Development of a risk classification system combining TN-categories and circulating EBV DNA for non-metastatic NPC in 10,149 endemic cases

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Abstract

Background: The objective of this study was to construct a risk classification system integrating cell-free Epstein-Barr virus (cfEBV) DNA with T- and N- categories for better prognostication in nasopharyngeal carcinoma (NPC).

Methods: Clinical records of 10,149 biopsy-proven, non-metastatic NPC were identified from two cancer centers; this comprised a training ($N = 9{,}259$) and two validation cohorts ($N = 890$; including one randomized controlled phase 3 trial cohort). Adjusted hazard ratio (AHR) method using a two-tiered stratification by cfEBV DNA and TN-categories was applied to generate the risk model. Primary clinical endpoint was overall survival (OS). Performances of the models were compared against American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) 8th edition TNM-stage classification and two published recursive partitioning analysis (RPA) models, and were validated in the validation cohorts.

Results: We chose a cfEBV DNA cutoff of ⩾2,000 copies for optimal risk discretization of OS, disease-free survival (DFS) and distant metastasis-free survival (DMFS) in the training cohort. AHR modeling method divided NPC into six risk groups with significantly disparate survival ($p < 0.001$ for all): AHR1, T1N0; AHR2A, T1N1/T2-3N0 cfEBV DNA $<$2,000 (EBV_{low}); AHR2B, T1N1/T2-3N0 cfEBV DNA ⩾2,000 (EBV_{high}) and T1-2N2/T2-3N1 EBV_{low}; AHR3, T1-2N2/T2-3N1 EBV_{high} and T3N2/T4N0 EBV_{low}; AHR4, T3N2/T4 N0-1 EBV_{high} and T1-3N3/T4N1-3 EBV_{low}; AHR5, T1-3N3/T4 N2-3 EBV_{high}. Our AHR model outperformed the published RPA models and TNM stage with better hazard consistency (1.35 *versus* 3.98–12.67), hazard discrimination (5.29 *versus* 6.69–13.35), explained variation (0.248 *versus* 0.164–0.225), balance (0.385 *versus* 0.438–0.749) and C-index (0.707 *versus* 0.662–0.700). In addition, our AHR model was superior to the TNM stage for risk stratification of OS in two validation cohorts ($p < 0.001$ for both).

Conclusion: Herein, we developed and validated a risk classification system that combines the AJCC/UICC 8th edition TN-stage classification and cfEBV DNA for non-metastatic NPC. Our new clinicomolecular model provides improved OS prediction over the current staging system.

Keywords: adjusted hazard ratio, Epstein-Barr virus DNA, nasopharyngeal carcinoma, risk stratification, TNM stage

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Introduction

Epstein-Barr virus (EBV) is invariably linked with the endemic form of nasopharyngeal carcinoma (NPC). In these EBV-associated NPC tumors, the virus-encoded genomic region is ubiquitously expressed in most tumor cells.[1,2] In addition to

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the detection of EBV within the tumor, small genomic fragments of the virus, which are presumably released by circulating NPC tumor cells, can be detected using ultrasensitive polymerase chain reaction (PCR)-based assays. Hence, several studies have examined and reported on the clinical utility of these circulating cell-free EBV DNA (cfEBV DNA) molecular assays for population screening of NPC[3] and disease surveillance.[4,5] Apart from its advantages for early detection, quantification of cfEBV DNA load has also been investigated as a biomarker of tumor burden, and circulating viral load has been shown to correlate to clinical stage of disease.[6] To this point, studies have shown that pretreatment cfEBV DNA load is complementary to conventional TNM staging for clinical prognostication,[7,8] which would suggest that this biomarker provides additional biological information that is not captured by T- and N-classification.

However, despite its potential prognostic significance, the existing American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) 8th edition TNM-stage classification does not consider pretreatment EBV DNA for risk stratification. This is related to several factors, including poor inter-laboratory concordance in cfEBV DNA quantification by the PCR assay, use of different EBV DNA thresholds for risk discretization and limited cohort sample sizes for robust model development.[2,9] On this note, Tang *et al.*[7] presented a prognostic nomogram for disease-free survival (DFS) using several known prognostics variables, including cfEBV DNA. In their nomogram, cfEBV DNA was considered as a continuous variable with an assigned weightage. Nonetheless, this is impractical for routine clinical use, since the system developed was non-intuitive, and it did not seem to impact on the clinical management of NPC. Recently, two published recursive partitioning analysis (RPA)-based risk stratification system classified NPC patients into five and four clinicomolecular risk groups using cfEBV DNA, T- and N-categories,[8,10] but owing to the limited sample sizes in both studies (1,529 cases [training 979 patients, validation 550 patients]; and 518 cases, respectively), the discordant models still require validation in larger cohorts.

Here, we aimed to construct a robust clinicomolecular model by combining pretreatment cfEBV DNA titer with T- and N-categories that is superior for risk stratification than the 8th edition TNM-stage classification, using a large dataset of 9,259 patients who were treated at an academic center. We also investigated the performance of our model in multiple internal (including patients from a prospective phase 3 randomized controlled trial) and external validation cohorts.

