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Distant Metastasis Risk Definition by Tumor Biomarkers Integrated Nomogram Approach for Locally Advanced Nasopharyngeal Carcinoma

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Abstract

Identifying metastasis remains a challenge for death control and tailored therapy for nasopharyngeal carcinoma (NPC). Here, we addressed this by designing a nomogram-based Cox proportional regression model through integrating a panel of tumor biomarkers. A total of 147 locally patients with advanced NPC, derived from a randomized phase III clinical trial, were enrolled. We constructed the model by selecting the variables from 31 tumor biomarkers, including 6 pathological signaling pathway molecules and 3 Epstein-Barr virus-related serological variables. Through the least absolute shrinkage and selection operator (LASSO) Cox proportional regression analysis, a nomogram was designed to refine the metastasis risk of each NPC individuals. Using the LASSO Cox regression model, we constructed a 9 biomarkers-based prognostic nomogram: Beclin 1, Aurora-A, Cyclin D1, Ki-67, P27, Bcl-2, MMP-9, 14-3-3σ, and VCA-IgA. The time-dependence receiver operating characteristic analysis at 1, 3, and 5 years showed an appealing prognostic accuracy with the area under the curve of 0.830, 0.827, and 0.817, respectively. In the validation subset, the concordance index of this nomogram reached to 0.64 to identify the individual metastasis pattern. Supporting by this nomogram algorithm, the individual metastasis risk might be refined personally and potentially guiding the treatment decisions and target therapy against the related signaling pathways for patients with locally advanced NPC.

Keywords

nasopharyngeal carcinoma, tumor biomarker, nomogram algorithm, classifier, metastasis

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Introduction

Nasopharyngeal carcinoma (NPC), an Epstein-Barr virus (EBV) infection-related head and neck squamous cell carcinoma, is highly prevalent in the Southeast Asian countries.[1,2] Radiotherapy alone or concurrent chemoradiotherapy has been used as the primary curative treatment for early-stage NPC with a 5-year disease-free survival rising to 78%.[3] However, although the introduction of neoadjuvant chemotherapy enhanced the local control rate, 20% to30% of patients still develop the distant metastasis, which is the main obstacle for improving the overall survival for NPC.[4] Thus, recognizing the high-risk distant metastasis individuals is crucial to provide these particular patients a rational personalized therapy and follow-up.

Currently, the tumor–node–metastasis (TNM) staging system is the primary tool utilized to predict outcomes and contributes largely to the treatment planning for patients with NPC. However, the patients sharing the same TNM stage have the heterogeneous outcome, indicating that other factors might contribute to the determination of prognosis. For decades, tumor biomarkers were demonstrated to facilitate the TNM classification for risk definition and provide insights to develop target therapy to improve the survival in many cancers. For example, the breast cancer subset exhibiting high amplification of the human epidermal growth factor (HER2/neu) receptor had an inferior overall survival.[5] Trastuzumab, a monoclonal antibody that was specifically designed to interfere with the HER2/neu receptor, was recommended to patients with HER2/neu overexpression, especially to those with metastatic disease.[6] However, the single prognostic tumor biomarker or TNM prognostication system was also found to have the limitation of low sensitivity and specificity to predict tumor recurrence individually.[7] For breast cancer, a 21-gene algorithm approach showed high predictive efficacy to stratify the individual patient as low risk and high risk to relapse (6.8% vs 30.5%) at the 10-year follow-up, validating a novel way to predict disease relapse risk individually. Importantly, this multigenes or tumor biomarkers (microRNA and proteins) integrated method had shown a powerful efficacy to predict individual prognosis precisely, not only for breast cancer but also for other cancers, such as colon cancer, nonsmall cell lung cancer, and NPC.[8-10]

Here, a nomogram model was developed to identify the individual risk to distant metastasis for patients with locally advanced NPC. Briefly, we integrated 3 EBV-related serological biomarkers and 31 clinicopathological tumor biomarkers in protein level. Further, the nomogram model was used to categorize the metastatic risk for each individual patients with NPC through Cox proportional hazards regression analysis and the least absolute shrinkage and selection operator (LASSO) method. Moreover, the predictive value between the nomogram model and current clinically used TNM staging system was compared to refine the individual metastatic risk for locally advanced NPC.

