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Article

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Establishment and Validation of Prognostic Nomograms for Endemic Nasopharyngeal Carcinoma

Lin-Quan Tang*, Chao-Feng Li*, Jing Li*, Wen-Hui Chen*, Qiu-Yan Chen, Lian-Xiong Yuan, Xiao-Ping Lai, Yun He, Yun-Xiu-Xiu Xu, Dong-Peng Hu, Shi-Hua Wen, Yu-Tuan Peng, Lu Zhang, Shan-Shan Guo, Li-Ting Liu, Ling Guo, Yi-Shan Wu, Dong-Hua Luo, Pei-Yu Huang, Hao-Yuan Mo, Yan-Qun Xiang, Rui Sun, Ming-Yuan Chen, Yi-Jun Hua, Xing Lv, Lin Wang, Chong Zhao, Ka-Jia Cao, Chao-Nan Qian, Xiang Guo, Yi-Xin Zeng†, Hai-Qiang Mai†, Mu-Sheng Zeng†

Affiliations of authors: Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine (LQT, CFL, JL, WHC, QYC, LZ, SSG, LTL, LG, YSW, DHL, PYH, HYM, YQX, RS, MYC, YJH, XL, LW, CZ, KJC, CNQ, XG, YXZ, HQM, MSZ), Department of Nasopharyngeal Carcinoma (LQT, QYC, LZ, SSG, LTL, LG, YSW, DHL, PYH, HYM, YQX, RS, MYC, YJH, XL, LW, CZ, KJC, CNQ, XG, HQM), Department of Information Technology (CFL), Department of Medical Statistics and Epidemiology, the School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong, China (LXY); ZhongShan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China (XPL, YH, YXXX, DPH, SHW, YTP).

*Authors contributed equally to this work.
†Authors contributed equally to this work.

Corresponding author: Mu-Sheng Zeng, MD, PhD, Department of Experimental Research, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou 510060, P. R. China (e-mail: zengmsh@mail.sysu.edu.cn).

Abstract

Background: This study aimed to establish an effective prognostic nomogram with or without plasma Epstein-Barr virus DNA (EBV DNA) for nondisseminated nasopharyngeal carcinoma (NPC).

Methods: The nomogram was based on a retrospective study of 4630 patients who underwent radiotherapy with or without chemotherapy at Sun Yat-sen University Cancer Center from 2007 to 2009. The predictive accuracy and discriminative ability of the nomogram were determined by a concordance index (C-index) and calibration curve and were compared with EBV DNA and the current staging system. The results were validated using bootstrap resampling and a prospective cohort study on 1819 patients consecutively enrolled from 2011 to 2012 at the same institution. All statistical tests were two-sided.

Results: Independent factors derived from multivariable analysis of the primary cohort to predict recurrence were age, sex, body mass index (BMI), T stage, N stage, plasma EBV DNA, pretreatment high sensitivity C-reactive protein (hs-CRP), lactate dehydrogenase (LDH), and hemoglobin level (HGB), which were all assembled into the nomogram with (nomogram B) or without EBV DNA (nomogram A). The calibration curve for the probability of recurrence showed that the nomogram-based predictions were in good agreement with actual observations. The C-index of nomogram B for predicting recurrence was 0.728 ($P < .001$), which was statistically higher than the C-index values for nomogram A (0.690), EBV DNA (0.680), and the current staging system (0.609). The C-index of nomogram B (0.730) and nomogram A (0.681) remained higher for predicting recurrence among patients treated with intensity-modulated radiotherapy ($P < .001$). The results were confirmed in the validation cohort.

Conclusions: The proposed nomogram with or without plasma EBV DNA resulted in more accurate prognostic prediction for NPC patients.

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Nasopharyngeal carcinoma (NPC) is endemic to Southern China and Southeast Asia, with a peak incidence of 50 cases per 100 000 (1) in these areas. Radiotherapy (RT) is the primary treatment, and concurrent chemoradiotherapy with or without adjuvant chemotherapy is the standard of care for advanced NPC (2–4). However, 20% to 30% of patients will develop local or systemic recurrences (5,6), most of which occur within the first two years after treatment (7). Because 10% to 20% of patients with local or systemic recurrence may be cured with additional treatment (8), it is necessary to identify patients with a high risk of recurrence earlier.

Currently, the American Joint Committee on Cancer (AJCC) TNM classification, based on anatomical information, is the most commonly used staging system and is the benchmark to establish treatment regimens for NPC patients. However, large variations are reported in the clinical outcomes of patients with the same stage receiving similar treatment strategies (9). This finding indicates that the present staging system is inadequate for predicting recurrence and does not reflect the biological heterogeneity of NPC patients. However, many other risk factors, such as age, sex, smoking status, body mass index (BMI), circulating Epstein-Barr virus (EBV) DNA, serum lactate dehydrogenase (LDH) (10), and high sensitivity C-reactive protein (hs-CRP) (11), have been demonstrated to influence recurrence in NPC patients and should be considered for predicting individualized prognosis. Specifically, plasma EBV DNA, which is now gradually being adopted in clinical applications and is currently considered to be the most attractive potential biomarker to complement the TNM classification (12), has been shown to be correlated with tumor burden (13), TNM stage (14), response to chemoradiotherapy (12,15,16), and survival in NPC patients (17–19). However, notably, there is still no effective way to combine plasma EBV DNA into the current TNM stage. Therefore, a comprehensive, easy-to-use tool that estimates individual risk by incorporating TNM stage, EBV DNA, and other risk factors could serve as a valuable decision-making tool for clinicians.

Nomograms are graphical depictions of predictive statistical models for individual patients (20), and they have been developed for various types of cancers (21–23). Because the use of nomograms has a demonstrated advantage over the traditional staging systems used to predict patient outcomes for many cancers, nomograms have been proposed as an alternative method or even as a new standard to guide treatment allocation for cancer patients (23,24). Hence, the present study aimed to develop a practical clinical tool by combining clinicopathologic factors, plasma EBV DNA, and other prognostic biomarkers. We also performed a test to determine whether this model provides a more accurate prediction of recurrence when compared with plasma EBV DNA alone and currently available staging systems.