Materials and methods

Patient selection

The study cohort comprised 10,149 patients with histologically proven, non-metastatic (M0) NPC from two academic institutions. This comprised a training cohort ($N = 9,259$) for model development, which was identified from the NPC-specific database embedded within the big-data intelligence platform at the Sun Yat-Sen University Cancer Center (SYSUCC) (Supplementary Materials, online only). An independent prospective cohort (NCT01245959, Supplementary Materials, online only) with 237 patients from the same center,[11] and an external cohort from the Wuzhou Red Cross Hospital (WZRCH, $N = 653$) were enrolled for validation. Overall, the training and validation cohorts were diagnosed and treated between 2009 and 2015. The inclusion and exclusion criteria, and detail procedures of patient selections were illustrated in Figure 1. The institutional ethical review boards of all included hospitals approved this retrospective analysis of anonymized data (IRB reference No.: [SYSUCC] YB2020-338-01; [WZRCH] LL2019-16). Informed consent was obtained for all patients from the SYSUCC-TPF trial cohort; while requirement for informed consent was waived by the ethical review boards for the SYSUCC-Training and WZRCH cohorts, given the retrospective nature of this study.

Diagnosis, treatment, and follow-up

All 10,149 patients were diagnosed, treated, and followed-up according to the respective institutional guidelines for NPC in the academic centers (Supplementary Materials, online only). All patients in each cohort were restaged by two radiation oncologists (GQZ and YS [SYSUCC-Training, SYSUCC-TPF]; JHL and TSG [WZRCH]) who are specialized in head and neck cancer in accordance with the AJCC/UICC 8th edition TNM-staging system,[12] with discordance resolved by consensus.

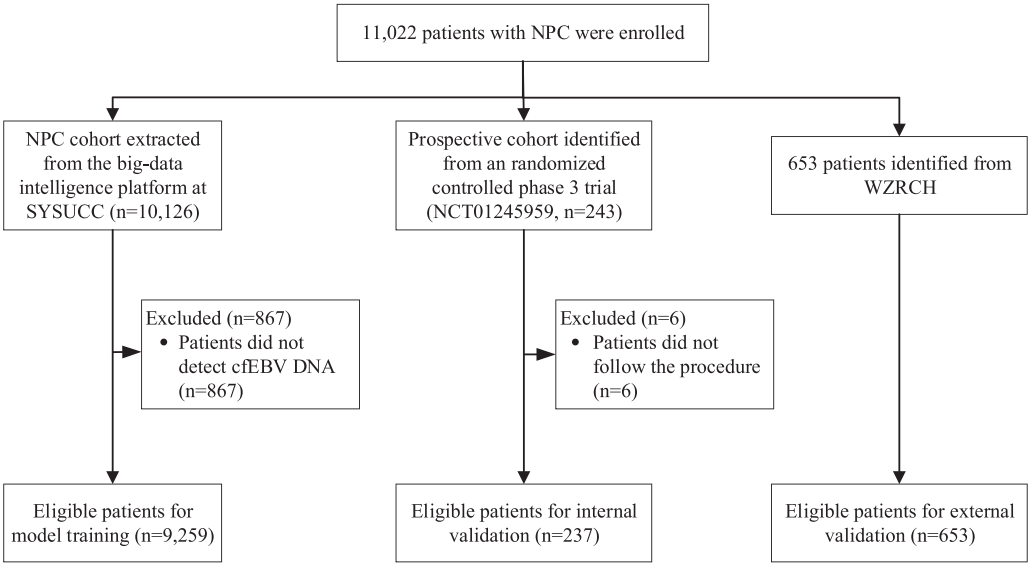

Figure 1. Flowchart showing the study design and patient selection process.

Statistical analysis

The primary endpoint was overall survival (OS), which was calculated from start of treatment to date of death from any cause, or date of last follow-up visit. Secondary endpoints were DFS, locoregionally recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS), calculated from start of treatment to date of first relapse, locoregional recurrence, and distant metastasis, respectively. Patients who were alive at end of study period were censored at the date of last follow-up visit. Survival analyses were performed using the Kaplan–Meier method and compared by the log-rank test. Cox proportional hazards regression was used for hazard ratio (HR) estimation in the multivariable analyses.

Determination of cfEBV DNA cutoff for risk discretization, and combinatorial cfEBV DNA and TN-category model construction

The relationship between cfEBV DNA titer and outcomes was calculated using Cox proportional hazards regression model through restricted cubic splines (RCS).[13–15] RCS allows threshold identification of cfEBV DNA on outcomes as described in previous studies.[14,15] Adjusted hazard ratios (AHRs) method was used to derive the risk classification model combining TN-status and cfEBV DNA. The performance of the AHR model in predicting OS was assessed and compared against the AJCC/UICC 8th edition TNM-stage classification and two published RPA models.[8,10,16] Detailed information on these processes is described in the Supplementary Materials.

Validation of proposed clinicomolecular risk stratification system in three independent cohorts

Validation of the proposed clinicomolecular risk stratification model (TN + cfEBV DNA) was performed in the SYSUCC-TPF and WZRCH cohorts by evaluating the performances of this model for prognostication of OS. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the accuracy of AHR model for survival prediction against AJCC/UICC 8th edition TNM-staging system. Decision curve analysis (DCA) was used to compare the efficacy of survival prediction between AHR model and TNM-stage classification.

