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ORIGINAL RESEARCH

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Neutrophil to apolipoprotein A-I ratio as an independent indicator of locally advanced nasopharyngeal carcinoma

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Abstract

Purpose: To explore the peripheral blood cells (neutrophil/monocyte/lymphocyte/platelet) to apolipoprotein AI or high-density lipoprotein-cholesterol ratio (NAR, MAR, LAR, PAR, NHR, MHR, LHR, and PHR) as independent prognostic indicators for stage III nasopharyngeal carcinoma (NPC).

Patients and methods: Between 2009 and 2014, 562 patients diagnosed with stage III NPC who were treated with a concomitant chemotherapy and intensity-modulated radiotherapy with cumulative cisplatin dose ≥200 mg/m^2 were included in this retrospective study. Routine blood and biochemical variables and baseline clinical characteristics (T and N stage, age, sex, and induction chemotherapy) were collected. After inserting 19 hematological parameters into a set, we applied the least absolute shrinkage and selection operator (LASSO) algorithm and restricted cubic splines regression to select valuable parameters for predicting 5-year overall survival (OS). Subsequently, univariate and multivariate survival analyses were used to assess independent indicators of 5-year OS, distant metastasis survival, regional recurrence-free survival (RRFS), and disease-free survival.

Results: NAR, MAR, serum lactated dehydrogenase (LDH), and Epstein-Barr virus (EBV)-DNA were selected using LASSO regression, and the optimal cut-off values for NAR, MAR, EBV-DNA, and, LDH were 4.39, 0.3, 1590 copies/mL, and 218.4 IU/L, respectively. In multivariate survival analysis, higher NAR was associated with both poor 5-year OS and RRFS (hazard ratio [HR], 1.88; 95% confidence interval [CI], 1.09-3.25, $P = .024$; HR, 3.13; 95% CI, 1.42-6.91, $P = .005$, respectively).

Conclusion: NAR could be an attractive indicator for evaluating the 5-year OS in patients with stage III NPC, which is closely related to inflammation and circulating lipid metabolism.

Level of Evidence: 4

KEYWORDS
apolipoprotein AI, nasopharyngeal carcinoma, neutrophil, prognostic, ratio

1 | INTRODUCTION

Based on the tumor node metastasis (TNM) system, nearly one-third of patients with stage III to IV nasopharyngeal carcinoma (NPC) have a poorer survival rate than those in the early stage and died within 5 years of the initial diagnosis.[1] This phenomenon prompted us to search for prognostic factors associated with this subgroup of patients to aid in formulating adequate treatment strategies for high-risk patients. The pathophysiology of NPC has several speculated risk factors, and the most widely accepted is Epstein-Barr virus (EBV) infection.[2,3] Recent advances in genetic molecular biotechnology have led to the discovery of newly proposed potential mechanisms for tumor development, such as chronic inflammation and metabolic imbalance.[4] Furthermore, numerous potential blood biomarkers have been found in head and neck cancers.[5] Previous studies reported that some routine blood parameters, such as the platelet- and neutrophil-lymphocyte ratio (PLR and NLR, respectively) and red blood cell distribution width (RDW), could be used to evaluate the chronic inflammatory state in each patient and predict survival in those with locally advanced NPC (LA-NPC).[6-10] However, studies on the predictive effect of routine blood biomarkers have reported inconsistent results. For example, increased NLR was shown to be a poor independent risk factor in NPC patients,[6,7] while other studies found no significant association of this marker with survival.[8,9] Inflammation and metabolic status indexes (apolipoprotein-AI [apoA-I], high-density lipoprotein-cholesterol [HDL-C]) were shown to be protective risk factors in patients with locally advanced stage III to IV NPC patients.[11-13] ApoA-I is the core apolipoprotein component in HDL-C,[14] and HDL-C/apoA-I exerts anti-inflammatory and antithrombotic functions by interacting with routine peripheral blood routine indexes (neutrophil, monocyte, lymphocyte, and platelet).[15-18] Therefore, we suspected that the ratio of routinely measured peripheral blood routine cells to apoA-I or HDL-C ratio (NAR, MAR, LAR, PAR, NHR, MHR, LHR, and PHR, respectively) may serve as new inflammation and lipid metabolic markers.