Methods

Patients

A retrospective observational study was conducted on a primary cohort of NPC patients who underwent radiotherapy with or without chemotherapy between January 2007 and December 2009 at the Sun Yat-sen University Cancer Center (SYSUCC), Guangzhou, China. Of the primary cohort comprising 5145 patients with NPC who received radiotherapy with or without chemotherapy, 515 patients were excluded from the analysis. The inclusion criteria and exclusion criteria of the primary cohort are detailed in the Supplementary Materials (available online).

Table 1. Patient demographics and clinical characteristics*

Characteristic	Primary cohort (n= 4630) No. (%)	Validation cohort (n= 1819) No. (%)
Age, y		
18–29	224 (4.8)	93 (5.1)
30–39	979 (21.1)	374 (20.6)
40–49	1519 (32.8)	651 (35.8)
50–59	1187 (25.6)	453 (24.9)
≥60	721 (15.6)	248 (13.6)
Sex		
Female	1231 (26.6)	483 (26.6)
Male	3399 (73.4)	1336 (73.4)
Histology, WHO type		
II	198 (4.3)	45 (2.5)
III	4432 (95.7)	1774 (97.5)
ECOG		
0	200 (4.3)	73 (4.0)
1	4410 (95.2)	1739 (95.6)
2	20 (0.4)	7 (0.4)
Clinical stage		
I	163 (3.5)	60 (3.3)
II	657 (14.2)	240 (13.2)
III	2513 (54.3)	991 (54.5)
IVa	955 (20.6)	369 (20.3)
IVb	342 (7.4)	159 (8.7)
Tumor stage		
T1	447 (9.7)	162 (8.9)
T2	984 (21.3)	335 (18.4)
T3	2179 (47.1)	910 (50.0)
T4	1020 (22.0)	412 (22.6)
Node stage		
N0	629 (13.6)	324 (17.8)
N1	1904 (41.1)	709 (39.0)
N2	1739 (37.6)	620 (34.1)
N3	358 (7.7)	166 (9.1)
Treatment		
RT alone	918 (19.8)	259.0 (14.2)
CCRT	1502 (32.4)	678.0 (37.3)
NACT + CCRT	2081 (44.9)	852.0 (46.8)
CCRT + AC	129 (2.8)	30.0 (1.6)
Radiotherapy technique		
2DRT/3DCRT	3056 (66.0)	412 (22.6)
IMRT	1574 (34.0)	1407 (77.4)
EBVDNA, copy/mL		
<1000	1746 (37.7)	823 (45.2)
1000–9999	1201 (25.9)	507 (27.9)
10 000–99 999	959 (20.7)	349 (19.2)
100 000–999 999	554 (12.0)	114 (6.3)
≥ 1 000 000	170 (3.7)	26 (1.4)
VCA-IgA		
<1:80	1116 (24.1)	602 (33.1)
1:80–1:320	2424 (52.4)	857 (47.1)
≥ 1:640	1090 (23.5)	360 (19.8)
EA-IgA		
<1:10	1865 (40.3)	814 (44.7)
1:10–1:20	772 (16.7)	295 (16.2)
≥1:40	1993 (43.0)	710 (39.0)
LDH, U/L		
<245	4348 (93.9)	1693 (93.1)
≥245	282 (6.1)	126 (6.9)
hs-CRP, g/mL		
<1.0	1927 (41.6)	659 (36.2)
1.0–3.0	1422 (30.7)	609 (33.5)

Table 1. *Continued*

Characteristic	Primary cohort (n= 4630) No. (%)	Validation cohort (n= 1819) No. (%)
≥3.0	1281 (27.7)	551 (30.3)
WBC, 109/L		
<4	162 (3.5)	62 (3.4)
4–10	4077 (88.1)	1596 (87.7)
≥10	391 (8.4)	161 (8.9)
Neutrophil, 10^9/L		
< 2.0	129 (2.8)	45 (2.5)
2.0–7.0	4154 (89.7)	1601 (88.0)
≥7.0	347 (7.5)	173 (9.5)
HGB, g/L		
<113	150 (3.2)	60 (3.3)
113–151	3238 (69.9)	1152 (63.3)
≥151	1242 (26.8)	607 (33.4)
PLT, 109/L		
<100	87 (1.9)	13 (0.7)
100–300	3812 (82.3)	1544 (84.9)
≥300	731 (15.8)	262 (14.4)
Body mass index, kg/m^2		
<18.5	365 (7.9)	170 (9.3)
18.5–22.9	2063 (44.6)	785 (43.2)
22.9–27.4	1851 (40.0)	718 (39.5)
≥27.5	351 (7.6)	146 (8.0)
Smoking		
No	2784 (60.1)	1119 (61.5)
Yes	1846 (39.9)	700 (38.5)
Family history of NPC		
No	4090 (88.3)	1618 (88.9)
Yes	540 (11.7)	201 (11.1)

* 2DRT = two-dimensional radiotherapy; 3DCRT = three-dimensional conformal radiotherapy; concurrent CCRT = concurrent chemoradiotherapy; CCRT+AC = concurrent chemoradiotherapy plus adjuvant chemotherapy; EA = early antigen; EBV DNA = Epstein-Barr virus DNA; ECOG = Eastern Cooperative Oncology Group; hs-CRP = high-sensitivity C-reactive protein; IgA = immunoglobulin A; IMRT = intensity-modulated radiotherapy; LDH = serum lactate dehydrogenase levels; HGB = hemoglobin; NACT+CCRT = neoadjuvant chemotherapy plus concurrent chemoradiotherapy; NPC = nasopharyngeal carcinoma; PLT = platelets; RT = radiation therapy; VCA = viral capsid antigen; WBC = white blood cell; WHO = World Health Organization.

From January 2011 to June 2012, an independent cohort of consecutive NPC patients was prospectively enrolled, using the same inclusion and exclusion criteria as the primary cohort. The patients were defined as the validation cohort of this study. All the patients in the primary and validation cohort were restaged according to the seventh AJCC TNM staging manual (25). This study was approved by the Clinical Research Ethics Committee of the SYSUCC, and all participants provided written informed consent prior to treatment.