All statistical tests were two-sided, and a p value of <0.05 was considered significant. Statistical analyses were performed in R version 3.4.4 (http://www.r-project.org/), Stata 14.2 software (StataCorp, College Station, TX), and SPSS 23.0 software (SPSS Inc, IL).

Results

Patient characteristics and treatment outcomes

The characteristics of patients from the training and validation cohorts are shown in Table 1.

Table 1. General characteristics of patients with nasopharyngeal carcinoma in the training and validation cohorts.

	Training cohort	Validation cohorts	
	SYSUCC-training (*n* = 9,259)	**SYSUCC-TPF (*n* = 237)**	**WZRCH (*n* = 653)**
Age (years)			
Median	45	41	48
IQR	38–53	35–48	42–56
Sex, *n* (%)			
Male	6,784 (73.3)	193 (81.4)	467 (71.5)
Female	2,475 (26.7)	44 (18.6)	186 (28.5)
WHO histologic type, *n* (%)			
Keratinizing	238 (2.6)	0 (0)	45 (6.9)
Nonkeratinizing	9,021 (97.4)	237 (100)	608 (93.1)
Tumor category, *n* (%)			
T1	1,533 (16.6)	5 (2.1)	96 (14.7)
T2	1,508 (16.2)	14 (5.9)	152 (23.3)
T3	4,294 (46.4)	145 (61.2)	146 (22.4)
T4	1,924 (20.8)	73 (30.8)	259 (39.6)
Node category, *n* (%)			
N0	1,449 (15.6)	0 (0)	44 (6.7)
N1	4,646 (50.2)	129 (54.4)	337 (51.6)
N2	2,004 (21.6)	91 (38.4)	181 (27.8)
N3	1,160 (12.5)	17 (7.2)	91 (13.9)
Stage, *n* (%)			
I	514 (5.6)	0 (0)	22 (3.4)
II	1,644 (17.8)	0 (0)	156 (23.9)
III	4,249 (45.9)	152 (64.1)	161 (24.7)
IVA	2,852 (30.8)	85 (35.9)	314 (48.1)
cfEBV DNA, copy/mL			
Median	2,050	5,630	500
IQR	0–17,000	652–33,200	<500–2,195
Chemotherapy, *n* (%)			
None	1,250 (13.5)	0 (0)	40 (6.1)
NACT ± ACT	906 (9.8)	0 (0)	53 (8.1)
CCRT ± ACT	3,412 (36.9)	122 (51.5)	263 (40.3)
NACT + CCRT ± ACT	3,691 (39.9)	115 (48.5)	297 (45.5)

ACT, adjuvant chemotherapy; CCRT, concurrent chemoradiotherapy; cfEBV DNA, cell-free Epstein-Barr virus DNA; IQR, interquartile range; NACT, neoadjuvant chemotherapy; SYSUCC, Sun Yat-Sen University Cancer Center; TPF, docetaxel/cisplatin/fluorouracil chemotherapy regimen; WHO, World Health Organization; WZRCH, Wuzhou Red Cross Hospital.

Median follow-up of these cohorts was 66.1 (interquartile range: 53.6–81.5) months, 82.1 (71.2–89.8) months, and 60.9 (47.0–67.8) months, respectively. The breakdown of the sites of relapses of these cohorts is detailed in Supplementary Table 1 (online only). Estimated 5-year OS rates were 86.1% (95% confidence interval [CI] 85.7%–86.5%) for SYSUCC-Training cohort, 81.4% (95% CI 78.9%–83.2%) for SYSUCC-TPF, 78.7% (77.0%–80.4%) for WZRCH cohort, respectively.

Prognostic effect of cfEBV DNA on survivals

We observed a consistent relationship between cfEBV DNA (log-scale) and OS, DFS, and DMFS (Supplementary Figure 1, online only), but not for LRFS in cfEBV DNA higher than 2,000 copies (log[cfEBV DNA) 3.32–3.34). This may be explained by the fact that our cohort was exclusively treated using intensity-modulated radiotherapy (IMRT), and thus lessened the association of conventional clinical prognostic variables with LRFS.[17,18] We also performed a sensitivity analysis to determine the optimal cutoff value for cfEBV DNA by testing for association with survival outcomes in subgroups dichotomized by 2,000, 20,000, 200,000, and 2,000,000 copies of cfEBV DNA (per 10-fold increase). Stable HRs were observed for OS, DFS, and DMFS with the different cfEBV DNA titers cutoffs (HR_{OS} 2.36 [2,000 copies] *versus* 2.33 [20,000], 2.33 [200,000], 2.88 [2,000,000] HR_{DFS} 2.17 *versus* 2.10, 2.09, 2.49; HR_{DMFS} 2.54 *versus* 2.44, 2.48, 2.68; Supplementary Figure 1, online only). We therefore conclude that cfEBV DNA of 2,000 copies is a stable and robust cutoff for risk stratification in non-metastatic NPC. This threshold was demonstrated to be valid for prognostication on multivariable analyses (Supplementary Table 2 and 3, online only).