Materials and Methods

Patient Selection

A total of 147 diagnosed and pathologically confirmed patients with NPC, derived from a randomized phase III clinical trial, were enrolled in this study.[11] Clinical stage was defined according to the NPC staging system of China.[1,12] The patients received induction chemotherapy plus concurrent adjuvant chemoradiotherapy (IC/CCRT) or induction chemotherapy plus radiotherapy (IC/RT) randomly. The inclusion/exclusion criteria of this randomized trial have been reported previously.[11,13] In brief, 147 patients were enrolled in this study; 7 patients were excluded, due to the history of previous anticancer therapy in 3 patients in IC/RT subgroup, and lost to 5-year follow-up in 4 patients (1 in IC/RT subgroup and 3 in IC/CCRT subgroup). In IC/CCRT subgroup, patients were treated with 2 cycles of floxuridine + carboplatin (floxuridine 750 mg/m^2, day 1-5; carboplatin area under the curve [AUC] = 6) chemotherapy and 1 week later with radiotherapy and concurrent carboplatin (AUC = 6) chemotherapy on day 7, 28, and 49, respectively. In IC/RT subgroup, patients received 2 cycles of floxuridine + carboplatin (floxuridine 750 mg/m^2, day 1-5; carboplatin AUC = 6) chemotherapy and underwent radiotherapy 1 week thereafter. In this study, a total radiation dose of 68 to 72 Gy with 2 Gy daily fractions and 5 days per week was conducted.

Immunohistochemical Staining and Measurements

Paraffin-embedded primary tumor tissues of the patients were collected to construct the tissue microassays according to the protocol published previously.[14] The candidate biomarkers were all reported to be involved in NPC carcinogenesis or prognosis. In the present study, 31 biomarkers were tested: 6 pathological signaling pathway molecules, consisting of cell cycle: Cyclin D1, 14-3-3σ, Aurora-A, CENP-H, Stathmin, P21, CDC2, P27, ERK, p-ERK, and Ki-67; migration- and invasion-related molecules: E-Cadherin, β-Catenin, N-Cadherin, Snail, Twist, c-Met, and nm23; tumor microenvironment-related molecules: HIF-1α, COX2, MMP-2, MMP-9, and TIMP-2; apoptosis- and autophagy-related molecules: Bax, Bcl-2, Survivin, AKT 1, Pontin, and Beclin 1; epigenetic-related molecule: EZH2, and EBV-related molecule: LMP 1 were tested by immunohistochemical (IHC) staining following the procedures published previously.[15,16,17] Here, we changed the specific primary antibody with non-immune serum immunoglobulins as a negative control (1:200 dilutions). Two independent observers blinded to the clinical information evaluated the IHC staining score of each biomarker. As previously reported, we semiquantitatively assessed each tumor biomarker expression level by measuring the staining intensity and extent.[18] In brief, we graded staining intensity as follows: negative (score 0), bordering (score 1), weak (score 2), moderate (score 3), and strong (score 4). The staining extent was ranked into 4 parts according to the percentage of positive staining cells in the field: 0% to 25% (score 1), 26% to 50% (score

2), 51% to 75% (score 3), and 76% to 100% (score 4). The overall score was obtained by multiplying the staining intensity and extent. The scores were assessed by 2 independent pathologists blinded to the clinical follow-up. If there was any controversy about the score, a third observer would be intervened to make a final judgment. Here, the consistency of the overall score reached to 96%.

Epstein-Barr virus-Related Serological Antibodies Assays

The serums of each patient were obtained before the oncological treatment. Three EBV-related serological biomarkers associated with tumorigenesis of NPC, including EA-IgA, VCA-IgA, and anti-enzyme rate of EBV DNase-specific neutralizing antibody, were measured through enzyme-linked immunosorbent assay method.[19]

Patients Follow-Up

The patients in this study were followed up rigorously according to the follow-up protocol. In general, patients were observed at 3-month intervals during the first 3 years after therapy and at 6-month intervals thereafter. The routine follow-up examinations including history recording, physical examination, complete blood and biochemical examinations, fiberoptic nasopharyngoscopy, magnetic resonance imaging of the whole neck, cranial nerve examination, chest radiography, abdominal sonography, and whole-body bone scintigraphy were carried out when clinically indicated. The distant metastasis-free survival (DMFS) was defined as the time from diagnosis to the date of distant metastasis or when censored at the latest date.

Statistical Analysis

We used univariate Cox regression model to explore the association between DMFS and each biomarker. The LASSO multivariate Cox regression model was used to select the biomarkers, and the Cox regression coefficients were used to generate the nomograms. Discrimination was evaluated by analyzing the AUC of the receiver operating characteristic curve (ROC curve) as well as the concordance index (C-index). To alleviate the overfitting issue, we used 1000 bootstrap samples to correct the C-index. The average C-index of the models developed on the bootstrap sample and applied to the original sample were calculated. The difference between these 2 C-indexes was used to optimize the model based on the original model. Nomogram and bootstrap resampling were done with the rms package of R software, and all the other statistical tests were done with R software version 3.2.2. A 2-side P value of $<.05$ was considered as statistically significant.