Diagnosis and Treatment

After a detailed history and a complete physical examination, blood was collected from the patients to determine the presence of EBV-specific VCA/IgA antibodies, EBV-specific EA/IgA antibodies, hs-CRP and LDH levels as well as white blood cell (WBC), neutrophil, hemoglobin (HCG), and platelet (PLT) counts. Before treatment, the following baseline clinical information was collected: sex, age, height, weight, family history of NPC, smoking status, and performance score by the Eastern Cooperative Oncology Group (ECOG). Other routine investigations included clinical examinations of the head and neck region, magnetic resonance imaging scanning of the suprasellar cistern to the collarbone, fiberoptic nasopharyngoscopy, chest radiography, abdominal sonography, whole-body bone scan or ^{18}F-Fluorodeoxyglucose positron emission tomography and computed tomography (PET/CT). All patients at the study institution were treated according to the principle of treatment for NPC patients at SYSUCC. Detailed information on treatment is presented in the Supplementary Materials (available online).

Table 2. Multivariable Analysis of the Primary Cohort*

Variable	Recurrence HR (95% CI)	P
Age, y		
18–29	Reference	
30–39	1.04 (0.73 to 1.48)	.88
40–49	1.16 (0.82 to 1.64)	.39
50–59	1.27 (0.90 to 1.80)	.18
≥60	1.83 (1.28 to 2.59)	<.001
Sex		
Female	Reference	
Male	1.57 (1.32 to 1.86)	<.001
Tumor stage		
T1	Reference	
T2	1.73 (1.21 to 2.50)	.003
T3	1.92 (1.37 to 2.70)	<.001
T4	2.12 (1.49 to 3.01)	<.001
Node stage		
N0	Reference	
N1	1.64 (1.24 to 2.17)	.001
N2	1.86 (1.40 to 2.47)	<.001
N3	2.15 (1.55 to 2.98)	<.001
EBVDNA, copy/mL		
<1000	Reference	
1000–9999	1.16 (0.93 to 1.43)	.20
10 000–99 999	2.32 (1.90 to 2.85)	<.001
100 000–999 999	3.20 (2.58 to 3.97)	<.001
≥1 000 000	4.33 (3.29 to 5.69)	<.001
LDH, U/L		
<245	Reference	
≥245	1.52 (1.23 to 1.87)	<.001
hs-CRP, g/mL		
<1.0	Reference	
1.0–3.0	1.110 (0.94 to 1.31)	.23
≥3.0	1.48 (1.25 to 1.74)	<.001
HGB,g/L		
<113	Reference	
113–151	0.67 (0.49 to 0.91)	.012
≥151	0.53 (0.33 to 0.75)	<.001
Body mass index, kg/m^2		
<18.5	Reference	
18.5–22.9	0.89 (0.71 to 1.11)	.300
22.9–27.4	0.62 (0.49 to 0.78)	<.001
≥27.5	0.69 (0.50 to 0.95)	.024

* Hazard ratios estimated by Cox proportional hazards regression. All statistical tests were two-sided. CI = confidence interval; EBV DNA = Epstein-Barr virus DNA; HGB = hemoglobin; HR = hazard ratio; hs-CRP = high-sensitivity C-reactive protein; LDH = serum lactate dehydrogenase levels; NPC = nasopharyngeal carcinoma.

Real-time Quantitative EBV DNA Polymerase Chain Reaction

Plasma EBV DNA concentrations were routinely measured by quantitative polymerase chain reaction (PCR) prior to treatment

as described in previous studies (26–28). The methodology for detecting plasma EBV DNA is described in detail in the Supplementary Materials (available online).

Follow-up and Outcome

Patients were observed at least once every three months during the first three years and then every six months thereafter until death after treatment. A detailed history and a complete physical examination were performed at each follow-up visit. Blood was collected for plasma EBV DNA and routine blood and biochemistry tests. Nasopharyngescopy, MRI of the head and neck, chest radiography, abdominal sonography, whole-body bone scan or PET/CT were routinely performed annually or when clinically indicated tumor relapse occurred. NPC recurrence was defined as the appearance of a newly detected local/regional recurrence or distant metastasis, confirmed by nasopharyngeal biopsy or two radiologic images with or without elevation of tumor biomarkers. Our primary endpoint was disease-free survival (DFS), which was calculated from the date of diagnosis to the date of the first relapse at any site, death from any cause, or the date of the last follow-up visit.

Statistical Analysis

Categorical variables were classified based on clinical findings, and continuous variables were transformed into categorical variables based on routine cutoff points in clinical application (10,29–32). A diagnostic plot using the null martingale residuals of the EBV DNA with and without other prognostic variables (Loess curves in red, Supplementary Figure 1, available online) was made to analyze the function form between EBV DNA and DFS (33). A martingale residual analysis indicates that the prognostic value of EBV DNA is linear. Moreover, previous studies have demonstrated that the relative risk for every 10-fold increase in plasma EBV DNA level to predict clinical events was 3.8 (95% confidence interval [CI] = 1.6 to 9.2) (34); thus, each 10-fold increase in plasma EBV DNA levels was chosen as the cutoff level in this study. Survival curves were depicted using the Kaplan-Meier method and compared by the log-rank test. Risk factors to predict recurrence selected for the derivation of prediction models were based on previous publications, which were routinely accessible in clinics with high measurement accuracy. Variables that reached a P value of .05 or less in the univariate analyses were subjected to multivariable Cox regression analysis.