Prognostic performance of current 8th edition TNM stage

The performance of the 8th edition TNM stage for prognostication in the training cohort is illustrated in Supplementary Figure 2 (online only); we tested the intra-group consistency of each TN-category in the SYSUCC-Training cohort by splitting the patients using TN-category and cfEBV DNA titers. Interestingly, we observed significant heterogeneity among patients with stage II to IVA NPC ($p < 0.001$ for all comparisons; Supplementary Figure 2, online only). In particular, stages III and IVA patients harbored the widest heterogeneity for OS between the subgroups, and thus we deduced that the current 8th edition TN-categories can be subdivided into finer groupings with improved homogeneity of OS within each risk group.

Development of a clinicomolecular risk stratification system using T- and N-categories and pretreatment cfEBV DNA titer

To this end, we constructed a new risk grouping model combining cfEBV DNA titer status and AJCC/UICC 8th edition TN-categories using the AHR modeling method. Figure 2 presents AHRs for OS by T- and N-categories with (Figure 2(a)) and without (Figure 2(b)) the inclusion of cfEBV DNA as a parameter, adjusted for age and gender. Overall, we observed an interaction between TN-categories and cfEBV DNA on AHR_{OS}; cfEBV DNA had a significant effect on risk of death across all the TN-categories, with the exception of T1N0. Next, we applied both the AHR values of each TN-category and the disease trajectories of the different stages of NPC to derive the final risk groupings. As a case in point, T1N1 tumors with EBV DNA $\geq$ 2,000 copies (AHR 7.598) would not be binned in a higher risk group than T1N2 tumors with EBV DNA $<$ 2,000 copies (AHR 3.022) and EBV DNA $\geq$ 2,000 copies (AHR 5.37), even though the AHR value of the former is higher. Using this logic, we were able to develop an AHR risk classification system that contains six risk groups (AHR1, 2A, 2B, 3, 4, and 5), with each TN-category being upgraded to a higher AHR risk based on EBV DNA $\geq$ 2,000 copies, except for T1N0 and T4N1. Our AHR model improved risk stratification of OS than the AJCC/UICC 8th edition TNM-stage classification (Figure 2(c)); 5-year OS was 98.9% (AHR1), 96.1% (AHR2A), 91.7% (AHR2B), 87.7% (AHR3), 78.5% (AHR4), and 72.3% (AHR5) ($p < 0.001$, Figure 2(d)), compared with that for stage I to stage IVA of 98.9%, 92.5%, 88.3%, and 76.6%, respectively ($p < 0.001$, Figure 2(e)). Importantly, our AHR risk classification system achieved good intra-group consistency among the subgroups for AHR1 to AHR5, except for the most unfavorable T4 N3 and cfEBV DNA $\geq$ 2,000 copies subgroup (Supplementary Figure 3).

Performances of clinicomolecular risk stratification system against 8th edition TNM and published RPA models

Next, we compared the AHR model against the AJCC/UICC 8th edition TNM-stage classification and two published RPA models.[8,10] Table 2

(a)

AHR	EBV DNA <2000copies/mL				EBV DNA ≥2000copies/mL			
	N0	N1	N2	N3	N0	N1	N2	N3
T1	Ref. (463)	3.065 (451)	3.022 (110)	9.742 (35)	1.115 (51)	7.598 (211)	5.37 (112)	19.419 (100)
T2	3.753 (190)	5.239 (446)	7.096 (135)	8.447 (49)	6.464 (39)	8.798 (307)	10.795 (169)	18.936 (173)
T3	1.999 (415)	6.406 (1192)	9.394 (349)	15.421 (114)	9.363 (90)	9.176 (1027)	19.135 (650)	20.848 (457)
T4	9.425 (115)	14.66 (369)	15.468 (120)	16.617 (45)	19.079 (86)	16.302 (643)	21.464 (359)	30.908 (187)

(b)

AHR	AJCC			
	N0	N1	N2	N3
T1	Ref. (514)	4.481 (662)	4.219 (222)	16.63 (135)
T2	4.192 (229)	6.641 (753)	9.03 (304)	16.307 (222)
T3	3.401 (505)	7.595 (2219)	15.438 (999)	19.535 (571)
T4	13.275 (201)	15.538 (1012)	19.768 (479)	27.629 (232)

(c)

AHR	EBV DNA <2000copies/mL				EBV DNA ≥2000copies/mL			
	N0	N1	N2	N3	N0	N1	N2	N3
T1	AHR1	AHR2A	AHR2B	AHR4	AHR1	AHR2B	AHR3	AHR5
T2	AHR2A	AHR2B	AHR2B	AHR4	AHR2B	AHR3	AHR3	AHR5
T3	AHR2A	AHR2B	AHR3	AHR4	AHR2B	AHR3	AHR4	AHR5
T4	AHR3	AHR4	AHR4	AHR4	AHR4	AHR4	AHR5	AHR5

AHR	AJCC			
	N0	N1	N2	N3
T1	Stage I			
T2		Stage II		
T3			Stage III	
T4				Stage IVA