Results

The clinical and pathological features are listed in Table 1, and the IHC staining of 31 biomarkers in the 147 patients with locally advanced NPC are shown in Figure S1. The median follow-up period was 60.8 months (range: 2.63-89.87 months) for all patients. Forty-two patients (28.6%) finally developed distant metastasis during the 5-year follow-up. The median DMFS was 62.4 and 60.4 months in IC/RT and IC/CCRT subgroups, respectively ($P > .05$). The 2-year and 5-year DMFS was 77.63% and 51.32% in the IC/RT subgroup, while 73.24% and 50.70% in the IC/CCRT subgroup, respectively (all $P > .05$).

As shown in Figure 1a, there is no significant difference regarding to each clinicopathological variables between the subsets with and without distant metastasis. The univariate analysis indicated that Beclin 1, Aurora-A, Bcl-2, P27, 14-3-3σ, and VCA-IgA were associated with DMFS rate (Table S1). Further, the LASSO Cox regression model was used to construct a prognostic classifier, selecting 9 candidate variables from the 34 tumor-related molecular biomarkers and 8 clinical variables associated with DMFS (Table 2 and Figure 1b). To provide a clinically quantitative tool to predict the individual distant metastasis probability, a nomogram classifier was constructed to predict 1-, 3-, and 5-year DMFS using the 9 candidate biomarkers (Figure 2a). The nomogram can assign the probability of DMFS by adding up the scores of the selected factors shown on the point scale. Specifically, the "risk score" was used to identify the distant metastasis risk for each patient based on their individual 9 biomarker levels, where "risk score $= 0.0524$*Beclin 1 $+ 0.1156$*Aurora-A $+ 0.0384$*Cyclin D1 $+ 0.0569$*Ki-67 $+ 0.0472$*Bcl-2 $+ 0.0709$*P27 $- 0.1492$*14-3-3σ $- 0.1288$*MMP-9 $+ 0.0004$*VCA-IgA."

As shown in Table 2, except 14-3-3σ, the rest of the 8 variables were all positively associated with DMFS. The "rpart" procedure in R software was used to generate the optimum cut-off score, where those patients with a risk score of 0.491 or higher was classified as high risk of distant metastasis and those with a risk score lower than 0.491 was ranked as low risk of distant metastasis. Importantly, the distribution of the DMFS was varied significantly between the subsets identified as high-risk group and low-risk group, where the 3-year DMFS was 33.3% for the high-risk group, and 82.4% for the low-risk group (hazard ratio [HR]: 7.527, 95% confidence interval [CI]: 3.978-14.242, $P < .0001$). Conversely, the DMFS was indistinguishable between TNM stage III and stage IV (Figure 2b).

Individually, the predictive accuracy of this classifier to predict personal metastasis pattern was also characterized. The time-dependence ROC analysis at 1, 3, and 5 years proved that the AUC of this classifier could reach to 0.830, 0.827, and 0.817, respectively (Figure 3a and b). In addition, the C-index for classifier was 0.768. Accounting for the overfitting issue, the bootstrap approach was further utilized. With 1000 bootstrap resamples, the average C-statistics of the models developed on the original data, the training data, and the testing data were 0.81, 0.90, and 0.73, respectively. Thus, the expected C-index for the present study should be 0.64 when applied to validation data set.

Figure 3c demonstrates the 5-year survival predicted by the nomogram in stage III and stage IV of the NPC staging system of China. A wide range of predicted survival could be

Table 1. Patient Characteristics and Correlation With Distant Metastasis-Free Survival.

