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ORIGINAL RESEARCH

Significance of Pre-Treatment CALLY Score Combined with EBV-DNA Levels for Prognostication in Non-Metastatic Nasopharyngeal Cancer Patients: A Clinical Perspective

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Background: The C-reactive protein-albumin-lymphocyte (CALLY) score is a novel indicator associated with inflammation, immunity, and nutrition, utilized for cancer prognostic stratification. This study aimed to evaluate the integrated prognostic significance of the pre-treatment CALLY score and Epstein-Barr virus (EBV) DNA levels in nasopharyngeal carcinoma (NPC) patients and to develop prognostic models.

Patients and Methods: A total of 1707 NPC patients from September 2015 to December 2017 were retrospectively enrolled. The cut-off point for the CALLY score, determined by maximum selected rank statistics, integrates with the published cut-off point for pre-EBV DNA to develop a comprehensive index. Subsequently, patients were randomly allocated in a 1:1 ratio into training and validation cohorts. Survival analysis was conducted using the Kaplan-Meier method with Log rank tests, and the Cox proportional hazards model was applied to identify independent prognostic factors for constructing predictive nomograms. The predictive ability of the nomograms were assessed through the concordance index (C-index), calibration curves, and decision curve analysis.

Results: By integrating CALLY scores and EBV-DNA levels, patients were categorized into three risk clusters. Kaplan-Meier curves reveal significant differences in overall survival (OS), distant metastasis-free survival (DMFS), and locoregional relapse-free survival (LRRFS) outcomes among different risk groups (all P values < 0.05). Multivariate analysis revealed that CALLY-EBV DNA index serves as an independent prognostic factor for the OS, DMFS, and LRRFS. The prognostic nomograms based on the CALLY-EBV DNA index provided accurate predictions for 1-year, 3-year, and 5-year OS, DMFS, and LRRFS. Additionally, compared to the traditional TNM staging system, the nomograms exhibited enhanced discriminatory power, calibration capability, and clinical applicability. All results were in agreement with the validation cohort.

Conclusion: The CALLY-EBV DNA index is an independent prognostic biomarker. The nomogram prediction models, constructed based on the CALLY-EBV DNA index, demonstrates superior predictive performance compared to the traditional TNM staging.

Keywords: CALLY score, EBV DNA, nasopharyngeal carcinoma, biomarker, prognostic model

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Introduction

Nasopharyngeal carcinoma (NPC) is a category of epithelial cell tumors with distinct etiological mechanisms and a broad spectrum of clinical manifestations, prevalent in the Southern regions of China and Southeast Asia.[1] Radiation therapy and concurrent chemoradiotherapy with/without neoadjuvant/adjuvant chemotherapy are the principal treatment modalities for non-metastatic NPC.[1,2] Nevertheless, it's important to note that approximately 20–30% of locally advanced NPC patients may experience local relapse.[3] Identifying high-risk patients before treatment and administering intensified therapies can potentially offer more personalized treatment options for specific patient populations. While the Tumor, Node, Metastasis (TNM) staging system is widely accepted as the best clinical predictor of the outcome of NPC patients, the heterogeneity among patients can lead to differences in prognosis even within the same stage.[4] This heterogeneity restricts its ability to discriminate patients with different prognoses and to guide therapeutic decisions. Relying solely on the anatomical staging system for prognosis prediction and personalized treatment is insufficient. Therefore, the exploration of new biomarkers to enhance the current traditional staging system remains an urgent priority.

The cancer-related inflammation in the tumor microenvironment, coupled with the state of malnutrition in the body, influences the occurrence, progression, distant metastasis, and treatment resistance of tumors, ultimately leading to a decreased quality of life in cancer patients.[5,6] And moreover, malignant solid tumors trigger an endogenous inflammatory response, establishing a tumor-promoting microenvironment that influences immune surveillance, fosters cancer progression, and impacts treatment responses.[7] Recently, several scoring systems derived from blood or biochemical tests, reflecting the body's inflammatory, nutritional, and immune status, are closely related to prognosis in NPC patients, including platelet-to-lymphocyte ratio (PLR),[8] neutrophil-to-lymphocyte ratio (NLR),[9] controlling nutritional status score (CONUT),[10] Glasgow Prognostic Score (GPS),[11] and prognostic nutritional index (PNI),[12] among others. These predictive indicators are routine assessments for all cancer patients, obtainable through pre-treatment blood tests, featuring advantages such as simplicity, wide availability, low cost-effectiveness, and ease of calculation. However, these indicators serve merely as a partial reflection of the patient's holistic health status, as the efficacy of ultimate treatment outcomes is intricately intertwined with a multitude of factors including the patient's inflammatory status, nutritional condition, immune function, tumor-related factors, etc. This underscores the need for a comprehensive and multifaceted approach in assessing patients'overall health and prognosis.

The C-reactive protein-albumin-lymphocyte (CALLY) score, a novel prognostic biomarker, was first introduced in 2021 by Müller, L et al,[13] for predicting survival outcomes in patients with hepatocellular carcinoma. The CALLY score encompasses C-reactive protein, serum albumin levels, and total lymphocyte count content, reflecting the body's inflammatory levels within the tumor microenvironment, nutritional status, and immune system condition, respectively.[13,14] Thereafter, the prognostic role of the CALLY score in various cancer types was explored, including non-small cell lung cancer,[15] gastric cancer,[16] colorectal cancer,[17] oral cavity cancer,[18] ovarian cancer,[19] and others. However, there is no existing research exploring the prognostic value of the CALLY score for NPC patients.

Currently, the TNM staging system remains a crucial determinant for risk stratification in treatment decisions and prognosis prediction for NPC patients.[4] Apart form TNM stage, the pre-EBV DNA, reflecting tumor burden, has emerged as a significant factor in the clinical diagnosis, risk classification, dynamic monitoring, and prognosis of NPC patients, serving as an important a supplement for the TNM staging system.[20,21] Patients with higher pre-EBV DNA copy numbers appear to have a greater risk of distant metastasis, necessitating more aggressive treatment. Nevertheless, these considerations often overlook the impact of hematological parameters on the treatment of NPC patients, potentially affecting treatment outcomes. In this study, we elucidated the complementary role between the CALLY score and pretreatment plasma EBV DNA levels. The CALLY-EBV DNA index, integrating with inflammatory-immunonutritive score and tumor-related factors, may be used to identify high-risk patients who could benefit from more aggressive treatment approaches or closer monitoring. Additionally, we have developed and validated novel predictive models based on the CALLY-EBV DNA index, and other clinical parameters to optimize NPC patient management, treatment decisions, and the development of future clinical guidelines.

Materials and Methods

Patients and Study Design

We retrospectively included and analyzed data from 1707 nasopharyngeal carcinoma (NPC) patients who were initially diagnosed at Sun Yat-sen University Cancer Center between September 2015 and December 2017. Inclusion criteria: (1) Newly diagnosed and pathologically confirmed NPC patients; (2) Age≥18 years; (3) Patients without distant metastasis; (4) ECOG scores ranging from 0 to 2. Exclusion criteria: (1) Patients with concurrent other malignancies or severe illnesses (severe acute or chronic diseases); (2) Patients with incomplete clinical and laboratory data; (3) Patients with missing follow-up data. The 8th edition of the AJCC TNM staging system was used for all patients. Participants were randomized 1:1 into training and validation cohorts for models development and validation. The study protocol was approved by the Ethics Committee of the Sun Yat-sen University Cancer Center and conducted in accordance with the ethical standards of the institution and the Helsinki Declaration. Due to the retrospective nature of the study and the anonymization of patient data, informed consent was waived.