A nomogram was formulated based on the results of multivariable Cox regression analysis. The selection of the final prediction model was performed with a backward step down selection process with the Akaike information criterion (35). The performance of the nomogram was evaluated by the concordance index (C-index) and assessed by comparing nomogram-predicted vs observed Kaplan-Meier estimates of survival probability, and bootstraps with 1000 resamples were applied to these activities. Comparisons between the nomogram, EBV DNA alone, and current staging systems in the entire population and in subgroups of patients treated with IMRT were performed with the rcorrp.cens function in the Hmisc package (36) in R and were tested by the C-index. A larger C-index indicated more accurate prognostic stratification. The total points of each patient in the validation cohort were calculated according to the established nomogram, and then Cox regression in this cohort was performed using the total points as a factor. Finally, the C-index and calibration curve were derived based on the regression analysis. All the related computerized programs for nomograms using R are listed in detail in the Supplementary Materials (available online). Statistical analyses to identify risk factors were performed using SPSS (Statistical Package for the Social Sciences) 17.0 for Windows (SPSS, Chicago, IL), and the nomogram

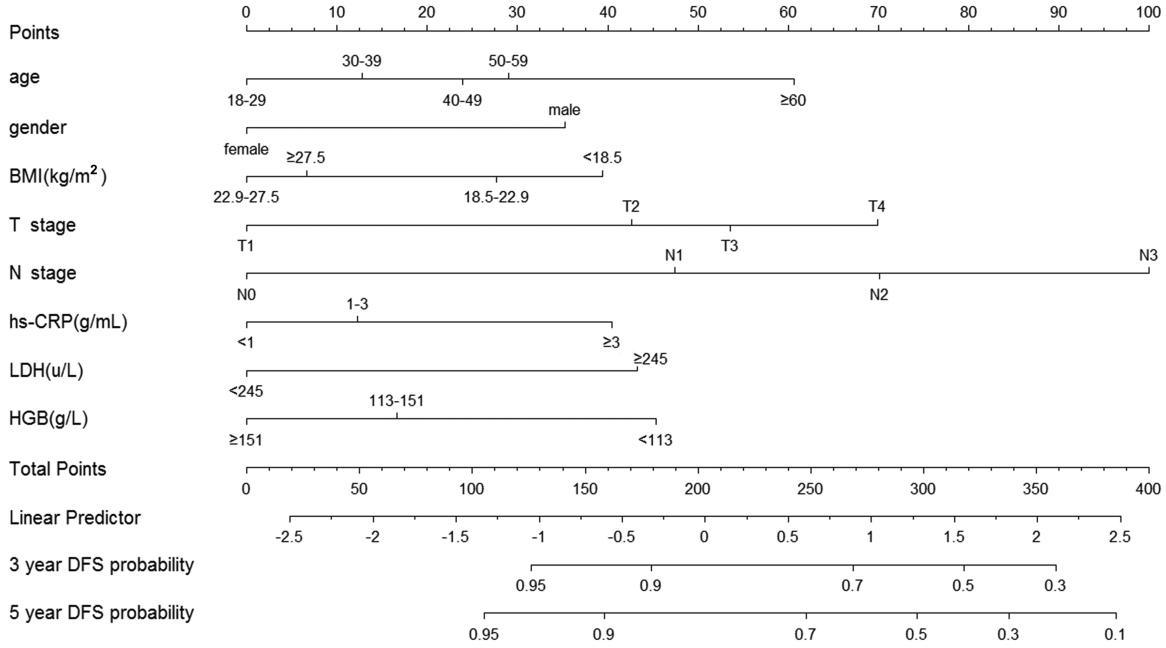

Figure 1. Nomogram A, including age, sex, body mass index, T stage, N stage, pretreatment hs-CRP, serum lactate dehydrogenase levels, and hemoglobin levels, for three- and five-year disease-free survival (DFS) in patients with nasopharyngeal carcinoma. The nomogram allows the user to obtain the probability of three- and five-year DFS corresponding to a patient's combination of covariates. As an example, locate the patient's T stage and draw a line straight upward to the "Points" axis to determine the score associated with that T stage. Repeat the process for each variable, and sum the scores achieved for each covariate, and locate this sum on the "Total Points" axis. Draw a line straight down to determine the likelihood of three- or five-year DFS. BMI = body mass index; DFS = disease-free survival; HGB = hemoglobin; hs-CRP = high-sensitivity C-reactive protein; LDH = serum lactate dehydrogenase levels.

was computed with the rms (37) package in R version 3.0.2 (http://www.r-project.org/). All statistical tests were two-sided, and *P* values of less than .05 were considered to be statistically significant.

Results

Patient Characteristics and Follow-up

The characteristics of the 4630 consecutive NPC patients in the primary cohort and 1819 patients in the validation cohort are listed in Table 1. After median follow-up times of 55.9 months (range = 1.3 to 90.8 months) and 33.5 months (range = 2.0 to 46.7 months) for the primary and validation cohorts, respectively, 338 and 92 patients developed local and/or regional recurrence, 602 and 208 developed distant metastasis, and 42 and 15 patients died without developing any relapse, respectively.

Tumor Relapse and Factors Associated With DFS in the Primary Cohort

The post-treatment one-, three-, and five-year recurrence rates were 6.2%, 16.0%, and 20.4%, respectively. Univariate analysis indicated that age, sex, ECOG performance, T stage, N stage, treatment modalities, radiotherapy technique, plasma EBV DNA, VCA-IgA, EA-IgA, LDH, hs-CRP, and HGB levels, BMI, and smoking status was associated with treatment failure of NPC (Supplementary Table 1, available online). Multivariable analyses continued to demonstrate that age, sex, BMI, T stage, N stage and plasma EBV DNA, pretreatment hs-CRP, LDH, and hemoglobin levels were independent risk factors for tumor recurrence (Table 2).

Nomogram Development With or Without EBV DNA

As EBV DNA measurement is not routinely available in the majority of centers, we first built nomogram A to predict three- and five-year DFS using the variables of age, sex, BMI, T stage, N stage and pretreatment hs-CRP, LDH, and hemoglobin levels without plasma EBV DNA (Figure 1). In Figure 2, A and B, the y-axes are observed survival estimated by the Kaplan-Meier method, the x-axes are predicted survival calculated by the nomogram, and the solid lines represent the ideal reference line for which predicted survival corresponds with actual survival. The calibration plot for the probability of DFS three years or five years after treatment showed optimal agreement between the prediction by nomogram A and actual observation for nomogram A. As the plasma EBV DNA was considered to be the most attractive potential biomarker to complement TNM stage, we continued to develop a new nomogram B with plasma EBV DNA and used the above-mentioned eight risk factors (Figure 3). The calibration plot also showed optimal agreement between the prediction by nomogram B and actual observation for nomogram B (Figure 4, A and B)