Characteristics (n = 147)	No (%)	Univariate, HR (95% CI)	*P* Value
Age, year (>43 vs ≤ 43)	51.7 vs 48.3	1.01 (0.58-1.77)	.97
Gender (male vs female)	78.9 vs 21.1	1.05 (0.54-2.05)	.89
T stage (T1-T2 vs T3-T4)	16.3 vs 83.7	0.64 (0.27-1.52)	.31
N stage (N0-N1 vs N2-N3)	46.3 vs 53.7	0.79 (0.45-1.41)	.43
Overall stage (III vs IVa)	53.7 vs 46.3	0.93 (0.53-1.63)	.80
Treatment (IC/RT vs IC/CRT)	51.7 vs 48.3	1.10 (0.62-1.94)	.75
14-3-3σ [a] (>7.0 vs ≤ 7.0)	33.3 vs 66.7	1.53 (0.81-2.89)	.19
AKT1[a] (> 5.0 vs ≤ 5.0)	44.2 vs 55.8	1.44 (0.79-2.62)	.23
Aurora-A[a] (≤6.0 vs > 6.0)	51.0 vs 49.0	0.36 (0.20-0.66)	<.01
Bax[a] (≤ 4.0 vs > 4.0)	61.9 vs 38.1	0.75 (0.42-1.31)	.30
Bcl-2[a] (>5.0 vs ≤ 5.0)	54.4 vs 45.6	0.78 (0.44-1.38)	.40
Beclin 1[a] (≤6.0 vs > 6.0)	60.5 vs 39.5	0.59 (0.33-1.06)	.07
β-Catenin[a] (>6.0 vs ≤ 6.0)	50.3 vs 49.7	0.79 (0.42-1.46)	.45
CDC2[a] (≤5.0 vs >5.0)	44.9 vs 55.1	0.71 (0.40-1.25)	.24
CENP-H[a] (≤5.0 vs > 5.0)	53.1 vs 46.9	0.73 (0.42-1.28)	.28
c-Met[a] (≤6.0 vs > 6.0)	50.3 vs 49.7	0.89 (0.51-1.56)	.68
COX2[a] (≤6.0 vs > 6.0)	57.1 vs 42.9	0.83 (0.47-1.47)	.53
Cyclin D1[a] (≤5.0 vs > 5.0)	56.5 vs 43.5	0.64 (0.36-1.13)	.12
E-Cadherin[a] (>5.0 vs ≤ 5.0)	56.5 vs 43.5	1.10 (0.62-1.92)	.75
ERK[a] (≤5.0 vs > 5.0)	53.1 vs 46.9	0.81 (0.46-1.43)	.47
EZH2[a] (≤9.0 vs > 9.0)	49.7 vs 50.3	0.82 (0.47-1.44)	.49
HIF-1α[a] (≤7.0 vs > 7.0)	58.5 vs 41.5	0.61 (0.34-1.07)	.08
Ki-67[a] (≤5.0 vs > 5.0)	49.6 vs 50.4	0.78 (0.44-1.36)	.37
LMP1[a] (>4.0 vs ≤ 4.0)	44.9 vs 55.1	0.94 (0.51-1.73)	.84
MMP-2[a] (≤6.0 vs > 6.0)	49.7 vs 50.3	0.64 (0.37-1.13)	.13
MMP-9[a] (>2.0 vs ≤ 2.0)	46.9 vs 53.1	1.60 (0.83-3.06)	.16
N-Cadherin[a] (≤5.0 vs > 5.0)	53.7 vs 46.3	0.69 (0.39-1.21)	.19
nm23[a] (≤ 5.0 vs >5.0)	40.8 vs 59.2	0.62 (0.34-1.12)	.11
P21[a] (≤4.0 vs > 4.0)	58.5 vs 41.5	0.77 (0.44-1.35)	.35
P27[a] (≤6.0 vs > 6.0)	42.2 vs 57.8	0.49 (0.28-0.85)	.01
p-ERK[a] (>3.0 vs ≤ 3.0)	43.5 vs 56.5	1.12 (0.58-2.16)	.73
Pontin[a] (>3.0 vs ≤ 3.0)	43.5 vs 56.5	0.86 (0.48-1.54)	.62
Snail[a] (> 4.0 vs ≤ 4.0)	54.4 vs 45.6	1.14 (0.65-2.00)	.65
Stathmin[a] (≤6.0 vs > 6.0)	42.9 vs 57.1	0.69 (0.40-1.22)	.20
Survivin[a] (>3.0 vs ≤ 3.0)	58.5 vs 41.5	1.10 (0.63-1.92)	.75
TIMP-2[a] (>6.0 vs ≤ 6.0)	46.9 vs 53.1	1.07 (0.61-1.88)	.81
Twist[a] (>3.0 vs ≤ 3.0)	55.8 vs 44.2	0.96 (0.55-1.70)	.90
EA-IgA[c] (≤1:40 vs > 1:40)	65.3 vs 34.7	0.89 (0.49-1.63)	.71
VCA-IgA[c] (≤1:160 vs > 1:160)	39.5 vs 60.5	0.86 (0.48-1.56)	.63
AER[c] (≤55.0% vs > 55.0%)	38.8 vs 61.2	0.63 (0.35-1.13)	.12

Abbreviations: AER, antienzyme rate; HR, hazard ratio; CI, confidence interval.

identified in stage III and stage IV. Besides, the range of predicted survival was wider for higher stages.

To further test the efficacy of the nomogram, the calibration curve of the nomogram 1 was plotted (Figure 3d). The *y*-axis was the observed DMFS estimated by the Kaplan-Meier method, and the *x*-axis was the predicted survival computed by the nomogram. As expected, the calibration plot showed a favorable agreement between the nomogram predicted DMFS and the actual observation. The tolerance of variation range was 10%.