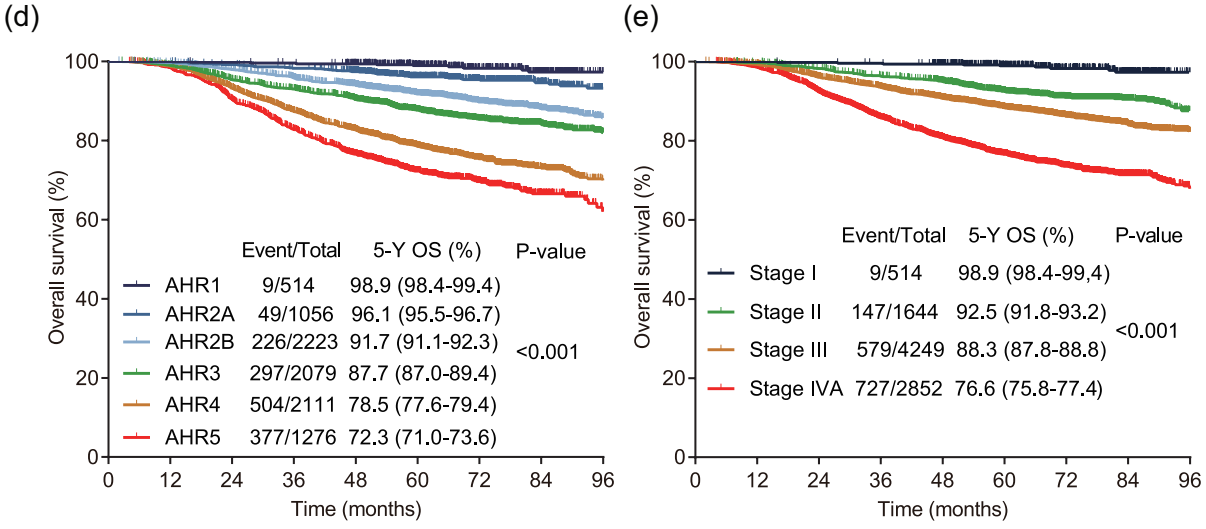

Figure 2. Development of an adjusted hazard ratio (AHR) risk classification system for M0 nasopharyngeal carcinoma (NPC). (a) AHR for overall survival (OS) for the different TN-categories and cfEBV DNA combined subgroups, adjusted for age and gender. (b) AHR for OS for the TN-categories alone, adjusted for age and gender. Numbers in parentheses in A and B refer to the sample sizes of the respective subgroups. (c) Risk groups derived by the proposed AHR classification system compared against the AJCC/UICC 8th edition TNM staging system. (d, e) Kaplan–Meier curves for OS stratified by the AHR risk classifications and TNM stage groups in the SYSUCC-Training cohort.

summarizes the performance of all the models in the SYSUCC-Training cohort. The AHR model was the most superior among all the different methods in terms of hazard consistency, hazard discrimination, explained variation, balance, C-index, Somers'D, Akaike information criterion, and Bayesian information criteria. The HRs for risk of death of the four risk classifications are presented in Supplementary Table 4 (online only); our AHR model outperformed TNM-stage classification and the published RPA models for prognostication. We therefore selected our AHR model for validation.

Validation of AHR model for prognostication in three independent cohorts

Figure 3 shows the OS outcomes of our AHR model in the two validation cohorts ($N=890$); AHR risk classification system yielded clear separation for the different AHR risk groups in SYSUCC-TPF and WZRCH cohorts. This validates the reproducibility

Table 2. Performance evaluation of AHR, RPA, and 8th edition AJCC/UICC TNM stage schema for nasopharyngeal carcinoma.

	Proposed model	Published models		
	AHR	**AJCC 8th**	**RPA_Guo**	**RPA_Lee**
Hazard consistency	**1.35**	8.25	3.98	12.67
Score	**0**	0.609	0.232	1
Rank	**1**	3	2	4
Hazard discrimination	**5.29**	6.69	11.90	13.35
Score	**0**	0.175	0.821	1
Rank	**1**	2	3	4
Explained variation	**0.248**	0.201	0.225	0.164
Score	**0**	0.561	0.275	1
Rank	**1**	3	2	4
Likelihood difference	119.33	**133.63**	131.10	92.37
Score	0.347	**0**	0.062	1
Rank	3	**1**	2	4
Balance	**0.385**	0.534	0.438	0.749
Score	**0**	0.408	0.146	1
Rank	**1**	3	2	4
Overall score	**0.347**	1.753	1.535	5.000
Overall rank	**1**	3	2	4
C-index[a]	**0.707**	0.677	0.700	0.662
Score	**0**	0.669	0.158	1.000
Rank	**1**	3	2	4
Somers'D	**0.413**	0.353	0.399	0.323
Score	**0**	0.669	0.158	1.000
Rank	**1**	3	2	4
AIC	**25195**	25387	25265	25510
Score	**0**	0.608	0.223	1
Rank	**1**	3	2	4
BIC	**25245**	25422	25308	25546
Score	**0**	0.589066	0.209497	1
Rank	**1**	3	2	4

AHR, adjusted hazard ratio; AIC, Akaike information criterion; AJCC, American Joint Committee on Cancer; BIC, Bayesian information criteria; RPA, recursive partitioning analysis; UICC, Union for International Cancer Control.
[a]Adjusted for age and gender.