Although induction chemotherapy (IC) plus concurrent chemoradiotherapy (CCRT) treatment regimen gain recurrence-free survival (RRFS) benefit for NPC patients with stage IVa, we noticed that the treatment benefit from IC plus CCRT is still unclear for patients with stage III, when compared with CCRT alone.[19,20] One randomized controlled trial (RCT) has reported a higher RRFS probability for IC plus CCRT treatment regime as compared with CCRT alone in NPC patients with stage III to IV,[19] while another RCT showed no significant RRFS benefit for IC plus CCRT plan in NPC patients with stage III.[20] Meanwhile, concomitant intensity-modulated radiation therapy with chemotherapy (chemo-IMRT) was recommended as a radical therapy for NPC patients with stage III to IV.[21] The cumulative cisplatin dose (CCD) ≥200 mg/m^2 throughout treatment was an independent favorable factor for survival.[22] As previous studies investigating the prognostic markers in the peripheral blood did not consider treatment plan as confounding factor. Thus, we aimed to evaluate the independent factors in the routine hematological parameter set (including the new markers: peripheral blood cells to apoA-I or HDL-C ratio) for stage III NPC patients who received concomitant chemo-IMRT with CCD ≥200 mg/m^2.

2 | MATERIALS AND METHODS

2.1 | Patients

This retrospective study was approved by the Institutional Review Board of the Sun Yat-sen University Cancer Center (No. YB2020-412-01). The present study followed the Declaration of Helsinki, and the need for informed consent was waived. The essential raw data were uploaded to the Research Data Deposit public platform (RDD, http://www.researchdata.org.cn), with the RDD approval number of RDDA2020001746. Patients diagnosed with NPC at the Department of Radiation Oncology, Sun Yat-sen University Cancer Center between December 2009 and December 2014 were retrospectively enrolled. The eligibility criteria were as follow: (i) stage III (T0-2N2M0, T3N0-2M0) according to the American Joint Committee on Cancer Staging System (seventh version); (ii) diagnosis of as non-keratinizing differentiated carcinoma or non-keratinizing undifferentiated carcinoma; (iii) patients treated with or without IC, and completed concomitant chemo-IMRT with CCD ≥200 mg/m^2; and (iv) hematological variables recorded before radical treatment. The exclusion criteria were as follows: (i) concomitant treatment with adjuvant chemotherapy; and (ii) previously diagnosed chronic comorbidities (including hypertension, diabetes, blood system disease, chronic hepatitis, or chronic kidney disease) or another malignant tumor. A flowchart is shown in Figure 1.

2.2 | Blood test and data collection

We reviewed the data available from the electronic medical records system. Complete patient data, including age, sex, T stage, N stage, EBV-DNA copy numbers before initial treatment, and IC usage, were recorded. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes. Routine peripheral blood cell parameters (neutrophils, lymphocytes, monocytes, platelets, and RDW) were analyzed using a Sysmex automated blood analyzer and related reagents (Sysmex, Kobe, Japan). Serum biochemical parameters, including serum lactate dehydrogenase (LDH), HDL-C, total bile acid (TBA), apoA-I, C-reactive protein (CRP), total bilirubin (TBIL), and serum albumin, were assayed using a HITACHI automatic biochemical analyzer and related reagents (HITACHI, Tokyo, Japan). NLR and PLR were calculated as the neutrophil/platelet count (10^9/L) to lymphocyte (10^9/L) ratio, respectively. The formula for the remaining combined indicators is listed below:

NAR: neutrophil count (10^9/L) to apoA-I (g/L) ratio.
MAR: monocyte count (10^9/L) to apoA-I (g/L) ratio.
LAR: lymphocyte count (10^9/L) to apoA-I (g/L) ratio.
PAR: platelet count (10^9/L) to apoA-I (g/L) ratio.
NHR: neutrophil count (10^9/L) to HDL-C (mmol/L) ratio.
MHR: monocyte count (10^9/L) to HDL-C (mmol/L) ratio.
LHR: lymphocyte count (10^9/L) to HDL-C (mmol/L) ratio.
PAR: platelet count (10^9/L) to HDL-C (mmol/L) ratio.
PNI: albumin level (g/L) + 0.005 × lymphocyte count (per mm^3).[23]