Discussion

Nasopharyngeal carcinoma of the undifferentiated histological subtype has a remarkable racial and geographical distribution in Southeast Asia, especially in Cantonese area of China. The tremendous advances of overall prognosis and treatment outcomes during the past few decades have been attributed to the improvement of disease screening and diagnosis, diagnostic imaging, radiotherapy techniques, use of combination systemic therapy, and dedicated clinical and biomarker surveillance.[20,21] Although the standard therapy for NPC has 78% 5-year survival rate, the metastasis hinders the achievement of a higher survival rate, the distant metastasis rate has remained high (occurring in approximately 30% of patients), and ultimately results in death. Therefore, more effective systemic therapy is needed to improve the overall survival for these patients.[3,22,23] If metastasis was diagnosed at an earlier stage, the salvage treatment would achieve more favourable outcome. Therefore, identifying the high-risk distant metastasis individuals is

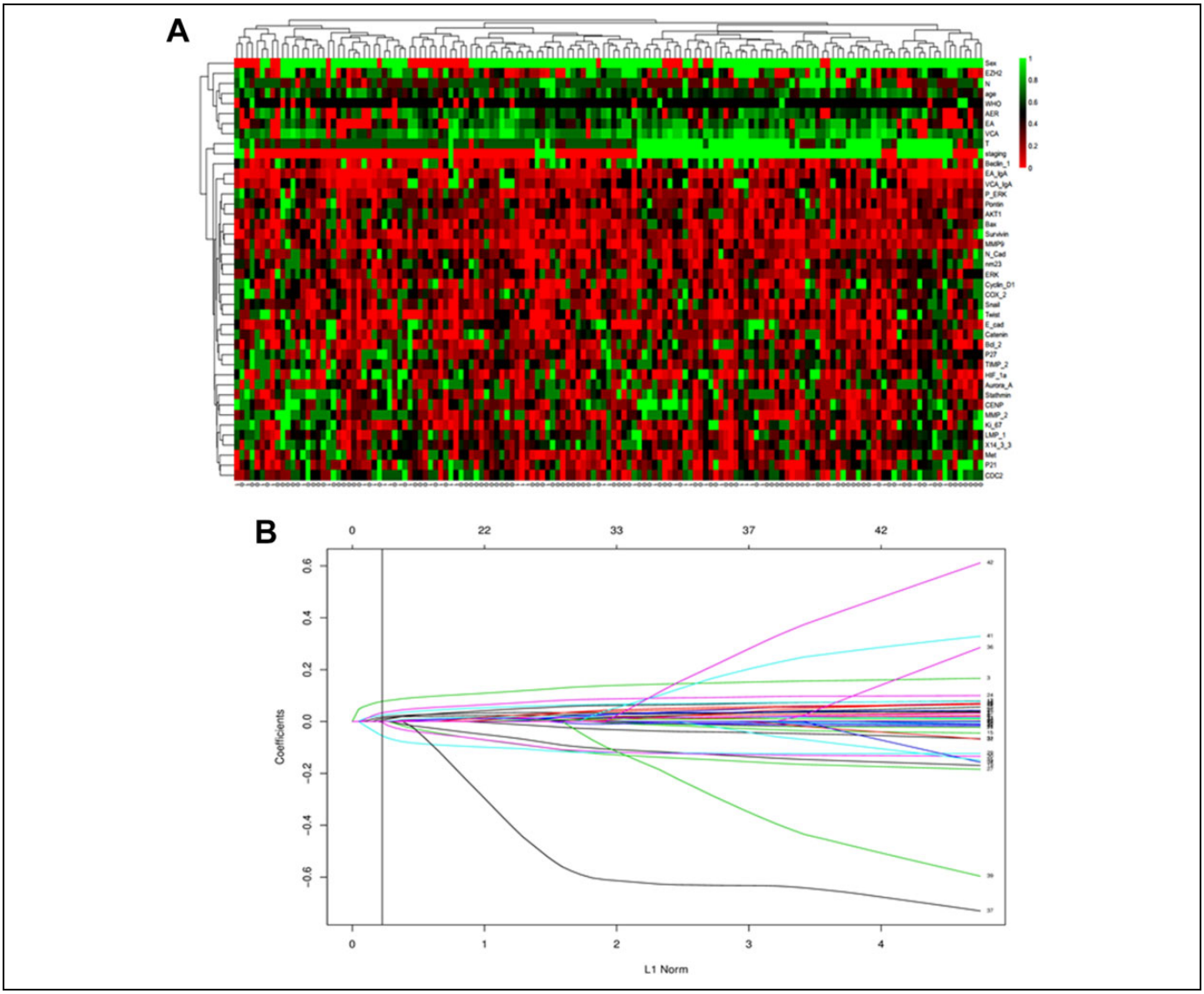

Figure 1. A, Heatmap of the 147 cancer tissues expressed 31 biomarkers. Every row represents an individual biomarker, and each column represents an individual sample. Pseudo colors indicate transcript levels from low to high on a scale from 0 to 1, ranging from a low association strength (bright, red) to high (bright, green). B, The least absolute shrinkage and selection operator coefficient profiles of 34 tumor-related molecular biomarkers and 8 clinicopathological variables associated with DMFS. A vertical line is drawn at the value chosen by 10-fold cross-validation. DMFS indicates distant metastasis-free survival.