Data Collection and Classification

We collected laboratory data from the week prior to treatment as well as clinicopathological data from the patient's medical records. Clinical information obtained from patient records included age, gender, smoking history, clinical stage, and treatment strategy. Laboratory data encompassed pre-treatment plasma EBV DNA levels, lymphocyte count, albumin (ALB) levels, and C-reactive protein (CRP) levels. Plasma EBV DNA levels (copies/mL) were measured by real-time quantitative polymerase chain reaction (PCR). The CALLY index is calculated using the following formula, which is consistent with the methodologies employed in previous studies:[13,17]

CALLY index = (albumin level in g/dL) × (lymphocyte count in /μL) / (CRP level in mg/dL) × 10^4.

Similar to the method used for selecting cut-off values of other research parameters,[22,23] the optimal CALLY score threshold was determined using maximally selected rank statistics and set at 5.58 (Figure S1). Based on previous research,[24,25] the threshold for pre-treatment plasma EBV DNA levels was set at 4000 copies/mL. CALLY scores > 5.58 and EBV DNA levels ≤ 4000 copies/mL were considered as low risk factors, while CALLY scores ≤ 5.58 and EBV DNA levels > 4000 copies/mL were considered as high risk factors. Consequently, CALLY scores combined with pre-treatment EBV DNA levels were divided into four groups: the Group 1 (G1), CALLY score > 5.58 and EBV DNA levels ≤ 4000 copies/mL; the Group 2 (G2), CALLY score ≤ 5.58 and EBV DNA ≤ 4000 copies/mL; the Group 3 (G3), CALLY score > 5.58 and EBV DNA > 4000 copies/mL; the Group 4 (G4), CALLY score ≤ 5.8 and EBV DNA > 4000 copies/mL.

Treatment Strategies

All patients were treated using IMRT (intensity-modulated radiation therapy) techniques with fractions of 1.8–2.27 Gy, administered 5 times a week, over a total period of 6–7 weeks. The delineation of target volumes depends on our institution's treatment protocol, consistent with Reports 50 and 62 from the International Commission on Radiation Units and Measurements. The cumulative radiation dose to the gross tumor volume in the nasopharynx (GTVnx) ranged from 60–75 Gy, while the involved neck region (GTVnd) received a cumulative radiation dose of 50–70 Gy. All potential areas of local target volumes and cervical lymph nodes were treated with doses of 50–64 Gy or higher. During the study, all patients were treated according to our institution's treatment guidelines (based on the 8th edition of the AJCC/UICC Cancer Staging Manual).[2] Specifically, for stage I disease, IMRT was recommended as the treatment modality. For stage II to IVA diseases, platinum-based concurrent chemoradiotherapy (CCRT) was advised, with the option to include or omit neoadjuvant/adjuvant chemotherapy. The predominant therapeutic strategy was concurrent chemoradiotherapy (CCRT) with cisplatin, wherein cisplatin was administered at a dosage of 80–100 mg/m² every three weeks, totaling 2–3 cycles.

Endpoints and Follow-Up

Following the completion of all interventions, patients were slated for post-treatment follow-up appointments, with intervals set at every 3 months for the initial 2 years, every 6 months spanning the 3rd to 5th years, and annually

thereafter. Every follow-up assessment consisted of a thorough physical examination, nasopharyngoscopy, head and neck MRI, chest X-rays or computed tomography scans, abdominal ultrasound or computed tomography scans, bone scans, or 18F-FDG PET/CT, along with measurements of plasma EBV DNA levels. The primary endpoint of this study was the overall survival (OS), defined as the time from the initial pathological diagnosis of nasopharyngeal cancer to death from any cause or the last follow-up. The secondary endpoints were distant metastasis-free survival (DMFS), defined as the interval between date of pathological diagnosis and data of first distant metastasis event or the last follow-up, and locoregional relapse-free survival (LRRFS), defined as the interval between date of pathological diagnosis and date of recurrence in the nasopharynx or cervical lymph nodes, or the last follow-up.

Sample Size Calculation and Statistical Analysis

For the sample size calculation, we utilized all available data from the database to ensure maximum statistical power and the generalizability of our findings. Furthermore, to ensure an adequate sample size and maintain statistical robustness, we employed the Kendall sample size calculation method, which guarantees that the number of enrolled non-missing cases is at least ten times the number of independent variables.

The comparison of categorical variables was conducted using either the chi-square test or Fisher's exact test, while continuous variables were transformed into binary variables using the optimal cutoff values determined by selected rank statistics. ROC curve was used to compare the predictive performance of different indicators. Survival analysis was conducted using Kaplan-Meier method, and the comparison was assessed through the Log rank test. Cox proportional hazard models were used to assess predictive value of each candidate covariate, and the corresponding hazard ratios (HR) were calculated. In the univariate Cox analysis, covariates with a p-value of less than 0.05 were further incorporated into the multivariate Cox analysis. Prognostic nomograms, incorporating all significant prognostic covariates identified in the multivariate analysis, were generated using "rsm" R package. The predictive power and clinical benefit of the prognostic nomograms was determined by concordance index (C-index), calibration plots, and decision curve analysis (DCA). Bootstrapping with 1000 resamples and 10-fold cross-validation was employed to minimize overfitting. The data analysis was performed using SPSS statistical software version 22.0 (SPSS, Chicago, Illinois, USA) or R software (http://www.R-project.org; version 4.2.1). Statistical significance was determined with a two-tailed P-value below 0.05.

Results

Patient Characteristics

The median follow-up period was 67.6 months (range, 3.0-84.6 months). Patients were randomized 1:1 to the training cohort (n=855, 50.1%) and the validation cohort (n=852, 49.9%). Table 1 displays the baseline clinical and pathological characteristics of the training and validation cohorts, which exhibited comparability between the two groups. The median age in the overall population was 45 years (range, 37–53 years), with a predominance of males (73.2%).

Prognostic Role of the CALLY Score and EBV DNA Levels and Establishment of CALLY-EBV DNA Index

Based on the cutoff values of CALLY score and EBV DNA levels, Kaplan-Meier curves demonstrate a significant correlation between CALLY score and EBV DNA levels with patient OS, DMFS, and LRRFS. Specifically, patients with the high CALLY scores or low EBV DNA levels exhibit higher OS, DMFS, and LRRFS compared to those with the low CALLY scores or high EBV DNA levels ($P < 0.05$, Figure 1A and B).

Furthermore, according to the EBV-DNA levels (4000 copies/mL) and CALLY scores threshold (5.58), patients were classified into four groups (G1, n=642; G2, n=483; G3, n=263; and G4, n=319). We further explored the survival differences among different groups. As illustrated in Figure 1C, we observed similar overall survival outcomes between G2 and G3 ($P = 0.618$), while there were significant survival differences between G1, G4 groups, and the other groups (all $P < 0.05$). Thus, we merged G2 and G3 into the middle-risk cluster and designated G1 and G4 as low-risk and high-risk clusters, respectively (Figure 1D). The high-risk cluster had a higher proportion of stage IVa patients compared to the middle-risk and low-risk groups, accounting for 58.6%, 40.2%, and 21.3%, respectively ($P < 0.001$) (Table S1).