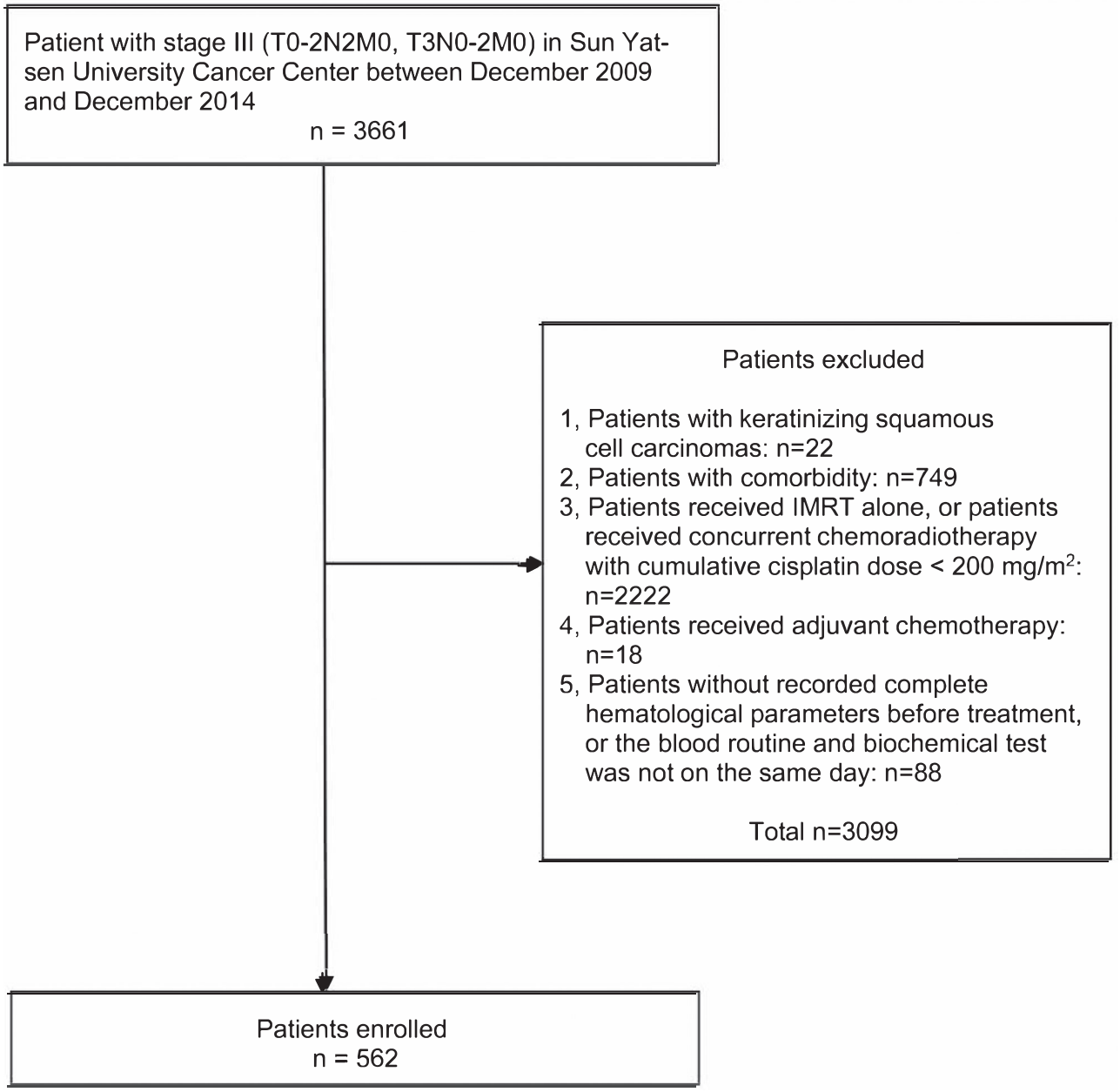


FIGURE 1 Flowchart of patient inclusion and exclusion process

2.3 | Treatment method and follow-up

Patients were treated with radical IMRT, and the detailed prescription dosages have been reported previously.[24] IC regimens contained four alternative regimens: TPF (docetaxel [60 mg/m^2] and cisplatin [60 mg/m^2] on day 1 with 5-fluorouracil [600-750 mg/m^2, over 120 hours]), PF (cisplatin [80 mg/m^2] on day 1 with 5-fluorouracil [750-1000 mg/m^2, over 120 hours]), TP (docetaxel [75 mg/m^2] and cisplatin [75 mg/m^2] on day 1), and GP (gemcitabine [1000 mg/m^2] on day 1 and day 8 with cisplatin [80 mg/m^2] on day 1). Each regimen was repeated every 3 weeks. Patients were treated with platinum (80 mg/m^2 or 100 mg/m^2 each 3 weeks, or 40 mg/m^2 weekly) during radiotherapy, and the CCD was calculated after concomitant chemo-IMRT. Patients underwent hematological (EBV-DNA copy numbers) and radiological examinations (magnetic resonance examination of the nasopharyngeal region and neck region, chest radiography, and abdominal ultrasound) every 3 months in the first 2 years and every 6 to 12 months afterwards. Follow-up period lasted until August 2020. The endpoint was a 5-year overall survival (OS) time, which was defined as the interval between the initial treatment to death, and the secondary endpoints were 5-year distant metastasis-free survival (DMFS), RRFS, local recurrence-free survival (LRFS), and disease-free survival (DFS), which were defined as the time from treatment to first distant metastasis, regional failure, local failure, and death for any cause, respectively.

2.4 | Statistical analysis

The least absolute shrinkage and selection operator (LASSO) regression algorithm was used to find the optimal prognostic parameters via cross-validation criterion.[25,26] Subsequently, the restricted cubic splines (RCS) regression analysis was applied to assess the monotonic linear relationship between the selected parameters and the 5-year OS.