Comparison of Predictive Accuracy Between Nomogram A, Nomogram B, EBV DNA, and Conventional Staging Systems

As shown in Table 2 and Supplementary Table 2 (available online), the C-index and hazard ratios of T stage, N stage, and plasma EBV DNA for recurrence were higher than the hazard ratios of the other factors. As shown in Figure 5, the AJCC TNM staging systems showed good prognostic stratification for patients in the stage I, stage II, stage III, stage IVa, and stage IVb groups, and EBV DNA showed good prognostic stratification with EBV DNA levels differing by 10-fold between the primary and validation cohorts. The predictive power for recurrence of NPC between nomogram A, nomogram B, plasma EBV DNA level, and conventional stage systems was compared. In the primary

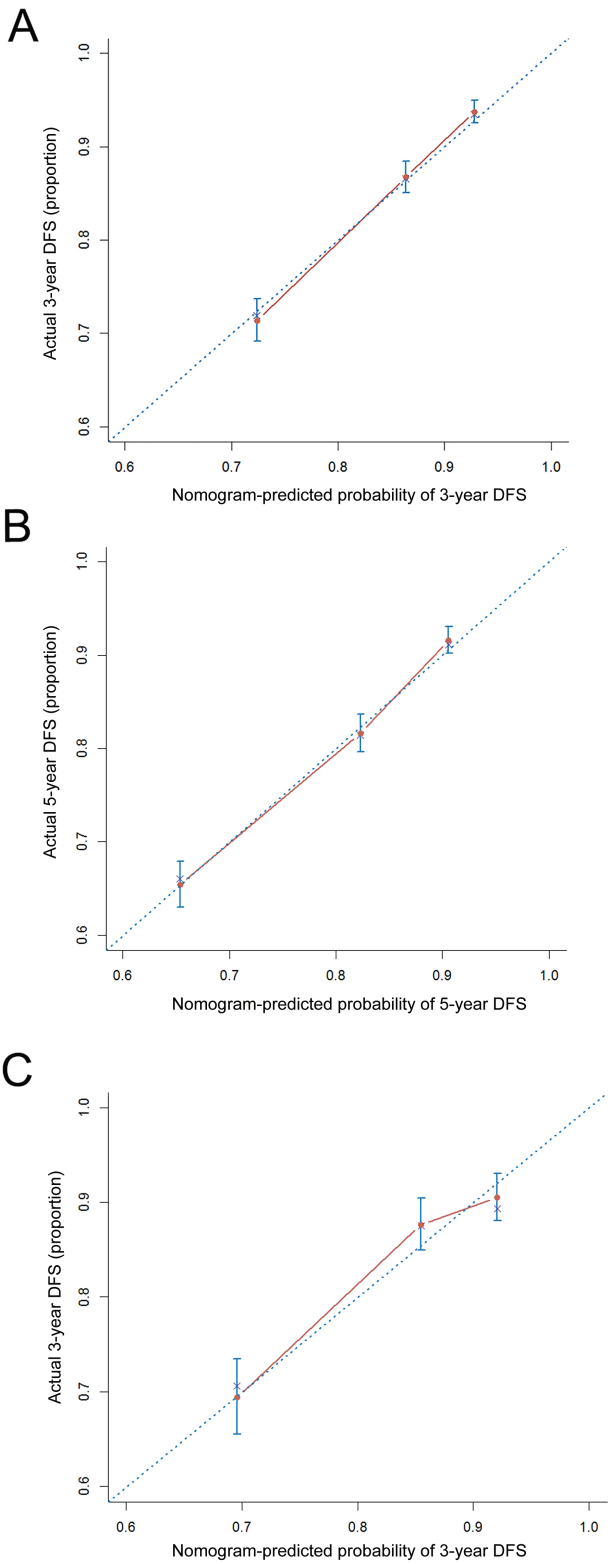

Figure 2. The calibration curve of nomogram A for predicting disease-free survival (DFS) at **(A)** three years and **(B)** five years in the primary cohort and at **(C)** three years in the validation cohort. Actual DFS is plotted on the y-axis; nomogram-predicted probability of DFS is plotted on the x-axis. DFS = disease-free survival.

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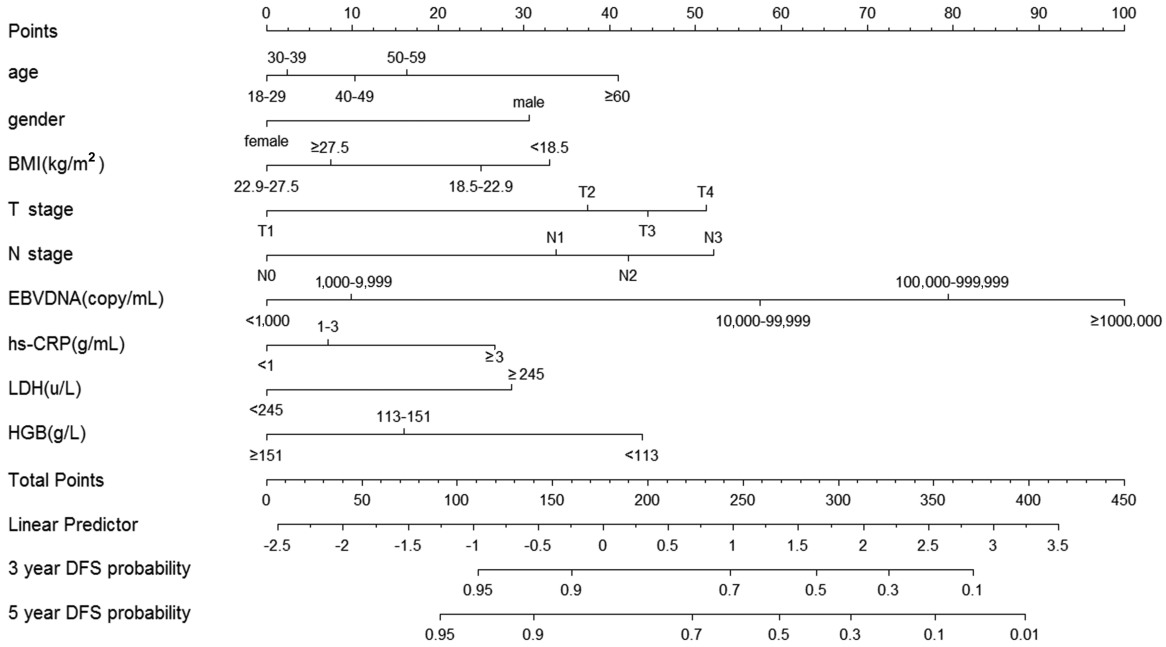