Table 1 Baseline Clinicopathological Characteristics Between the Training and Validation Cohorts

Characteristics	Training Cohort N=855(50.1%)	Validation Cohort N=852(49.9%)	Total N=1707(100%)	*P*-value
Age				0.298
<45 years	413(48.3)	433(50.8)	846(49.6)	
≥45 years	442(51.7)	419(49.2)	861(50.4)	
Sex				0.143
Female	216(25.3)	242(28.4)	458(26.8)	
Male	639(74.7)	610(71.6)	1249(73.2)	
Smoking history				**0.042**
No	589(68.9)	625(73.4)	1214(71.1)	
Yes	266(31.1)	227(26.6)	493(28.9)	
T stage				0.401
T1	111(13.0)	121(14.2)	232(13.6)	
T2	110(12.9)	129(15.1)	239(14.0)	
T3	413(48.3)	399(46.8)	812(47.6)	
T4	221(25.8)	203(23.8)	424(24.8)	
N stage				0.260
N0	106(12.4)	107(12.6)	213(12.5)	
N1	407(47.6)	435(51.1)	842(49.3)	
N2	199(23.3)	195(22.9)	394(23.1)	
N3	143(16.7)	115(13.5)	258(15.1)	
Clinical stage [#]				0.399
I	30(3.5)	32(3.8)	62(3.6)	
II	112(13.1)	125(14.7)	237(13.9)	
III	384(44.9)	400(46.9)	784(45.9)	
IVa	329(38.5)	295(34.6)	624(36.6)	
EBV DNA				**0.022**
≤ 4000 copies/mL	541(63.3)	584(68.5)	1125(65.9)	
>4000 copies/mL	314(36.7)	268(31.5)	582(34.1)	
LY(×10^9/L) [a]	1.90(1.50–2.30)	1.90(1.50–2.30)	1.90(1.50–2.30)	0.581
ALB [a]	45.0(43.0–46.8)	45.25(43.3–47.1)	45.1(43.1–47.0)	0.103
CRP [a]	1.44(0.63–3.44)	1.34(0.52–3.1)	1.40(0.58–3.28)	0.770
CALLY score				0.076
High	435(50.9)	470(55.2)	905(53.0)	
Low	420(49.1)	382(44.8)	802(47.0)	
Treatment strategy				0.996
IMRT alone	41(4.8)	42(4.9)	83(4.9)	
CRT	76(8.9)	78(9.2)	154(9.0)	
CCRT±AC	359(42.0)	355(41.7)	714(41.8)	
IC+CCRT±AC	379(44.3)	377(44.2)	756(44.3)	

Notes: [a]Continuous variables are presented as median [interquartile range]. [#]According to the eighth edition of UICC/AJCC staging system. Values in bold are significant ($P < 0.05$).

Abbreviations: EBV-DNA, Epstein-Barr virus DNA; LY, lymphocyte; ALB, albumin; CRP, C-reactive protein; CALLY, C-reactive protein-albumin-lymphocyte; IC, induction chemotherapy; IMRT, intensity modulated radiation therapy; CRT, chemoradiotherapy; CCRT, concurrent chemoradiotherapy; AC, adjuvant chemotherapy.

In addition, we evaluated whether the CALLY-EBV DNA index holds greater prognostic value than the CALLY score and EBV DNA levels alone for predicting the prognosis of NPC patients. The results derived from the ROC curves indicated that the CALLY-EBV DNA index exhibited superior predictive accuracy for OS in NPC patients, with an AUC of 0.628 (95% CI, 0.593–0.663). This was significantly higher than the AUC value of 0.585 for the CALLY score (95% CI, 0.551–0.619) with a *P*-value of 0.001, and also surpassed the AUC value of 0.588 for EBV-DNA levels (95% CI,

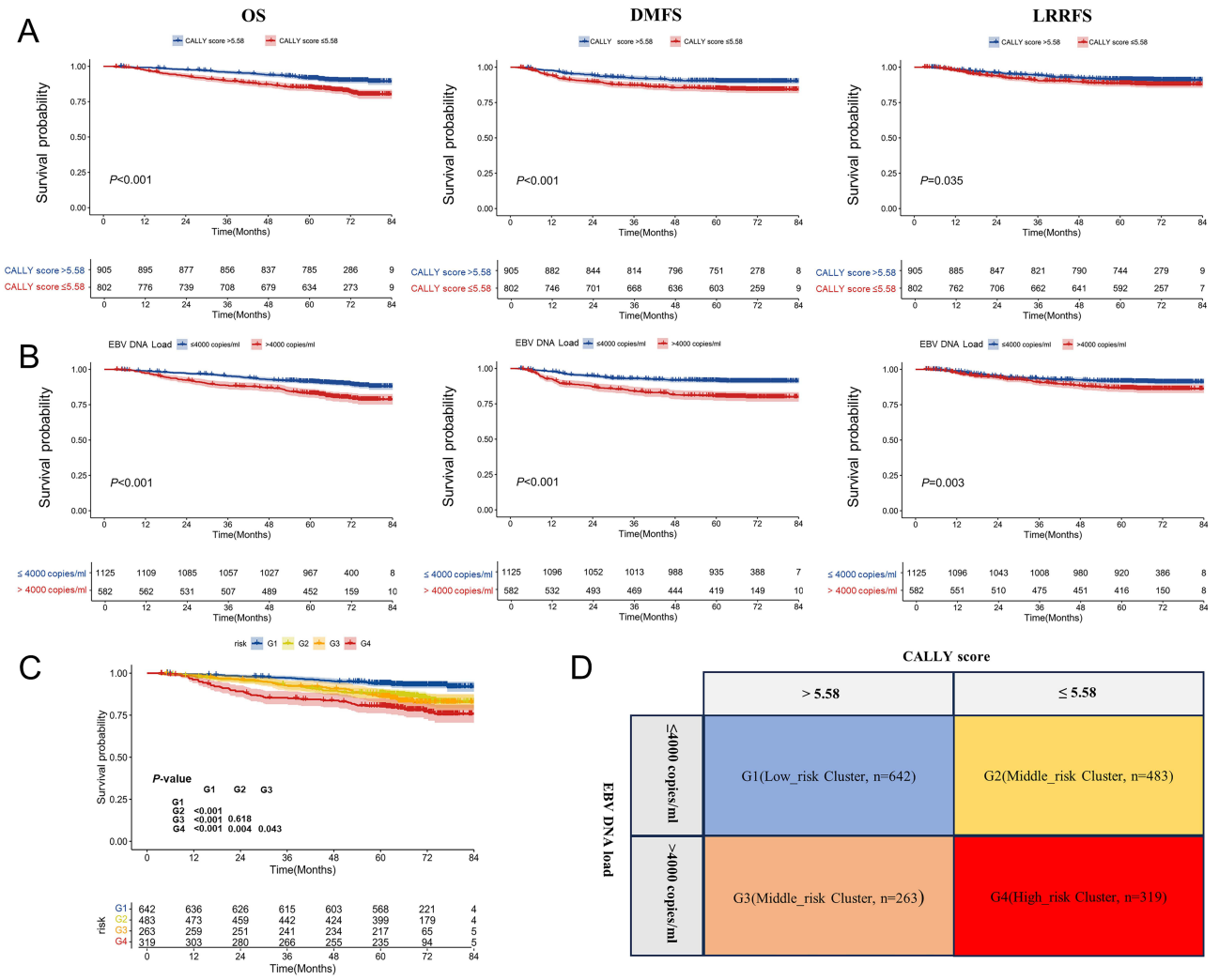

Figure 1 (**A**). Kaplan-Meier survival curves of OS, DMFS and LRRFS stratified by pre-treatment CALLY score. (**B**). Kaplan-Meier survival curves of OS, DMFS and LRRFS stratified by pre-treatment EBV DNA levels. (**C**). Kaplan-Meier survival curves of OS, DMFS and LRRFS for the four groups stratified by pre-treatment CALLY score and EBV DNA levels. (**D**). Diagrammatic grid for the three risk clusters. *P* values were calculated using the Log rank test.
Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; LRRFS, locoregional relapse-free survival; EBV-DNA, Epstein-Barr virus DNA; CALLY, C-reactive protein -albumin-lymphocyte.