[27] If a variable in the former step met a monotonic linear relationship with 5-year OS, the optimal cut-off value for this parameter was calculated using time-dependent receiver operating characteristic (ROC) curve analysis[28] and then dichotomized using the optimal cut-off value. We used the 5-year OS status as a binary variable to construct a logistic regression equation. Delong's test was performed to compare the discriminability of the different parameters. Subsequently, a binary variable set was obtained. To find the prognosis-related parameters in this set, univariate analysis was performed using the Kaplan-Meier method for 5-years OS, DMFS, RRFS, LRFS, and DFS, respectively, and a log-rank test was performed to evaluate the differences between groups. Under the premise of assuming proportional hazards, the multivariate Cox regression model was applied to assess independent survival prognostic factors for endpoints, and the Schoenfeld residuals plot was used to check whether the variables met the proportional hazard assumption.[29] For clinical considerations, we also included T and N stage, sex, age, and IC treatment, which were generally considered to be correlated with survival rate in patients with stage III in multivariate Cox regression analysis.[20,21] Previous studies revealed no significant difference in survival rate between non-keratinizing differentiated type and non-keratinizing undifferentiated type.[30-32] Therefore, we did not include pathologic subtype as a covariate in multivariate COX analysis. As the NLR indicator could reflect the immune status in each patient, we chose NLR as the reference variable when comparing the predictive performance of the investigated indicators. The ROC curve and DeLong's test were calculated using a MedCalc software, and other statistical analyses were performed using R (ver. 3.6.1). A two-tailed *P* value of no more than .05 was considered statistically significant.