Figure 3. Nomogram B, including the risk factors of nomogram A and plasma EBV DNA, for three- and five-year disease-free survival (DFS) in patients with nasopharyngeal carcinoma. The nomogram allows the user to obtain the probability of three- and five-year DFS corresponding to a patient's combination of covariates. As an example, locate the patient's T stage and draw a line straight upward to the "Points" axis to determine the score associated with that T stage. Repeat the process for each variable, and sum the scores achieved for each covariate, and locate this sum on the "Total Points" axis. Draw a line straight down to determine the likelihood of three- or five-year DFS. BMI = body mass index; DFS = disease-free survival; EBV DNA = Epstein-Barr virus DNA; HGB = hemoglobin; hs-CRP = high-sensitivity C-reactive protein; LDH = serum lactate dehydrogenase levels.

cohort, the C-index for DFS prediction was 0.609 (95% CI = 0.592 to 0.625) by the current staging system, statistically significantly lower than the C-index by nomogram A, with a value of 0.690 (95% CI = 0.674 to 0.701, $P < .001$). The C-index of nomogram B was 0.728 (95% CI = 0.712 to 0.744), which was higher than the C-indices of nomogram A, EBV DNA alone and the current staging system, with values of 0.690 (95% CI = 0.674 to 0.701, $P < .001$), 0.680 (95% CI = 0.663 to 0.697, $P < .001$), and 0.609 (95% CI = 0.592 to 0.625, $P < .001$), respectively. Intriguingly, the C-index for DFS prediction using the EBV DNA level alone was also superior to the C-index using the current staging system ($P < .001$) (Table 3).

The results indicated that nomograms with or without EBV DNA displayed better accuracy in predicting recurrence compared with the current staging system.

Comparison of Predictive Accuracy for DFS Between Nomogram A, Nomogram B, EBV DNA, and Conventional Staging Systems in Patients Treated With IMRT

IMRT has gradually replaced 2D-CRT as the primary means of radiotherapy in clinical practice, having superior loco-regional control and improved long-term survival for patients with NPC (38). Therefore, we continued to test whether the prognostic discrimination of a nomogram could apply to patients treated with IMRT. The C-index of nomogram B was 0.730 (95% CI = 0.701 to 0.760, $P < .01$), which was higher than the C-indices of nomogram A, EBV DNA level alone, and the current staging system, with values of 0.681 (95% CI = 0.650 to 0.712), 0.685 (95% CI = 0.654 to 0.715), and 0.611 (95% CI = 0.582 to 0.641), respectively (Table 3). The C-indices for DFS by nomogram A were statistically significantly higher than the C-index by the current staging system ($P < .001$). Statistically significant differences regarding C-indices between EBV DNA level and the current staging system were also observed for the subgroup populations. With the addition of plasma EBV DNA into nomogram B, the added value of EBV DNA on nomogram A was 0.038 for the entire population ($P < .001$) and 0.049 for the subgroup patients treated with IMRT ($P = .002$).

Validation of the Predictive Accuracy of Nomograms for DFS

In the validation cohort, the post-treatment one- and three-year recurrence rates were 6.5% and 17.5%, respectively. The C-index of nomogram B (0.709, 95% CI = 0.680 to 0.739) for predicting DFS in the validation cohort was higher than the C-indices of EBV DNA level alone (0.668, 95% CI = 0.639 to 0.697, $P = .009$), and the current staging system (0.619, 95% CI = 0.589 to 0.649, $P < .001$). A calibration curve showed good agreement between prediction and observation in the probability of three-year DFS in nomogram A and nomogram B (Figures 2C and 4C). With the addition of plasma EBV DNA into nomogram B, the C-index of 0.687 for nomogram A was up to the value of 0.709 for nomogram B for all patients in the validation cohort. The added value of EBV DNA on nomogram A was 0.022 for the entire population ($P = .09$) and 0.023 for the subgroup patients treated with IMRT ($P = .14$), although with a trend toward a statistically significant difference both for the entire population and subgroup patients treated with IMRT. Similar results were obtained for the subgroup of patients treated with IMRT in the validation cohort (Table 3; Supplementary Figures 2 and 3, available online).

Discussion

To the best of our knowledge, this is the first study to describe the development and validation of a prognostic nomogram