0.553–0.623) with a *P*-value of 0.004 (Figure S2). These findings suggest that the CALLY-EBV DNA index may serve as a more robust biomarker for the prognostic stratification of patients with NPC.

Prognostic Value of the Different Risk Stratifications for OS, DMFS, and LRRFS

In the primary cohort, the 5-year OS rates for the low-risk, middle-risk, and high-risk groups were 93.7%, 87.1%, and 80.1%, respectively, with corresponding DMFS rates of 93.8%, 85.2%, and 80.2%, and LRRFS rates of 93.0%, 88.9%, and 86.0%. In the training cohort, the 5-year OS rates for the low-risk, middle-risk, and high-risk groups were 92.8%, 85.2%, and 77.9%, with DMFS rates of 94.9%, 85.4%, and 77.0%, and LRRFS rates of 95.2%, 90.2%, and 91.0%, respectively. In the validation cohort, the 5-year OS rates for the low-risk, middle-risk, and high-risk groups were 94.7%, 89.5%, and 82.7%, with DMFS rates of 85.3%, 84.9%, and 84.3%, and LRRFS rates of 92.9%, 90.7%, and 84.6%, respectively.

The Kaplan-Meier curves demonstrate significant differences in OS, DMFS, and LRRFS outcomes between different risk groups in both the primary and training cohorts ($P < 0.05$), with the exception of no discernible survival distinction between the middle- and high-risk groups for LRRFS ($P = 0.908$, Figure 2A and B). In the validation cohort, patients in

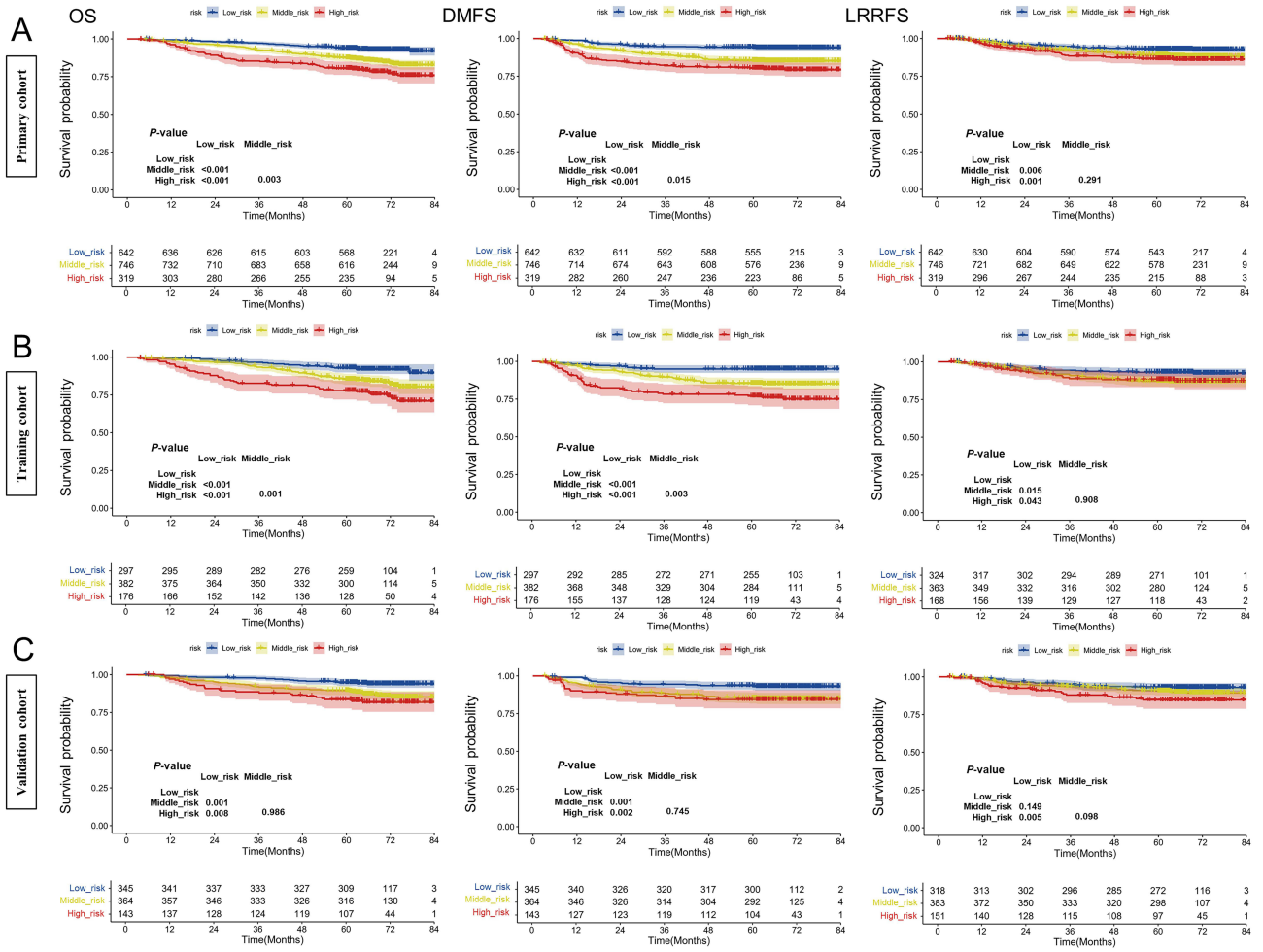

Figure 2 Kaplan–Meier survival curves of OS, DMFS and LRRFS for the three risk clusters in the primary cohort (**A**), training cohort (**B**), and validation cohort (**C**).
Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; LRRFS, locoregional relapse-free survival. *P* values were calculated using the Log rank test.

the low-risk group demonstrated significantly better OS, DMFS and LRRFS compared to the middle-risk and high-risk groups ($P < 0.05$), except for a non-significant survival difference between the low- and middle-risk groups in terms of LRRFS ($P = 0.149$, Figure 2C). Regrettably, in the validation cohort, no significant differences in OS, DMFS, and LRRFS were observed between the middle-risk and high-risk groups (OS, $P = 0.986$; DMFS, $P = 0.745$; LRRFS, $P = 0.098$; Figure 2C).

Univariate and Multivariate Cox Regression Analyses

Cox regression analyses were performed in both the training and validation cohorts. Variables with statistically significant differences ($P < 0.05$) in the univariate Cox model were incorporated into the multivariate Cox regression model. The results from the training cohort revealed independent associations of age, gender, T stage, N stage, and the CALLY-EBV DNA index with OS (Table 2). Additionally, N stage and the CALLY-EBV DNA index exhibited independently associated with DMFS (Table 3), while T stage, N stage, and the CALLY-EBV DNA index independently correlated with LRRFS (Table 4). Similarly, the multivariate Cox regression models in the validation cohort demonstrated that the CALLY-EBV DNA index maintained independent associations with OS (Table S2), DMFS (Table S3), and LRRFS (Table S4).