3 | RESULTS

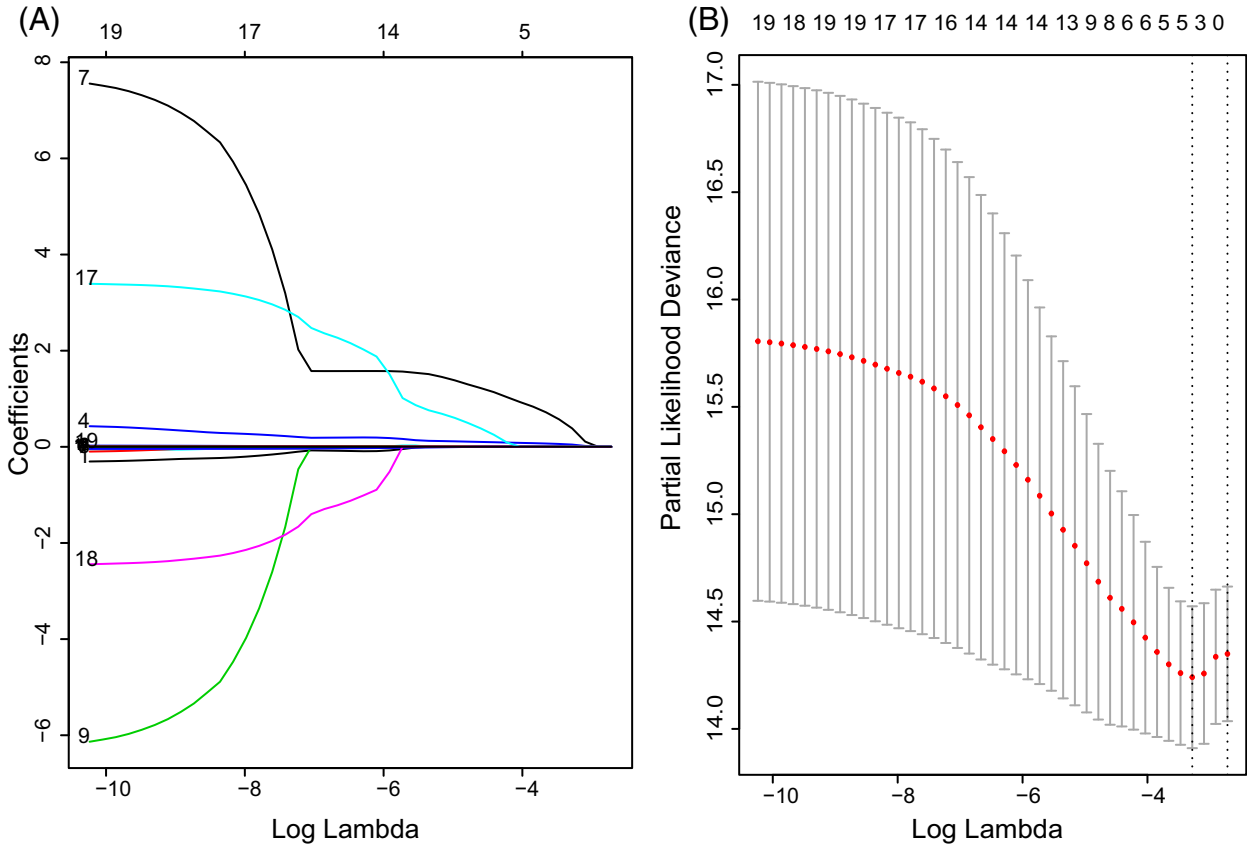
3.1 | Characteristics of study participants

A total of 562 patients were eligible to be included in the present retrospective cohort. When considering all patients, the median follow-up period was 76.97 (range, 8.33-114.30) months. As shown in Table 1, the median age in the entire patient cohort was 43 (range, 37-49) years; 64 (11.40%) patients were diagnosed with T1-2 and 498 (88.60%) patients with T3, 371 (66.0%) with N0-1, and 191 (34.0%) with N2. Four hundred and eight (72.6%) patients were treated with concomitant chemo-IMRT alone, and 154 (27.4%) patients underwent IC plus concomitant chemo-IMRT. Sixty-three (11.2%) patients died during follow-up, and the number of patients who experienced disease recurrence, regional recurrence, local recurrence, and distant metastasis were 107 (19.0%), 33 (5.9%), 36(6.4%), and 59 (10.5%), respectively.