Table 2. Selected Variables According to the Cox Proportional Hazards Regression Model.

Variables	HR	95% CI	*P* Value
Beclin 1	1.05	1 to 1.11	.051
Aurora-A	1.12	1.04 to 1.21	.002
Cyclin D1	1.04	0.98 to 1.11	.236
Ki-67	1.06	0.98 to 1.14	.134
Bcl-2	1.05	0.98 to 1.13	.192
P27	1.07	0.99 to 1.16	.082
14-3-3σ	0.86	0.78 to 0.95	.002
MMP-9	0.88	0.73 to 1.06	.187
VCA-IgA	1.00	1 to 1	.270

Abbreviations: HR, hazard ratio; CI, confidence interval.

crucial to provide these approximately 30% of remaining patients a rational personalized therapy and follow-up.

In clinical practice, the TNM staging system is the major tool for clinical decision-making and prognostic determinant for patients with tumor, including NPC. However, a great variation was also observed for the individuals sharing the same cancer stage, suggesting that the TNM staging system is not enough to determine patient outcome. On the other hand, recently, accumulated studies highlighted the use of single or integrated biomarkers to predict the outcome of patients with NPC. For example, enhanced expression of N-cadherin and β-catenin protein was positively correlated with NPC lymph node metastasis and predicted a poorer prognosis in patients with NPC. The impact of serum EBV antibodies on