Table 2 Univariate and Multivariate Analysis of OS in the Training Cohort

Characteristics	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age				
<45 years	Reference		Reference	
≥45 years	1.752(1.227–2.500)	**0.002**	1.627(1.133–2.337)	**0.008**
Sex				
Female	Reference		Reference	
Male	1.856(1.164–2.960)	**0.005**	1.619(1.008–2.600)	**0.046**
Smoking history				
No	Reference			
Yes	1.237(0.864–1.771)	0.245		
T stage				
T1	Reference		Reference	
T2	2.280(0.792–6.562)	0.127	1.957(0.677–5.656)	0.215
T3	3.706(1.491–9.209)	**0.005**	3.020(1.208–7.550)	**0.018**
T4	5.934(2.367–14.871)	**<0.001**	4.562(1.803–11.546)	**0.001**
N stage				
N0	Reference		Reference	
N1	1.713(0.814–3.607)	0.156	1.335(0.629–2.834)	0.452
N2	2.437(1.133–5.243)	**0.023**	1.738(0.795–3.803)	0.166
N3	3.570(1.656–7.696)	**0.001**	2.389(1.073–5.320)	**0.033**
Treatment strategy				
IMRT alone	Reference			
CRT	0.460(0.148–1.429)	0.179		
CCRT±AC	0.887(0.381–2.066)	0.781		
IC+CCRT±AC	1.104(0.478–2.547)	0.817		
CALLY-EBV DNA index				
Low-risk	Reference		Reference	
Middle-risk	2.159(1.349–3.455)	**0.001**	1.616(0.996–2.621)	0.052
High-risk	3.564(2.167–5.861)	**<0.001**	2.074(1.204–3.574)	**0.009**

Notes: Values in bold are significant (P < 0.05).

Abbreviations: OS, overall survival; EBV-DNA, Epstein-Barr virus DNA; CALLY, C-reactive protein-albumin-lymphocyte; IC, induction chemotherapy; IMRT, intensity modulated radiation therapy; CRT, chemoradiotherapy; CCRT, concurrent chemoradiotherapy; AC, adjuvant chemotherapy; HR, hazard ratio; CI, confidence interval.

Table 3 Univariate and Multivariate Analysis of DMFS in the Training Cohort

Characteristics	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age				
<45 years	Reference			
≥45 years	1.181(0.812–1.716)	0.384		
Sex				
Female	Reference		Reference	
Male	1.711(1.044–2.804)	**0.033**	1.634(0.994–2.687)	0.053
Smoking history				
No	Reference			
Yes	1.224(0.829–1.808)	0.308		

(*Continued*)

Table 3 (Continued).

Characteristics	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
T stage				
T1	Reference			
T2	1.014(0.403–2.555)	0.976		
T3	1.888(0.937–3.804)	0.075		
T4	2.025(0.969–4.231)	0.061		
N stage				
N0	Reference		Reference	
N1	2.242(0.887–5.666)	0.088	1.837(0.723–4.668)	0.201
N2	2.994(1.153–7.775)	**0.024**	2.058(0.783–5.412)	0.143
N3	6.213(2.442–15.809)	**<0.001**	3.714(1.421–9.710)	**0.007**
Treatment strategy				
IMRT alone	Reference			
CRT	0.840(0.201–3.517)	0.812		
CCRT±AC	1.564(0.485–5.041)	0.454		
IC+CCRT±AC	2.117(0.664–6.750)	0.205		
CALLY-EBV DNA index				
Low-risk	Reference		Reference	
Middle-risk	2.995(1.692–5.302)	**<0.001**	2.527(1.413–4.519)	**0.002**
High-risk	5.442(3.012–9.833)	**<0.001**	3.520(2.023–7.024)	**<0.001**

Notes: Values in bold are significant ($P < 0.05$).

Abbreviations: DMFS, distant metastasis-free survival; EBV-DNA, Epstein-Barr virus DNA; CALLY, C-reactive protein-albumin-lymphocyte; IC, induction chemotherapy; IMRT, intensity modulated radiation therapy; CRT, chemoradiotherapy; CCRT, concurrent chemoradiotherapy; AC, adjuvant chemotherapy; HR, hazard ratio; CI, confidence interval.

Table 4 Univariate and Multivariate Analysis of LRRFS in the Training Cohort

Characteristics	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age				
<45 years	Reference			
≥45 years	0.961(0.633–1.460)	0.853		
Sex				
Female	Reference			
Male	1.271(0.772–2.093)	0.345		
Smoking history				
No	Reference			
Yes	1.222(0.786–1.899)	0.373		
T stage				
T1	Reference		Reference	
T2	1.839(0.680–4.973)	0.230	1.742(0.641–4.737)	0.276
T3	1.567(0.657–3.740)	0.311	1.456(0.606–3.497)	0.401
T4	3.641(1.539–8.613)	**0.003**	3.391(1.405–80,181)	**0.007**
N stage				
N0	Reference		Reference	
N1	1.234(0.577–2.641)	0.588	1.515(0.730–3.043)	0.158
N2	1.956(1.392–3.294)	**0.036**	2.054(1.172–3.816)	**0.021**
N3	2.609(1.689–4.760)	**0.017**	2.678(1.376–5.309)	**0.038**

(*Continued*)

Table 4 (Continued).

Characteristics	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Treatment strategy				
IMRT alone	Reference			
CRT	0.774(0.218–2.744)	0.692		
CCRT±AC	1.043(0.371–2.929)	0.937		
IC+CCRT±AC	1.193(0.428–3.327)	0.736		
CALLY-EBV DNA index				
Low-risk	Reference		Reference	
Middle-risk	1.839(1.115–3.033)	**0.017**	1.913(1.272–2.876)	**0.022**
High-risk	1.979(1.169–3.467)	**0.023**	2.171(1.194–3.610)	**0.040**

Notes: Values in bold are significant ($P < 0.05$).

Abbreviations: LRRFS, locoregional relapse-free survival; EBV-DNA, Epstein-Barr virus DNA; CALLY, C-reactive protein-albumin-lymphocyte; IC, induction chemotherapy; IMRT, intensity modulated radiation therapy; CRT, chemoradiotherapy; CCRT, concurrent chemoradiotherapy; AC, adjuvant chemotherapy; HR, hazard ratio; CI, confidence interval.

Development and Validation of Novel Prognostic Models Based on the Different Risk Stratifications

To quantitatively predict the prognosis of NPC patients, personalized nomograms for predicting the 1-year, 3-year, and 5-year OS, DMFS, and LRRFS of NPC patients were established using selected independent prognostic parameters identified through multivariate Cox analysis. The OS nomogram incorporates age, sex, T stage, N stage, and CALLY-EBV DNA index parameters (Figure 3A). The DMFS nomogram includes N stage and CALLY-EBV DNA index parameters, while the LRRFS nomogram includes T stage, N stage, and CALLY-EBV DNA index parameters (Figure 3B and C). Each variable was transformed into point scores based on the corresponding Cox regression coefficient, and the sum of these values was positioned on a total point scale to derive the probability of survival. Additionally, calibration curves revealed that the nomograms accurately predicted survival rates for 1-year, 3-year, and 5-year periods, closely aligning with the observed survival rates in both the training and validation cohorts (Figure 4A and B).