TABLE 1 Patient characteristics (frequency and percentage/median [interquartile range])

Features	Frequency (percentage) or median [interquartile range]
Blood index	
NLR	2.17 [1.64, 2.88]
NAR	3.44 [2.66, 4.38]
MAR	0.35 [0.26, 0.45]
LDH (IU/L)	175.60 [154.07, 201.38]
CRP (mg/L)	1.56 [0.74, 3.18]
apoA-I (g/L)	1.23 [1.11, 1.36]
EBV-DNA (copies/L)	2.58 [0.00, 19.00]
T stage	
T1 + 2 stage	64 (11.40)
T3 stage	498 (88.60)
N stage	
N0 + 1 stage	371 (66.00)
N2 stage	191 (34.00)
Age, y	43 [37, 49]
Sex	
Male	405 (72.10)
Female	157 (27.90)
Induction chemotherapy	
Yes	154 (27.40)
No	408 (72.60)
Death	
Yes	63 (11.20)
No	499 (88.80)
Distant metastasis	
Yes	59 (10.50)
No	503 (89.50)
Regional recurrence	
Yes	33 (5.90)
No	529 (94.10)
Local recurrence	
Yes	36 (6.40)
No	526 (93.60)
Disease recurrence	
Yes	107 (19.00)
No	455 (81.00)

Note: Peripheral blood cells (neutrophil/monocyte) to apolipoprotein-AI (apoA-I) ratio (NAR, MAR, respectively).
Abbreviations: CRP, C-reactive protein; EBV-DNA, Epstein-Barr virus; LDH, serum lactate dehydrogenase; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.



(C)

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NLR       .
PLR       .
RDW       .
NAR       0.0469340997
LAR       .
PAR       .
MAR       0.3838568109
NHR       .
MHR       .
PHR       .
LHR       .
PNI       .
TBA       .
LDH       0.0010200867
TBIL      .
CRP       .
HDL       .
apoA      .
EBV.DNA   0.0003435476
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FIGURE 2 Peripheral blood features were selected by the LASSO (least absolute shrinkage and selection operator) regression. (A) The coefficient profile of hematology parameters selected by LASSO regression. (B) Partial likelihood deviance for the LASSO coefficient profiles. The first vertical dash line with the optimal lambda ($\lambda = 0.0375$), corresponding to the minimum partial likelihood deviance. (C) Four features with non-zero coefficients were selected from 19 features, the minimum λ is 0.0375, via 10-fold cross-validation. CRP, C-reactive protein; EBV-DNA, Epstein-Barr virus-DNA; LDH, serum lactate dehydrogenase; NLR, neutrophil to lymphocyte ratio; PNI, prognostic nutritional index; PLR, platelet to lymphocyte ratio; Peripheral blood cells (neutrophil/monocyte/lymphocyte/platelet) to apolipoprotein A1 (apoA-I) or HDL-cholesterol (HDL-C) ratio (NAR, MAR, LAR, PAR, NHR, MHR, LHR, and PHR, respectively); RDW, red blood cell distribution width; TBA, total bile acid; TBIL, total bilirubin

3.2 | Variable selection and Cut-off values

In total, 19 features (NLR, PLR, RDW, NAR, LAR, PAR, MAR, NHR, MHR, PHR, LHR, PNI, TBA, LDH, TBIL, CRP, HDL, apoA-I and EBV-DNA) were included in the LASSO regression algorithm (Figure 2A,B). After calculation, four variables (including NAR, MAR, EBV-DNA, and LDH) with non-zero coefficients were selected using the LASSO regression with an optimal lambda of .0375 (Figure 2C).

