic extension, the level of the lymph node, and the elective neck area (bilateral levels IIa, IIb, III, and Va are routinely covered for all N0 patients, whereas ipsilateral levels IV, Vb, and supraclavicular fossae were also included for the N1-3 patients). The prescribed doses were 66–70 Gy to the planning target volume (PTV), 60 Gy to PTV1, 54 Gy to PTV2, and 60–66 Gy to the PTV of the involved

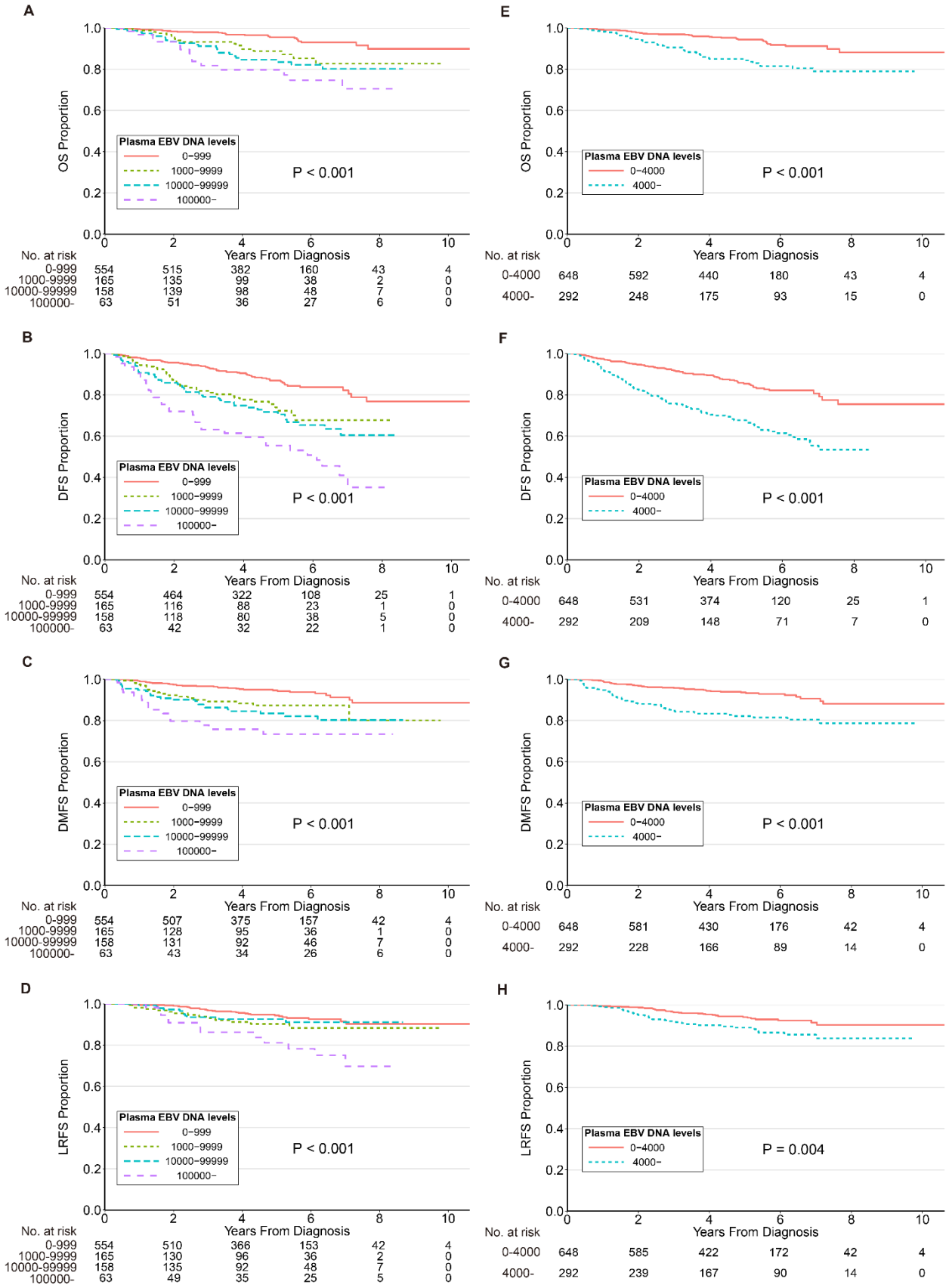
cervical lymph nodes in 28 to 33 fractions. All patients were treated once daily with five fractions weekly. And the intensity modulated radiotherapy quality assurance (QA) was performed before radiation treatment for all the NPC patients. Details of the radiation therapy techniques used at the SYSUCC were described in a previous study. (1. Zhao C, Han F, Lu LX, et al. Intensity modulated radiotherapy for local-regional advanced nasopharyngeal carcinoma. Ai Zheng 2004; 23(11 Suppl):1532-1537; 2. Ma J, Liu L, Tang L, et al. Retropharyngeal lymph node metastasis in nasopharyngeal carcinoma: prognostic value and staging categories. Clin Cancer Res 2007; 13(5):1445-1452.)