Subsequently, we compared the discriminative ability and clinical applicability between the nomograms and traditional TNM staging. The generated prognostic models demonstrated superior discriminative ability compared to traditional TNM staging in both the training and validation cohorts, as indicated by higher C-index values (OS: 0.707 vs 0.632, $P < 0.001$; DMFS: 0.678 vs 0.623, $P < 0.001$; LRRFS: 0.682 vs 0.623, $P < 0.001$ in the training cohort; OS: 0.740

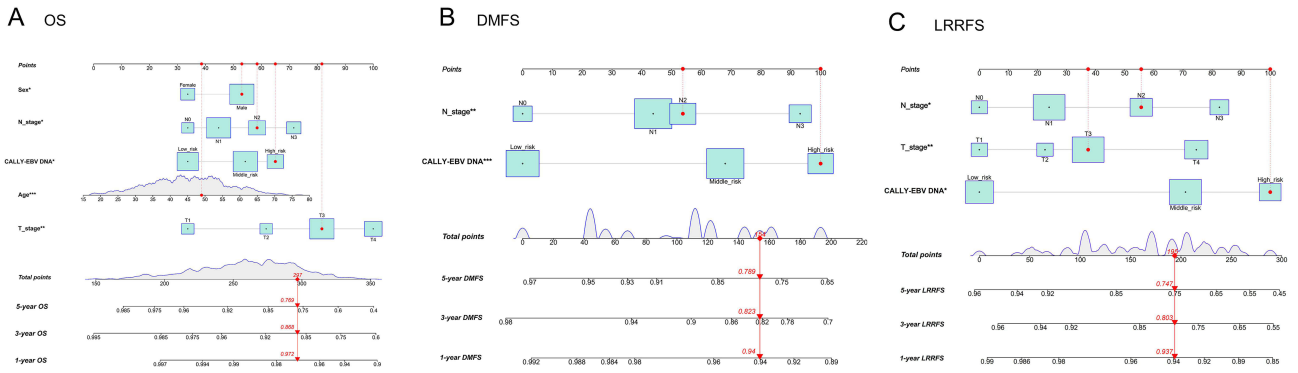

Figure 3 Nomograms incorporate the CALLY-EBV DNA index and clinicopathological factors to predict the 1-, 3-, and 5-year OS (**A**), DMFS (**B**), and LRRFS (**C**) of NPC patients.

Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; LRRFS, locoregional relapse-free survival; NPC, Nasopharyngeal carcinoma; EBV-DNA, Epstein-Barr virus DNA; CALLY, C-reactive protein -albumin-lymphocyte.

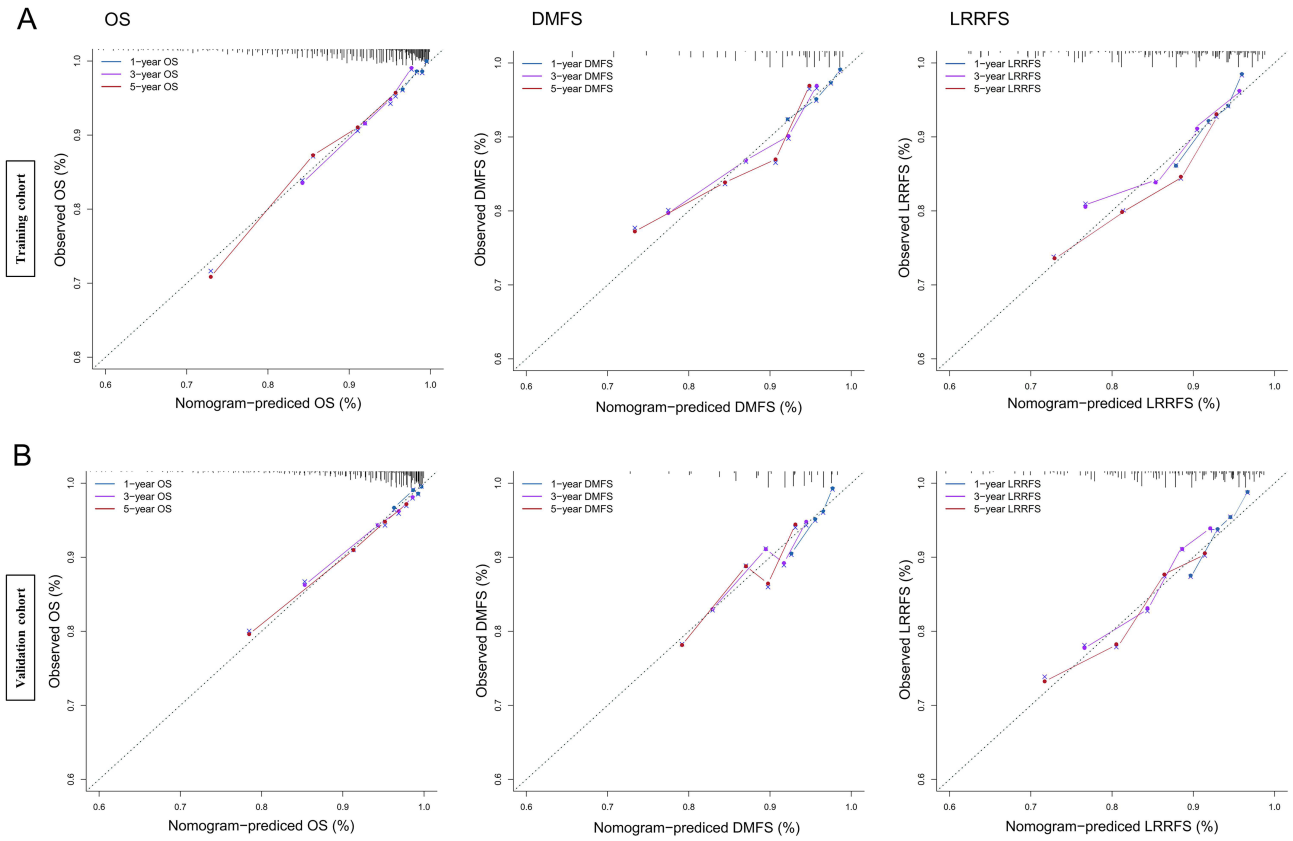

Figure 4 The calibration curves indicate the consistency between nomogram-predicted survival and actual outcomes for predicting 1-, 3-, and 5-year OS, DMFS, and LRRFS of NPC patients in training cohort (**A**) and validation cohort (**B**).
Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; LRRFS, locoregional relapse-free survival; NPC, nasopharyngeal carcinoma.

vs 0.631, $P < 0.001$; DMFS: 0.627 vs 0.598, $P = 0.009$; LRRFS: 0.656 vs 0.606, $P < 0.001$ in the validation cohort; Table 5). Additionally, DCA demonstrated that the nomograms exhibited higher net benefit and improved clinical applicability compared to the traditional TNM staging in both cohorts, indicating its superior ability to predict OS, DMFS, and LRRFS among NPC patients (Figure 5A and B).