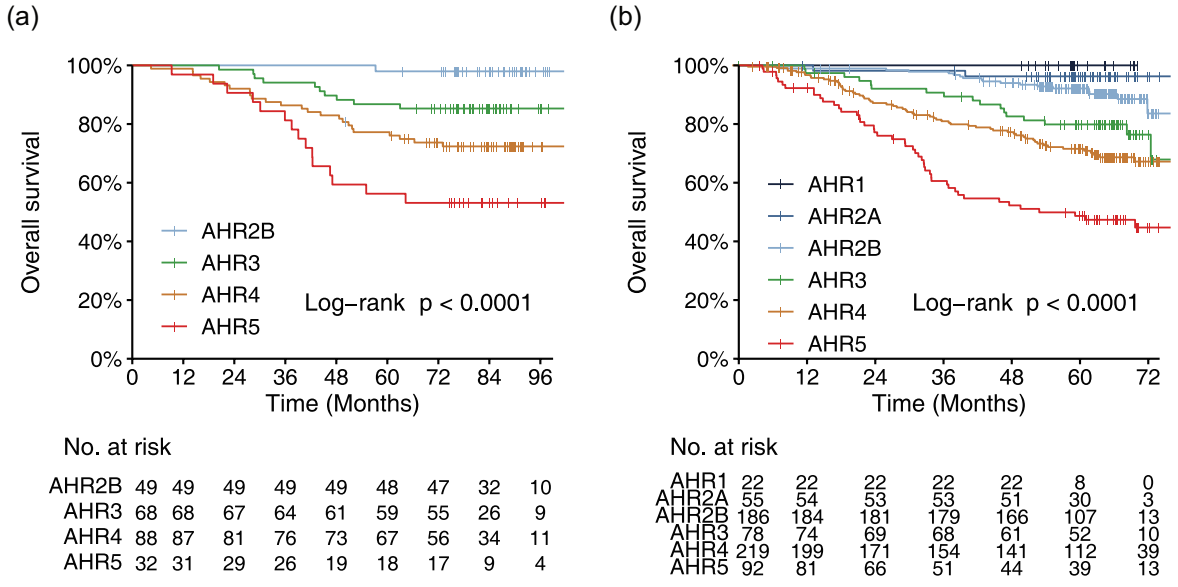

Figure 3. Validation analyses (overall survival) of the proposed AHR classification system in three independent cohorts. WZRCH: Wuzhou Red Cross Hospital. Values of *p* were derived by the log-rank test. (a) SYSUCC-TPF cohort; (b) WZRCH cohort.

Table 3. Distribution of patients in the AHR groups, compared with the 8th edition TNM classification system in the training cohort.

8th UICC/AJCC	*N*	Risk group
Stage I	514 (5.6%)	AHR1: 514 (100%)
Stage II	1,644 (17.8%)	AHR2A: 641 (39.0%)
		AHR2B: 696 (42.3%)
		AHR3: 307 (18.7%)
Stage III	4,249 (45.9%)	AHR2A: 415 (9.8%)
		AHR2B: 1,527 (35.9%)
		AHR3: 1,657 (39.0%)
		AHR4: 650 (15.3%)
Stage IVA	2,852 (30.8%)	AHR3: 115 (4.0%)
		AHR4: 1,461 (51.3%)
		AHR5: 1,276 (44.7%)

AHR, adjusted hazard ratio; AJCC, American Joint Committee on Cancer; UICC, Union for International Cancer Control.

of the AHR groupings in external cohorts, with independent cfEBV DNA testing and different clinical and cfEBV DNA parameters. In addition, AHR model achieved superior accuracy for survival prediction than TNM-staging system in SYSUCC-Training (AUC_{AHR} 0.681 [95% CI 0.668–0.695] *versus* AUC_{TNM} 0.641 [0.627–0.655]; Supplementary Figure 4A), SYSUCC-TPF (AUC_{AHR} 0.726 [0.657–0.794] *versus* AUC_{TNM} 0.666 [0.590–0.741]; Supplementary Figure 4B), and WZRCH (AUC_{AHR} 0.731 [0.689–0.773] *versus* AUC_{TNM} 0.683 [0.643–0.723]; Supplementary Figure 4 C), which were also validated by DCA analyses (Supplementary Figure 4D-F).

Finally, we present the clinical impact of our AHR model against the existing AJCC/UICC 8th edition TNM classification (Table 3). Our AHR risk stratification system was able to re-classify patients from every TN-stage group (other than for stage I) in the SYSUCC-Training cohort, thereby highlighting the intra-group heterogeneity in terms of OS-likelihood by the current stage classification system.

Discussion

Conventional TNM-stage classification represents a sound system for the clinical stratification of patients to inform on prognosis for NPC. Nonetheless, it is limited by the simplistic consideration of primary tumor extent and regional nodal burden, which may not capture the biological complexity of NPC.[19–21] Novel prognostic tools integrating clinical and molecular (cfEBV DNA) parameters for NPC are not yet implemented in