Follow-up information

After completion of the treatment, the patients were subsequently examined every three months for the first three years and then every six months thereafter until death. At each follow-up visit, detailed history was collected, and a complete physical examination was performed. Specifically, nasopharyngoscopy, magnetic resonance imaging (MRI) scanning of the head and neck, chest radiography, abdominal sonography, and whole-body bone scanning using single-photon emission computed tomography (SPECT) or ^{18}F-fluorodeoxyglucose positron emission tomography and computed tomography (PET/CT) were performed annually or when patients had clinical symptoms indicating tumour relapse.

Supplementary Figures Legends

Supplementary Figure 1 Kaplan-Meier survival curves for overall survival, disease-free survival, metastasis-free survival, and locoregional relapse-free survival for four groups by magnitude (A, B, C, and D, respectively) and two groups according to a cutoff of 4000 copies per millilitre (E, F, G, and H, respectively). DFS, disease-free survival; DMFS, distant metastasis-free survival; EBV, Epstein-Barr virus; LRFS, locoregional relapse-free survival; OS, overall survival.


A
OS Proportion
Plasma EBV DNA levels
0–999
1000–9999
10000–99999
100000–
P < 0.001
Years From Diagnosis
No. at risk
0-999 554 515 382 160 43 4
1000-9999 165 135 99 38 2 0
10000-99999 158 139 98 48 7 0
100000- 63 51 36 27 6 0
E
OS Proportion
Plasma EBV DNA levels
0–4000
4000–
P < 0.001
Years From Diagnosis
No. at risk
0-4000 648 592 440 180 43 4
4000- 292 248 175 93 15 0
B
DFS Proportion
Plasma EBV DNA levels
0–999
1000–9999
10000–99999
100000–
P < 0.001
Years From Diagnosis
No. at risk
0-999 554 464 322 108 25 1
1000-9999 165 116 88 23 1 0
10000-99999 158 118 80 38 5 0
100000- 63 42 32 22 1 0
F
DFS Proportion
Plasma EBV DNA levels
0–4000
4000–
P < 0.001
Years From Diagnosis
No. at risk
0-4000 648 531 374 120 25 1
4000- 292 209 148 71 7 0
C
DMFS Proportion
Plasma EBV DNA levels
0–999
1000–9999
10000–99999
100000–
P < 0.001
Years From Diagnosis
No. at risk
0-999 554 507 375 157 42 4
1000-9999 165 128 95 36 1 0
10000-99999 158 131 92 46 7 0
100000- 63 43 34 26 6 0
G
DMFS Proportion
Plasma EBV DNA levels
0–4000
4000–
P < 0.001
Years From Diagnosis
No. at risk
0-4000 648 581 430 176 42 4
4000- 292 228 166 89 14 0
D
LRFS Proportion
Plasma EBV DNA levels
0–999
1000–9999
10000–99999
100000–
P < 0.001
Years From Diagnosis
No. at risk
0-999 554 510 366 153 42 4
1000-9999 165 130 96 36 2 0
10000-99999 158 135 92 48 7 0
100000- 63 49 35 25 5 0
H
LRFS Proportion
Plasma EBV DNA levels
0–4000
4000–
P = 0.004
Years From Diagnosis
No. at risk
0-4000 648 585 422 172 42 4
4000- 292 239 167 90 14 0

Supplementary Table 1. Correlations analysis between EBV DNA levels and TNM stages.

EBV DNA levels [b]	Correlation coefficients [a]		
	T stage	N stage	Clinical stage
Continuous variable	0.234	0.330	0.307
Four pre-specified groups	0.215	0.318	0.288
Two groups	0.183	0.297	0.255

[a] Tested using Spearman method in all patients whose plasma EBV DNA were measured and all P < 0.001.

[b] Four pre-specified groups were based on magnitude and two groups were conventionally adopted a cutoff of 4000 copies per milliliter in endemic area.