Discussion

To the best of our knowledge, this is the first study to combine pretreatment plasma EBV DNA levels and the CALLY score for prognostic assessment in NPC patients. The study revealed that the combination of low plasma EBV DNA levels and high CALLY score is independently associated with better patient outcomes. Based on this integrated index,

Table 5 Comparison of the C-Index Among the Above Models for OS, DMFS, and LRRFS in the Training and Validation Cohorts

Prediction models		OS			DMFS			LRRFS		
		C-index	95% CI	*P*-value	C-index	95% CI	*P*-value	C-index	95% CI	*P*-value
Training cohort	Nomogram	0.707	0.665–0.748	Reference	0.678	0.631–0.724	Reference	0.682	0.640–0.724	Reference
	TNM stage	0.632	0.591–0.673	**<0.001**	0.623	0.579–0.666	**<0.001**	0.623	0.584–0.662	**<0.001**
Validation cohort	Nomogram	0.740	0.690–0.791	Reference	0.627	0.574–0.679	Reference	0.656	0.619–0.693	Reference
	TNM stage	0.631	0.581–0.680	**<0.001**	0.598	0.547–0.649	**0.009**	0.606	0.571–0.641	**<0.001**

Notes: *P* values were calculated using the ANOVA test. Values in bold are significant ($P < 0.05$).
Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; LRRFS, locoregional relapse-free survival; C-index, concordance index; HR, hazard ratio; CI, confidence interval.

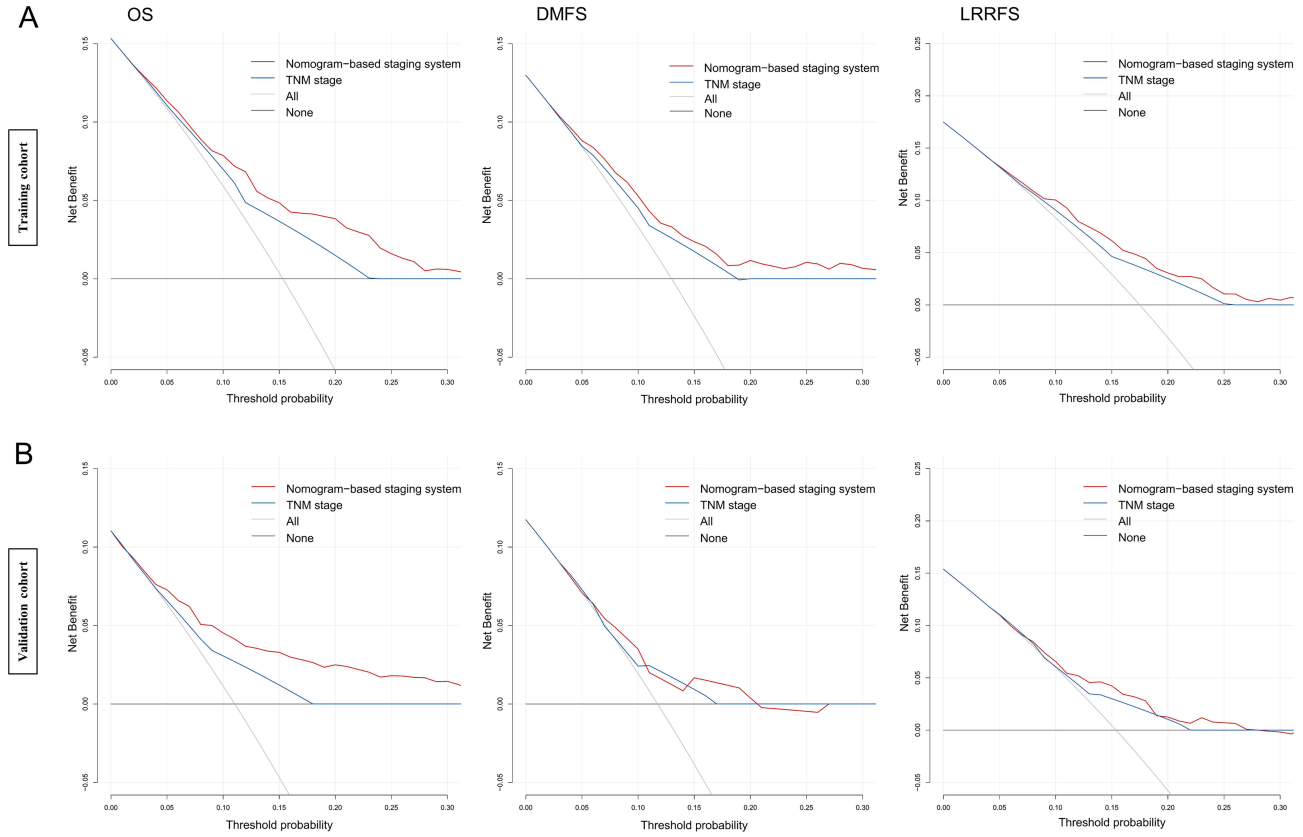

Figure 5 Decision curve analysis demonstrates the net benefit rate and clinical applicability by comparing the current nomograms with the traditional TNM staging for OS, DMFS, and LRRFS of NPC patients in the training cohort (**A**) and validation cohort (**B**).
Abbreviations: OS, overall survival; DMFS, distant metastasis-free survival; LRRFS, locoregional relapse-free survival; NPC, nasopharyngeal carcinoma.

we constructed personalized survival prediction models, which demonstrated excellent predictive performance compared to the traditional TNM staging.

NPC exhibits significant biological heterogeneity, with patients at the same stage receiving similar treatment regimens but experiencing markedly different outcomes.[4] Recently, highly precise genetic analyses and liquid biopsies have been employed to elucidate the in-depth molecular mechanisms of NPC, aiming to distinguish its heterogeneity and determining its prognosis.[26,27] However, these tests are criticized for their high cost and complex testing procedures, making it challenging for widespread clinical application. Since the introduction of the term "biomarkers",[28] an increasing amount of researches had explored and developed numerous simplified, affordable, and clinically accessible prognostic biomarkers for NPC. Recently, several immune-inflammatory or nutritional prognostic indexes, including lymphocyte-to -C-reactive protein ratio (LCR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), CONUT score, and PNI, have been generated and implemented in various cancers, including NPC.[10,12,29–31] The CALLY, serving as indicators of inflammatory response, nutritional status, and immune condition, has recently been proposed for predicting the prognosis of several cancers.[15–19] In clinical practice, the CALLY index could serve as a stratification tool to identify patients who may benefit from more aggressive or personalized therapeutic interventions.[16,32] For instance, patients with a high CALLY index, indicating a more pronounced inflammatory state, malnutrition, or compromised immune function, might be considered for immunonutrition therapy in addition to standard oncological treatments. This approach could potentially enhance their immune response to cancer and improve their OS and treatment tolerance. Furthermore, the CALLY index could be employed to monitor the response to therapy and guide subsequent treatment adjustments.[17,33] Patients with a decreasing CALLY index during the course of treatment may exhibit a favorable response, suggesting the need to continue or intensify the current treatment plan. Conversely, patients with an increasing CALLY index might require alternative therapeutic approaches or additional supportive care to

address the underlying inflammation, nutritional deficiencies, or immune dysfunction. In this study, we conducted the first assessment of the predictive value of the pre-treatment CALLY score on the prognosis of NPC patients, revealing a significant correlation between CALLY score and patient's OS, DMFS, and LRRFS. This discovery provides a foundation for stratifying the prognosis of NPC and personalized treatment strategies.