the clinic, partly because of model impracticality and limited sample size of these studies.[7,8,10] To address this unmet need, we adopted a big-data approach by assembling the largest dataset reported to date of 10,149 NPC cases, all of whom had pretreatment cfEBV DNA quantification and diagnostic staging that were centrally performed. We defined a robust cutoff of ⩾2,000 EBV DNA copies for risk discretization and applied an intuitive two-tiered classification schema to integrate cfEBV DNA titer and conventional T- and N-categories in the SYSUCC-Training cohort of 9,259 NPC patients. Apart from using a biostatistical approach of classifying patients, we also considered the clinical principles of the disease that underpin the development of the current AJCC/UICC 8th edition TNM-stage classification, and divided patients into six risk groupings that are more homogeneous in terms of risk of death within each subgroup. We identified that the AHR model was most superior for prognostication against the published RPA models,[8,10] and the TNM-stage classification. Our proposed AHR risk stratification criteria showed comparable performance for prognostication of OS in two separate cohorts; this is impressive considering that these cohorts varied in terms of clinical characteristics and treatment parameters. Moreover, the ability to stratify patients in the validation cohorts was observed, despite using cfEBV DNA readings that were derived using assays performed at different institutions (the SYSUCC was harmonized with the international standard testing method[8]). Notably, our model was also validated in a subset of 237 high-risk patients from a prospective clinical trial of induction TPF that was exclusively conducted in high-risk, locoregionally advanced NPC patients (5-year OS ranging from 56.3% [AHR5] to 98.0% [AHR2B]). Based on these findings, we have presented a new risk classification system combining conventional TN-categories and baseline cfEBV DNA titer for non-metastatic NPC that outperforms the existing stage classification system using the largest dataset reported to date.

Contrary to cfEBV DNA quantification at the mid-point and conclusion of treatment,[22] the proposition to incorporate pretreatment cfEBV DNA for prognostication in NPC is contentious for several reasons; this includes the reporting of different cutoffs for risk discretization, which is further compounded by the possibility of inter-laboratory variation.[2,9,23] To address these issues, we relied on a large dataset of 9,259 cases for which cfEBV DNA was quantified at a single clinical laboratory. Our PCR-based assay had high sensitivity (>90% detection at 500 copies) and limited within-run (<10%) and between-day (<20%) variation, and was recently validated under the premise of a global harmonization effort.[8] Next, we observed a similar linear dose (cfEBV DNA load)-response relationship for HR_{OS}, HR_{DFS}, HR_{DMFS}, and coincidentally derived comparable cutoffs of 3.32–3.34 lg(cfEBV DNA) for HR > 1.0 for the respective endpoints. The choice of 2,000 copies as a threshold is further corroborated by our sensitivity analysis showing the stability of HR_{OS} (2.33–2.88) when using cutoffs ranging from 2,000 to 2,000,000 copies. Taken together, our data addressed the perennial issues that hinder the mainstream incorporation of pretreatment cfEBV DNA for prognostication.

Ultimately, our work begs the question regarding the implications of our new and more refined risk classification system on the treatment of NPC patients. Currently, the National Comprehensive Cancer Network (NCCN) guidelines propose concurrent chemoradiotherapy (CCRT), CCRT + adjuvant chemotherapy (ACT) or neoadjuvant chemotherapy (NACT) + CCRT as reasonable treatment options for TNM stage II to IVA patients, but the choice of appropriate chemotherapy intensity to combine with RT remains contentious.[24–27] While it extends beyond the scope of our study findings, we propose that the new system using cfEBV DNA and TNM stage potentially helps to optimize clinical trial design and patient recruitment to better streamline treatment recommendation for NPC patients. Here, we proposed clinical trials to compare efficacy of RT alone *versus* CCRT for patients with AHR 2A to establish an optimal treatment strategy for this low-risk subgroup to avoid over-treatment, while considering trials comparing CCRT *versus* NACT + CCRT/CCRT + ACT among patients with AHR 2B and AHR 3 to ensure adequate intensity of treatment for this intermediate-risk subgroup. In addition, it is notable that the survival of patients with AHR 4 and AHR5 remains unsatisfactory; we propose conducting of clinical trials for investigations of new drugs or therapies to improve the prognosis of these patients.

Several caveats of our findings ought to be highlighted. Foremost, treatment regimens were not included as covariates for AHR model construction. This was because interventions are not baseline attributes, and there was no control over the allocation of interventions (such as randomization), which would confound any comparative analyses

between the different AHR groups. It is also based on this reasoning that we did not investigate the association of our AHR risk groups with treatment efficacy. This analysis is beyond the scope of our study, especially given the potential treatment biases. Prospective clinical trials are needed to investigate the appropriate treatment strategy for each AHR risk group. Next, we acknowledged the clinical heterogeneity between our training and validation cohorts. Of note, the cfEBV DNA levels in the WZRCH cohort were lower than the levels observed in the SYSUCC-Training cohort (median cfEBV DNA: 500 copies *versus* 2,050 copies). This variation could be explained by protocol variations between laboratories, but regardless, we were able to show that the cutoff of 2,000 copies was still able to identify two risk groups with disparate survival in the WZRCH cohort (Supplementary Figure 5A–D). This indirectly supports the robustness of our proposed cfEBV DNA cutoff of 2,000 copies, even with different cfEBV DNA molecular assays.

In conclusion, we successfully defined an optimal cfEBV DNA cutoff at baseline and combined the biomarker with conventional TN-categories to construct a new AHR risk classification system for M0 NPC. Our model stratifies patients into six risk groups with improved intra-group homogeneity for OS compared with the existing AJCC/UICC 8th edition TNM-staging system. This new system could be the basis for future strategies of clinical trial designing and patient recruitment for better streamlining treatments in NPC patients.