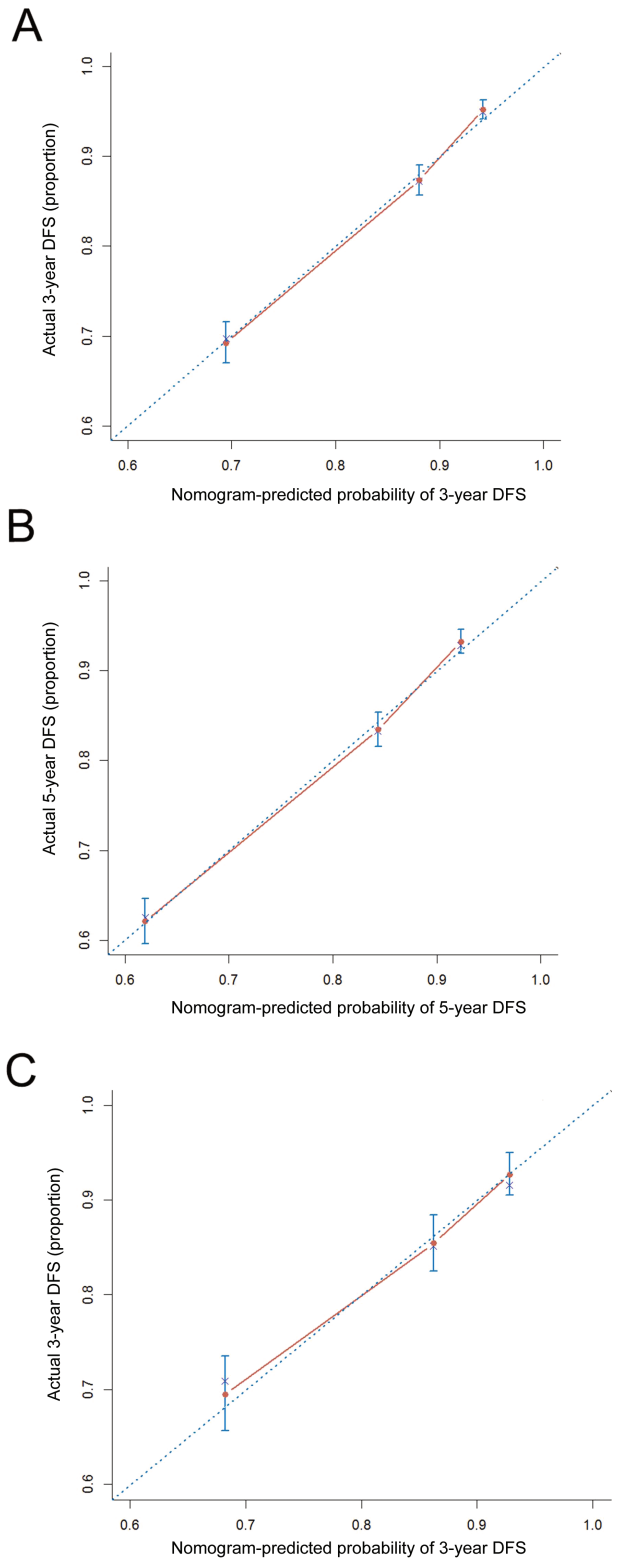

Figure 4. The calibration curve of nomogram B for predicting disease-free survival (DFS) at **(A)** three years and **(B)** five years in the primary cohort, and at **(C)** three years in the validation cohort. Actual DFS is plotted on the y-axis; nomogram-predicted probability of DFS is plotted on the x-axis. DFS = disease-free survival.

combining plasma EBV DNA levels and other risk factors to predict recurrence for NPC patients. Nomogram A, which took into account age, sex, BMI, T stage, N stage, pretreatment hs-CRP, LDH, and hemoglobin levels, and nomogram B, which included plasma EBV DNA and the other eight risk factors in question, had better predictive accuracy than the current conventional staging system. There are several controversies regarding the conventional staging system: The current staging system is purely based on the anatomical extent of the disease, staging systems do not completely reflect the biological heterogeneity of NPC patients, and other risk factors are not taken into account in current staging systems. These issues could affect the predictive accuracy of conventional systems for NPC patients. Intriguingly, according to our findings, regardless of the entire population or subgroup patients treated by IMRT, the C-indices of both nomogram A and nomogram B were higher than the current staging system in the primary and validation cohorts, and the method addressed the concerns mentioned above.

In the 21st century, the level of plasma EBV DNA has been demonstrated to be a useful biomarker for the clinical management of NPC, and it is considered to be the most attractive potential biomarker for NPC patients (12). Chan et al. (18) have shown that with a cutoff point of 4000 copies/mL, patients with early-stage disease were segregated by EBV DNA levels into a poor-risk subgroup with survival similar to that of stage III disease and a good-risk subgroup with survival similar to stage I disease. Lin et al. (19) also demonstrated that EBV DNA levels that were either higher than 1500 copies/mL prior to treatment or detectable after treatment were both predictive of disease recurrence and overall survival for NPC patents. However, there is still no effective way to incorporate EBV DNA content into the TNM classification. Interestingly, this study incorporated EBV DNA levels, clinicopathologic factors, and other biomarkers into the TNM staging system and found that the predictive accuracy of nomogram B was superior to EBV DNA levels alone, which makes the EBV DNA content more applicable in clinical practice.

As EBV DNA measurement is not routinely available in the majority of centers and the methodology is not globally standardized, interestingly, according to our findings; nomogram A developed without EBV DNA also showed greater predictive accuracy compared with the current staging system. This outcome indicated that nomogram A was still useful for the centers that do not have EBV DNA measurement available.

With the current advances in technology, the NCCN guideline has recommended that IMRT be the preferred radiotherapy technology for NPC patients in clinical practice. Therefore, it is important to analyze the performance of nomograms in the patients treated with IMRT separately. Irrespective of primary and validation cohorts, the C-indices of nomogram B and nomogram A in predicting recurrence for patients treated with IMRT were statistically significantly higher than the value of conventional classification. This result indicated that nomograms still have significant clinical value for NPC patients in the IMRT era for the center with or without EBV DNA measurement available. With the addition of plasma EBV DNA into nomogram B, the added value of EBV DNA on nomogram A was 0.038 and 0.022 for the entire population, and 0.049 and 0.023 for the subgroup patients treated with IMRT for the primary and validation cohort, respectively. There was a trend toward a statistically significant difference in the validation cohort, probably because these patients have a short follow-up time.

This study had several limitations. First, patient comorbidities were not included in the nomograms. Severe comorbidities affect survival or the selection of initial treatment, and these factors will affect disease recurrence to some extent. Although several studies (39,40) have demonstrated that comorbidities were correlated with the survival of NPC