Nevertheless, the molecular mechanisms that underlie the association between the CALLY score and prognosis remain incompletely elucidated. The physiological and pathological change of serum albumin, lymphocytes, and CRP in tumors aid in comprehending the mechanisms underlying the prognostic significance of the CALLY score in NPC. Serum albumin serves as a convenient and easily interpreted nutritional indicator, with higher levels correlating to improved nutritional status.[34] Hypoalbuminaemia is a mortality prognostic factor for several cancers, indicating a state of progressive malnutrition.[34,35] Pathologically, malnutrition leads to a deficiency in the energy and substances required for the body, gradually compromising essential metabolic activities, ultimately resulting in a condition colloquially referred to as "starvation" in NPC patients.[35] And moreover, malnutrition can detrimentally impact patient prognosis by compromising host immunologic function and cell-mediated immunity, underscoring its crucial role in the host's capacity to mitigate cancer risk.[36] Lymphocytes play an crucial role in the immune surveillance and defensive mechanisms of tumors.[37] These immune cells, with the ability to produce cytokines like tumor necrosis factor-alpha (TNF-α) and interferon-γ, contribute to better prognosis by impeding proliferation, migration and invasion as well as inducing cytotoxic cell death of cancer cells.[37,38] In the tumor microenvironment, CD4+ T helper cells, through the secretion of cytokines like interferon-γ and IL-2, facilitate the mobilization and activation of CD8+ cytotoxic T cells.[39] These CD8+ cells, in turn, exert antitumor effects by directly inducing the destruction of cancer cells through the release of granzyme and perforin.[40] Considering that the peripheral blood lymphocyte count is indicative of an individual's cytotoxic immune function, an increased presence of tumor-infiltrating cytotoxic T cells and T helper cells has been associated with a more favorable prognosis in solid tumors.[41] Cancer-associated inflammation is widely acknowledged as a hallmark of cancer, essentially governing all stages of malignant tumors, from susceptibility and early onset to development, metastatic spread, and eventual mortality.[42] The serum level of CRP, an acute phase protein, increases following the secretion of IL-6 by T cells and macrophages and serves as an indicator of the systemic inflammatory response.[43] The proinflammatory cytokine IL-6 is frequently elevated across different cancer types, including NPC,[44] and the elevated IL-6 levels have been associated with the activation of the JAK2/STAT3 signaling pathway, potentially promoting cancer cell proliferation and metastasis, and correlating with poor survival outcomes.[45] The increase in CRP in response to the upregulation of IL-6 could serve as a mechanism connecting elevated serum CRP levels to an adverse prognosis. Certainly, tumor-associated inflammatory responses, host's nutritional status, and the host's immune condition do not exist in isolation but often interact with each other, collectively impacting patient prognosis.[46] Therefore, identifying high-risk patients with infections, malnutrition, and immunodeficiency is crucial, and promptly implementing measures such as anti-infective and nutritional interventions to enhance immune function is essential for improving clinical outcomes.

In recent years, considerable efforts have been dedicated to exploring prognostic factors associated with tumors in patients with NPC.[47] Among these factors, one of utmost importance is the pretreatment plasma EBV DNA level.[48] Many clinicians consider that pretreatment plasma EBV DNA, originating partly from tumor cell death, reflects the gross tumor burden in NPC, with its levels closely associated with TNM classification and overall disease stage.[48,49] Furthermore, EBV infection closely correlates with the malignant transformation and tumorigenesis observed in EBV-related NPC, and plasma EBV DNA serves as a critical indicator of EBV load in NPC.[24] As a clinically valuable biomarker, pretreatment EBV DNA has been well-established for the diagnosis, risk stratification, dynamic monitoring, and prognosis of NPC.[50] In clinical treatment decisions, pre-EBV DNA levels can serve as a biomarker to guide the intensity and duration of therapy.[51] Patients with high pretreatment EBV DNA levels, indicating a larger tumor burden and potentially more aggressive disease, may benefit from more intensive treatment regimens, such as the addition of induction chemotherapy before definitive radiotherapy or concurrent chemoradiotherapy.[47] Moreover, the dynamic monitoring of EBV DNA levels during the course of treatment can provide real-time information on the tumor's response to therapy.[49] A decline in EBV DNA levels

may indicate a favorable response, prompting continuation of the current treatment plan. Conversely, persistently high or increasing EBV DNA levels may signal treatment resistance, necessitating a change in therapy or the exploration of alternative treatment options. The incorporation of pretreatment plasma EBV DNA levels into the TNM staging system, as proposed by recent studies, could lead to more accurate risk stratification and prognostic categorization.[51,52] This refinement could facilitate the identification of high-risk patient populations that might benefit from more aggressive or experimental therapeutic interventions, such as novel targeted therapies or immunotherapies.[47] Previous researches, in the quest for convenient prognostic markers, often narrowly focused on a singular aspect, either concentrating solely on host factors like the patient's overall inflammatory status and nutritional condition or exclusively on tumor-related characteristics, thereby overlooking a comprehensive consideration.[8–10,12,48] In this research, we integrated tumor-related factors, pretreatment plasma EBV DNA, with host factors, represented by the CALLY score reflecting patients' systemic inflammatory, nutrition, and immune status. The combination of these factors, mutually complementing each other, allows for improved stratification of NPC patients and provides more comprehensive prognostic information than individual nutritional indexes.

Despite substantial evidence supporting the utility of the TNM staging system in assessing disease progression and prognosis risk in NPC patients, significant heterogeneity exists in the prognostic outcomes among patient groups with identical stages undergoing similar treatment regimens.[4] We contend that the limitations of TNM staging in prognostic prediction stem from its exclusive reliance on postoperative pathology without accounting for fundamental difference, such as gender and age, along with pertinent cancer prognostic factors like inflammation levels, nutritional status, and immune function.[10,48] In our study, we combined pretreatment plasma EBV DNA levels and the CALLY index to develop and validate nomograms to complement the traditional TNM stage for predicting OS, DMFS, and LRRFS in NPC patients. To be simple, patients with high-level EBV DNA and low-level CALLY scores are identified as a high-risk group, showing significantly poorer survival rates compared to other groups. More importantly, the nomograms demonstrated a significantly higher prognostic value than the traditional TNM stage alone, as evidenced by the C-index comparison. Additionally, DCA results indicated that the nomograms yield a higher net benefit for predicting clinical utility in OS, DMFS, and LRRFS compared to the 8th edition TNM staging system. We are confident that our nomograms can supplement the TNM stage drawbacks, offering a more individualized and accurate prognosis assessment for patients with NPC.

However, this study has several limitations. Firstly, the research solely assessed baseline hematological and EBV DNA levels, and dynamic evaluations might provide a more accurate evaluation of the prognostic predictive value of this index. Secondly, all NPC patients in this study were enrolled from a single Asian institution, and the study results may not be applicable to other regions, necessitating extensive multicenter external validation. Thirdly, the choice and alteration of treatment regimens throughout the patient's care, along with changes in patient characteristics, can significantly impact their overall survival prognosis. Lastly, as this is a retrospective study, patients with incomplete data were excluded from the study, potentially introducing some bias. In conclusion, large-scale prospective studies from multiple institutions are still necessary for validating the prognostic value of this risk score.

Conclusion

Taken together, the CALLY-EBV DNA index, derived from CALLY scores and pre-treatment plasma EBV DNA levels, has integrated inflammatory, nutritional, immune scores, and tumor-related factors. Serving as a convenient and cost-effective biomarker, it independently predicts the prognosis of NPC patients. Utilizing the CALLY-EBV DNA index, we established easily applicable nomogram predictive models, demonstrating superior predictive capability compared to traditional TNM staging systems. Further external validation of the CALLY-EBV DNA index and prognostic models based on the CALLY-EBV DNA index remains essential.

Data Sharing Statement

The data that support the findings of this study are available for reasonable request from the corresponding author.

Ethics Statement

The study was approved by the Ethics Committee of the Sun Yat-sen University Cancer Center.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there are no conflicts of interest in this work.

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