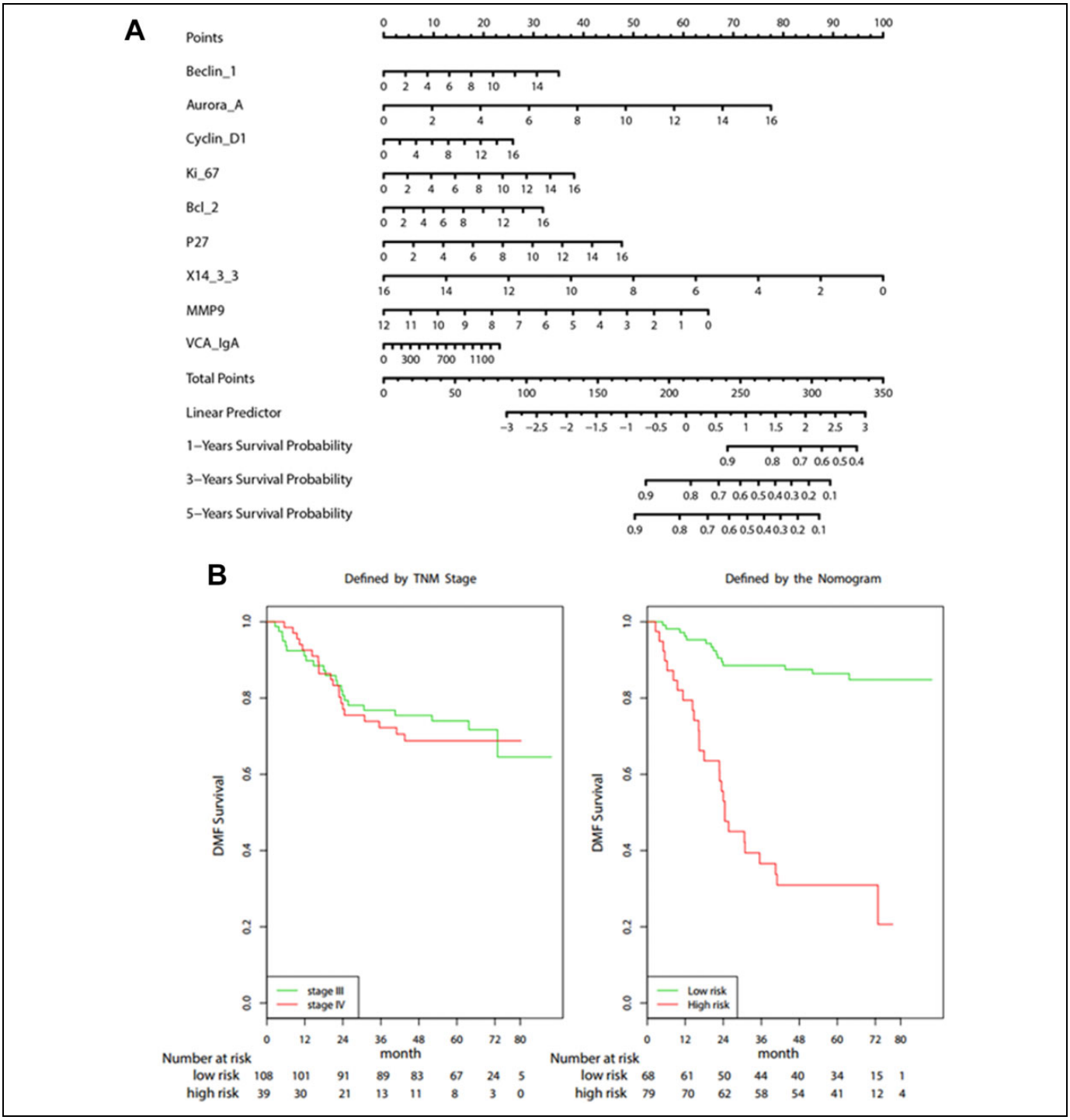

Figure 2. A, Nomogram for predicting 1-, 3- and 5-year DMFS in patients with nasopharyngeal carcinoma. B, Kaplan-Meier survival curves of DMFS: TNM staging system and the Cox proportional hazards model. DMFS indicates distant metastasis-free survival; TNM, tumor–node–metastasis.

the prognosis of patients with nonmetastatic NPC from a population with a high prevalence of both EBV infection and NPC.[24,25] However, the use of a single or several tumor biomarkers is limited due to its low predictive efficacy.[26,27] Thus, it is reasonable to combine TNM staging system and tumor biomarkers to predict the prognosis of patients with tumor. Here, we explored a panel of molecular biomarkers integrated nomogram mathematic approach to identify individual metastasis risk for locally advanced NPC. By measuring the expression levels of 31 biomarkers related to 6 pathological signaling pathways at tumor specimen, and 3 EBV-related biomarkers at serum level, we constructed a