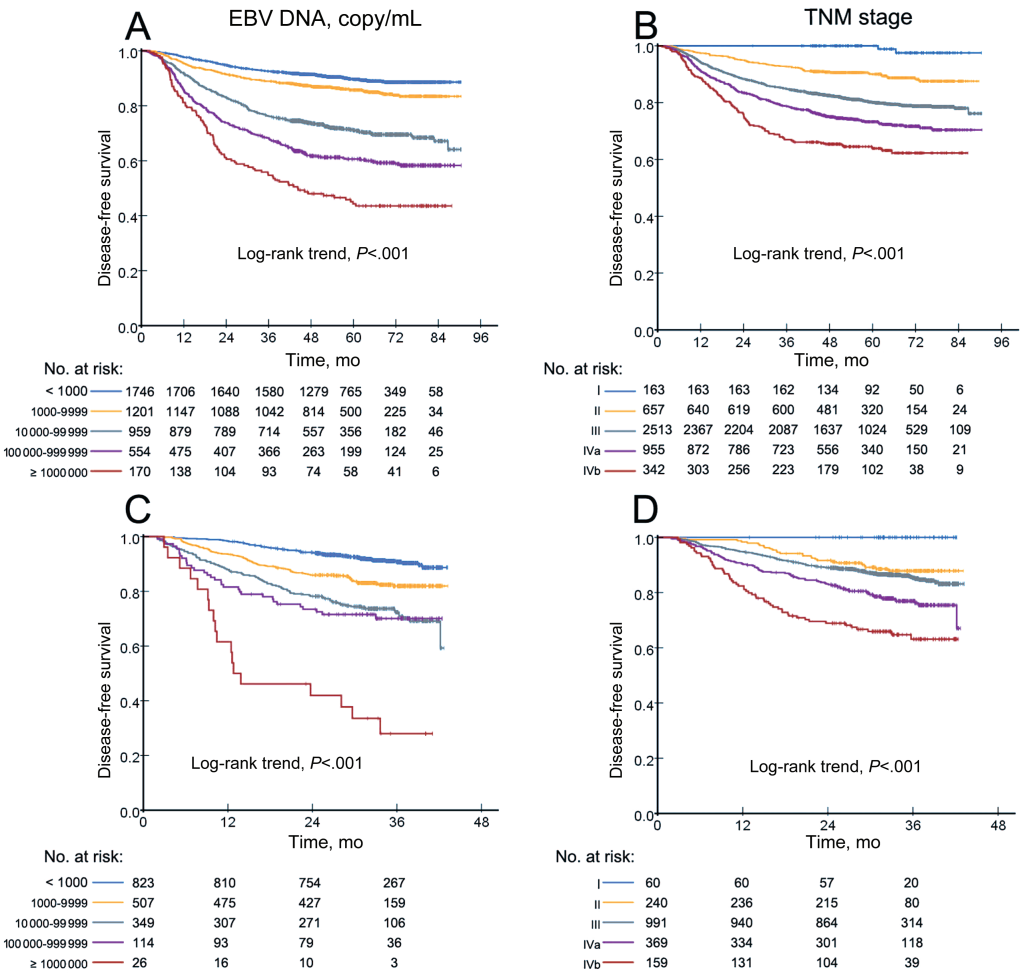

Figure 5. Kaplan-Meier survival curves of disease-free survival in the primary cohort: **A)** Epstein-Barr virus DNA. **B)** American Joint Committee on Cancer (AJCC) TNM staging system, validation cohort. **C)** Epstein-Barr Virus DNA. **D)** AJCC TNM staging system.

Table 3. The C-index of Nomogram A, Nomogram B, EBV DNA, and TNM stage for prediction of DFS in the primary cohort and validation cohort*

Factor	Primary cohort		Prospective validation cohort	
	C-index (95% CI)	P†	C-index (95% CI)	P†
All patients				
Nomogram B	0.728 (0.712 to 0.744)		0.709 (0.680 to 0.739)	
Nomogram A	0.69 (0.674 to 0.701)		0.687 (0.656 to 0.717)	
EBV DNA	0.68 (0.663 to 0.697)		0.668 (0.639 to 0.697)	
TNM stage	0.609 (0.592 to 0.625)		0.619 (0.589 to 0.649)	
Nomogram B vs Nomogram A		<.001		.09
Nomogram B vs EBV DNA		<.001		.009
Nomogram A vs TNM stage		<.001		<.001
EBV DNA vs TNM stage		<.001		.002
Subgroup patients treated with IMRT				
Nomogram B	0.73 (0.701 to 0.760)		0.715 (0.681 to 0.748)	
NomogramA	0.681 (0.650 to 0.712)		0.692 (0.656 to 0.727)	
EBV DNA	0.685 (0.654 to 0.715)		0.673 (0.638 to 0.708)	
TNM stage	0.611 (0.582 to 0.641)		0.609 (0.573 to 0.644)	
Nomogram B vs Nomogram A		.002		.14
Nomogram B vs EBV DNA		.008		.022
Nomogram A vs TNM stage		<.001		<.001
EBV DNA vs TNM stage		<.001		<.001

* Nomogram A: including eight risk factors (age, sex, BMI, T stage, N stage, pretreatment hs-CRP, LDH, and hemoglobin level); Nomogram B: including the risk factor of nomogram A plus plasma EBV DNA. C-index = concordance index; CI = confidence interval; EBV DNA = Epstein-Barr virus DNA; NPC = nasopharyngeal carcinoma.
† *P* values are calculated based on normal approximation using function rcorrp.cens in Hmisc package.

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patients, it is difficult to create categorized variables and to quantify risk because of the diversity of comorbidities. Therefore, incorporating comorbidities into the nomogram should be validated in future studies. Second, the nomogram was established based on data obtained exclusively from one center in an endemic area and the measurement plasma measurement EBV DNA still needs to be globally standardized. The third limitation was that the follow-up time was shorter in the validation cohort, and close monitoring and five-year follow-up data are still required for these patients. The fourth limitation was the definition of DFS as the date of the first diagnosis of NPC to the date of the first relapse at any site, death from any cause, or the date of the last follow-up visit. To ensure the validity of the analysis, the time from first diagnosis to the definitive treatment must be short and without much variability compared with the length of follow up. The definition of DFS in future validation studies should be more accurately defined as from the date the patient was rendered to have disease-free status. Finally, whether this nomogram can be applied to young patients (age < 18 years old) or patients with biopsy-proven World Health Organization type I disease remains to be determined.

In conclusion, we developed and validated nomograms with or without plasma EBV DNA predicting three- and five-year DFS for NPC patients in the endemic area. The proposed nomogram in this study provided statistically significantly better discrimination than the current TNM classification, and it offers a useful tool for predicting recurrence, providing patient counseling and timing surveillance, and clinical assessments. To generalize the use of this nomogram, validation with data from low-risk areas and other institutions is required.

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Notes

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We declare no conflicts of interest.

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