Supplementary Table 2. Results of Stratification Analysis with Propensity Score Methods between Treatment Regimens and EBV DNA Levels with Clinical Stage [a]

Factor	Clinical stage II-IVA-B		Clinical stage II-III		Clinical stage IVA-B	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
EBV DNA level, copies/ml						
IPTW method						
Distant metastasis-free survival						
All measured patients	1.276(0.792-2.053)	0.316	1.374(0.596-3.166)	0.456	2.796(1.167-6.700)	0.021
<4000 cohort	1.073(0.491-2.343)	0.860	0.953(0.369-2.463)	0.921	1.324(0.235-7.467)	0.751
⩾4000 cohort	2.895(1.361-6.158)	0.005	2.042(0.554-7.522)	0.283	3.671(1.482-9.092)	0.004
Overall survival						
All measured patients	1.254(0.816-1.925)	0.302	1.319(0.530-3.283)	0.553	2.245(0.590-8.542)	0.236
<4000 cohort	0.867(0.366-2.055)	0.747	1.124(0.397-3.184)	0.826	0.384(0.040-3.700)	0.407
⩾4000 cohort	2.711(1.058-6.949)	0.037	1.177(0.382-3.629)	0.777	4.464(1.766-11.280)	<0.001
Disease-free survival						
All measured patients	1.207(0.866-1.684)	0.267	1.398(0.801-2.439)	0.238	1.934(0.770-4.861)	0.161
<4000 cohort	1.028(0.588-1.798)	0.923	1.086(0.568-2.076)	0.802	0.498(0.143-1.736)	0.274
⩾4000 cohort	2.308(1.220-4.369)	0.010	1.784(0.775-4.104)	0.174	3.527(1.692-7.349)	<0.001
Locoregional relapse-free survival						
All measured patients	1.077(0.615-1.885)	0.795	1.251(0.546-2.868)	0.596	0.352(0.047-2.615)	0.308
<4000 cohort	1.000(0.398-2.507)	0.999	1.220(0.446-3.339)	0.699	N.S. [b]	- [b]
⩾4000 cohort	0.921(0.247-3.424)	0.902	1.349(0.355-5.126)	0.661	N.S.	-
PSM method						
Distant metastasis-free survival						
All measured patients	1.091(0.670-1.778)	0.726	1.032(0.484-2.200)	0.935	6.521(1.398-30.428)	0.017
<4000 cohort	1.557(0.655-3.698)	0.316	1.174(0.450-3.063)	0.743	N.S.	-
⩾4000 cohort	3.121(1.118-8.718)	0.030	1.428(0.335-6.092)	0.630	N.S.	-
Overall survival						
All measured patients	1.338(0.821-2.179)	0.242	1.408(0.640-3.099)	0.395	4.637(0.951-22.613)	0.058

<4000 cohort	1.903(0.731-4.954)	0.187	1.868(0.721-4.841)	0.198	N.S.	-
⩾4000 cohort	3.061(1.068-8.770)	0.037	1.353(0.302-6.057)	0.693	N.S.	-
Disease-free survival						
All measured patients	1.255(0.877-1.796)	0.214	1.226(0.751-2.003)	0.415	4.410(1.385-14.044)	0.012
<4000 cohort	1.421(0.780-2.589)	0.250	1.167(0.618-2.205)	0.634	N.S.	-
⩾4000 cohort	2.146(1.050-4.385)	0.036	1.163(0.478-2.830)	0.739	4.236(1.170-15.335)	0.028
Locoregional relapse-free survival						
All measured patients	1.215(0.687-2.149)	0.503	1.227(0.602-2.500)	0.573	0.503(0.052-4.861)	0.552
<4000 cohort	1.045(0.436-2.505)	0.921	1.091(0.450-2.646)	0.847	N.S.	-
⩾4000 cohort	1.200(0.331-4.349)	0.781	1.081(0.232-5.040)	0.921	N.S.	-

[a] There were significant interaction effects between treatment regimens and EBV DNA levels with clinical stage for distant metastasis-free survival, but not for overall, disease-free, or locoregional relapse-free survivals, and detailed interaction information was presented in figure 3. [b] Due to small numbers of cases in these subgroups, the confident intervals were extremely wide but non-significant (N.S.). CI, confidence interval; EBV, Epstein-Barr virus; HR, hazard ratio; IPTW, inverse probability of treatment weighting; PSM, propensity score matching.