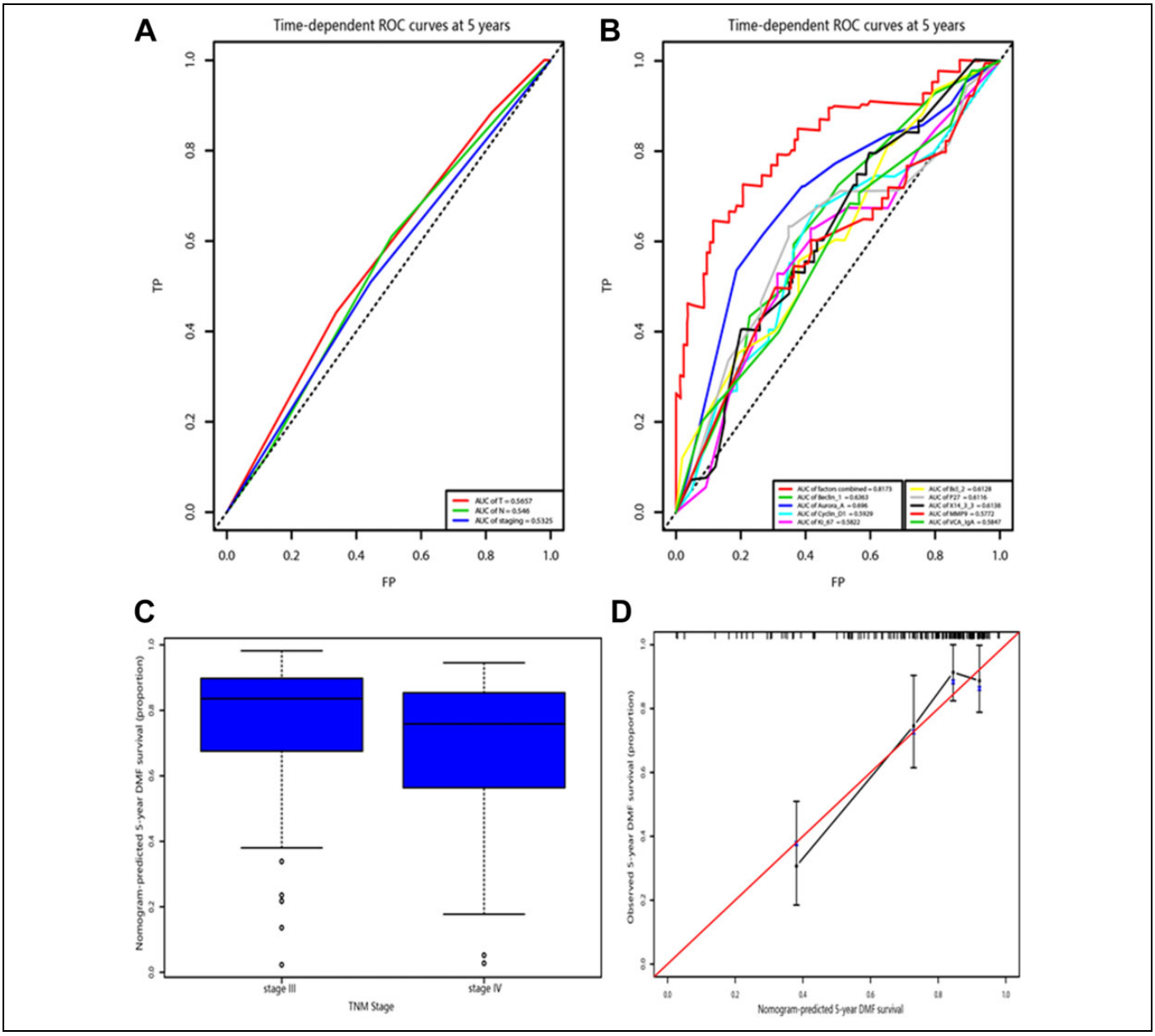

Figure 3. A, Receiver operating characteristic curve for the TNM stage. B, Receiver operating characteristic curve for the model and the selected biomarkers. C, The box plot represents the distribution of nomogram-predicted 5-year survival according the TNM staging system. D, Calibration of the nomogram. The *x*-axis represents the nomogram-predicted survival, and the *y*-axis represents actual survival and 95% CIs measured by Kaplan-Meier analysis. CI indicates confidence interval; DMFS, distant metastasis-free survival; TNM, tumor–node–metastasis.

simple graphical nomogram which might effectively define the risk of metastasis personally.

Indeed, our LASSO Cox proportional regression analysis allowed us to integrate multiple biomarkers into 1o nomogram, which had a higher predictive accuracy than the traditional TNM staging system, where the 3-year DMFS was 33.3% for the high-risk group, and 82.4% for the low-risk group (HR: 7.527, 95% CI: 3.978-14.242, $P < .0001$). Furthermore, patients with the same cancer stage were able to be stratified into different risk groups through this nomogram.[28,29]

Nasopharyngeal carcinoma is a heterogeneous disease since the outcomes vary between the patients with similar clinical and pathological features. And metastasis is a major hallmark for malignant tumors which accounts for the majority of cancer-related deaths including NPC.[30,31] Application of this nomogram to recognize high- and low-risk metastatic population has a vast benefit. First, it complements and segregates the TNM staging system for prognostication instead of the predictive limitation seen in TNM staging system or biomarkers when used alone. Second, the risk stratification can guide treatment decision for patients to be treated by radiotherapy (RT) alone or RT with chemotherapy or IC/CCRT or other personalized therapy based on the risk definition. By this way, patients with high risk of metastasis could benefit from intensive therapy-like

targeted therapy, intensity-modulated radiation therapy, whereas those with low risk could avoid unnecessary side effects.[11,32,33] Although further large scale, prospective, multicenter studies are needed to test these strategies for clinical use. Third, the follow-up protocol could be improved to cut excess economic cost. Moreover, the methods for measuring biomarker expression and EBV-related antibodies are practicable and efficient making our result more adaptable to clinical practice.

While our proposed nomogram has the potential to precisely identify the individual metastasis risk, the method may pose some limitations. For example, our study is limited in terms of generalizability since all our data obtained from patients in China and the distribution of clinical characteristics may vary among other areas. Besides, there is no external validation of the model, additional sets of independent samples will be needed to confirm our findings.

In conclusion, our study demonstrated promising multibiomarkers integrated nomogram in predicting NPC metastasis risk and may potentially guide the treatment decisions and target therapy against the related signaling pathways for patients with locally advanced NPC.

Authors' Note

The authors Xinjuan Fan, Ya Xie, Haiyang Chen, and Xiaobo Guo equally contributed to this work and are coprimary authors. X. J. F., Y. X., X. B. W., and J. Z. designed the study. H. Y. C., Y. M., X. L. P., Y. H., and M. L. drafted the manuscript. F. H., S. L., Y. Z. Y., M. H. H. and J. X. provided the oversight of provider accrual for the study. X. B. G. performed statistical analyses. All authors reviewed the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect tothe research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

Ethics Statement

The study was approved by the Sixth Affiliated Hospital of Sun Yat-sen University (Approval No. 2019ZSLYEC-111), and each participant signed an informed consent at their recruitment. The whole research was conducted in accordance with the approved guidelines.

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