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Conflict of interest statement

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Supplemental material

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References

1. Lin JC, Wang WY, Chen KY, *et al.* Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. *N Engl J Med* 2004; 350: 2461–2470.
2. Li YQ, Khin NS and Chua MLK. The evolution of Epstein-Barr virus detection in nasopharyngeal carcinoma. *Cancer Biol Med* 2018; 15: 1–5.
3. Chan KCA, Woo JKS, King A, *et al.* Analysis of plasma Epstein-Barr virus DNA to screen for nasopharyngeal cancer. *N Engl J Med* 2017; 377: 513–522.
4. Lo YM, Chan LY, Chan AT, *et al.* Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. *Cancer Res* 1999; 59: 5452–5455.
5. Wang WY, Twu CW, Lin WY, *et al.* Plasma Epstein-Barr virus DNA screening followed by ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography in detecting posttreatment failures of nasopharyngeal carcinoma. *Cancer* 2011; 117: 4452–4459.
6. Lo YM, Leung SF, Chan LY, *et al.* Plasma cell-free Epstein-Barr virus DNA quantitation in patients with nasopharyngeal carcinoma. Correlation with clinical staging. *Ann N Y Acad Sci* 2000; 906: 99–101.
7. Tang LQ, Li CF, Li J, *et al.* Establishment and validation of prognostic nomograms for endemic nasopharyngeal carcinoma. *J Natl Cancer Inst* 2016; 108: djv291.
8. Guo R, Tang LL, Mao YP, *et al.* Proposed modifications and incorporation of plasma Epstein-Barr virus DNA improve the TNM staging system for Epstein-Barr virus-related nasopharyngeal carcinoma. *Cancer* 2019; 125: 79–89.
9. Leung SF, Zee B, Ma BB, *et al.* Plasma Epstein-Barr viral deoxyribonucleic acid quantitation complements tumor-node-metastasis staging prognostication in nasopharyngeal carcinoma. *J Clin Oncol* 2006; 24: 5414–5418.
10. Lee VH, Kwong DL, Leung TW, *et al.* The addition of pretreatment plasma Epstein-Barr virus DNA into the 8th edition of nasopharyngeal cancer TNM stage classification. *Int J Cancer* 2019; 144: 1713–1722.
11. Sun Y, Li WF, Chen NY, *et al.* Induction chemotherapy plus concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in locoregionally advanced nasopharyngeal carcinoma: a phase 3, multicentre, randomised controlled trial. *Lancet Oncol* 2016; 17: 1509–1520.
12. Pan JJ, Ng WT, Zong JF, *et al.* Proposal for the 8th edition of the AJCC/UICC staging system for nasopharyngeal cancer in the era of intensity-modulated radiotherapy. *Cancer* 2016; 122: 546–558.
13. Therneau TM and Grambsch PM. *Modeling survival data: extending the Cox model*. New York: Springer, 2000.
14. Molinari N, Daures JP and Durand JF. Regression splines for threshold selection in survival data analysis. *Stat Med* 2001; 20: 237–247.
15. Heinzl H and Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. *Comput Methods Programs Biomed* 1997; 54: 201–208.
16. Xu W, Shen XW, Su J, *et al.* Refining evaluation methodology on TNM stage system: assessment on HPV-related oropharyngeal cancer. *Austin Biom and Biostat* 2015; 2: 1014.
17. Sun X, Su S, Chen C, *et al.* Long-term outcomes of intensity-modulated radiotherapy for 868 patients with nasopharyngeal carcinoma: an analysis of survival and treatment toxicities. *Radiother Oncol* 2014; 110: 398–403.
18. Setton J, Han J, Kannarunimit D, *et al.* Long-term patterns of relapse and survival following definitive intensity-modulated radiotherapy for non-endemic nasopharyngeal carcinoma. *Oral Oncol* 2016; 53: 67–73.
19. Ganz P, Heidecker B, Hveem K, *et al.* Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. *JAMA* 2016; 315: 2532–2541.
20. Fraser M, Sabelnykova VY, Yamaguchi TN, *et al.* Genomic hallmarks of localized, non-indolent prostate cancer. *Nature* 2017; 541: 359–364.
21. Li YY, Chung GT, Lui VW, *et al.* Exome and genome sequencing of nasopharynx cancer

identifies NF-κB pathway activating mutations. *Nat Commun* 2017; 8: 14121.

22. Lv JW, Chen YP, Zhou GQ, *et al*. Liquid biopsy tracking during sequential chemo-radiotherapy identifies distinct prognostic phenotypes in nasopharyngeal carcinoma. *Nat Commun* 2019; 10: 3941.
23. Le QT, Zhang Q, Cao H, *et al*. An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. *Clin Cancer Res* 2013; 19: 2208–2215.
24. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. Head and neck cancer, version 1, https://www.nccn.org/professionals/physician_gls/f_guidelines.asp (2021, accessed 9 November 2020).
25. Chen YP, Ismaila N and Chua MLK, *et al*. Chemotherapy in combination with radiotherapy for definitive-intent treatment of stage II-IVA nasopharyngeal carcinoma: CSCO and ASCO guideline. *J Clin Oncol* 2021; 39: 840–859.
26. Chua MLK, Wee JTS, Hui EP, *et al*. Nasopharyngeal carcinoma. *Lancet* 2016; 387: 10022.
27. Zhang Y, Chen L, Hu GQ, *et al*. Gemcitabine and cisplatin induction chemotherapy in nasopharyngeal carcinoma. *N Engl J Med* 2019; 381: 1124–1135.