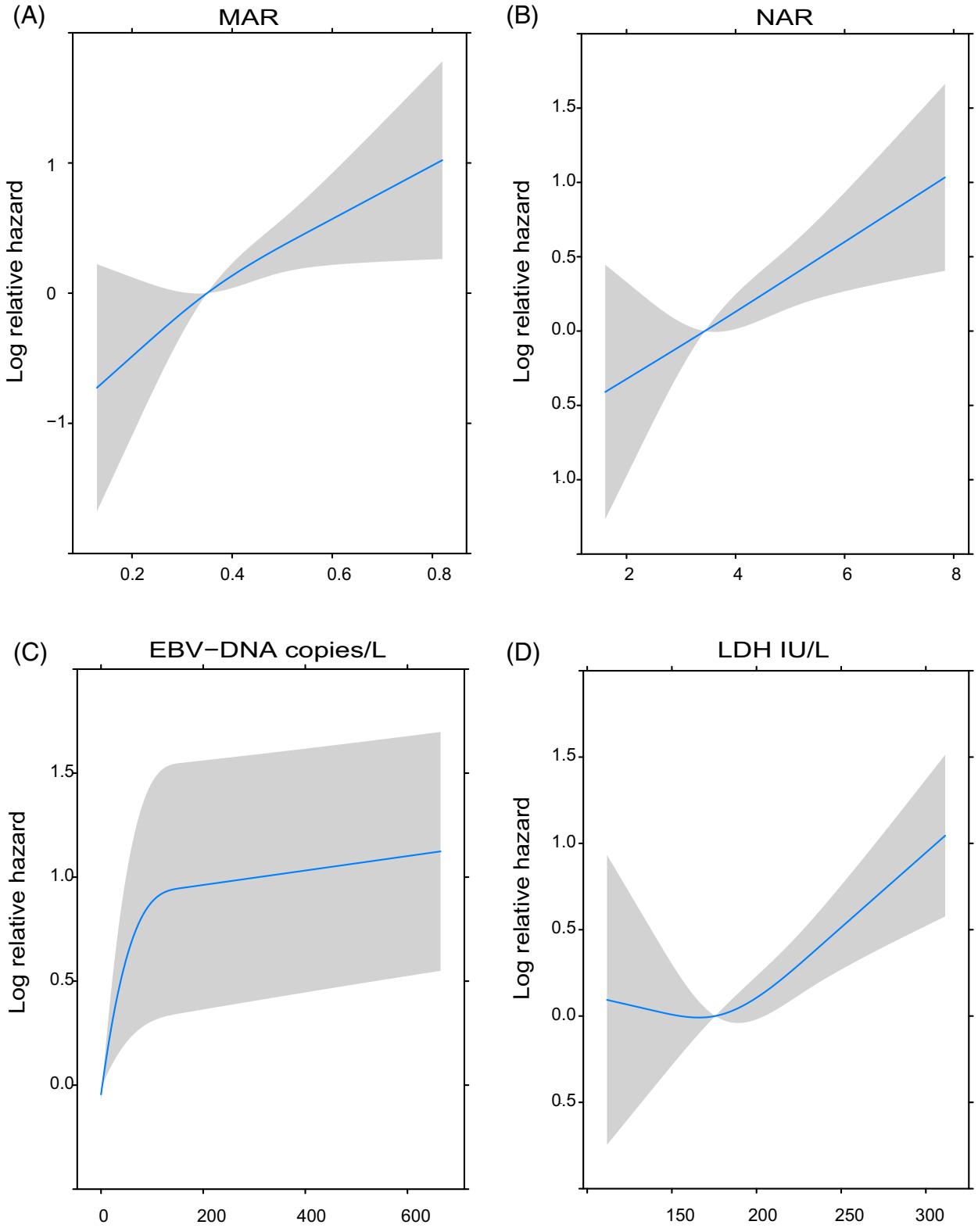

FIGURE 3 Association between (A) the monocyte to the apolipoprotein A-I ratio (MAR), (B) the neutrophil to apolipoprotein A-I ratio (NAR), (C) the Epstein-Barr virus (EBV)-DNA copy numbers, (D) serum lactate dehydrogenase (LDH) value and log relative hazard in restricted cubic spline plot

The RCS showed that NAR, MAR, EBV-DNA, and LDH all had a monotonic and nonlinear relationship with 5-year OS (Figure 3). Using the time-dependent ROC curves, the optimum binary cut-off values and corresponding areas under the curve (AUC) for NAR, MAR, EBV-DNA, and LDH were 4.39, 0.3, 1590 copies/mL, and 218.4 IU/L, respectively (Figure 4). Notably, the AUC values

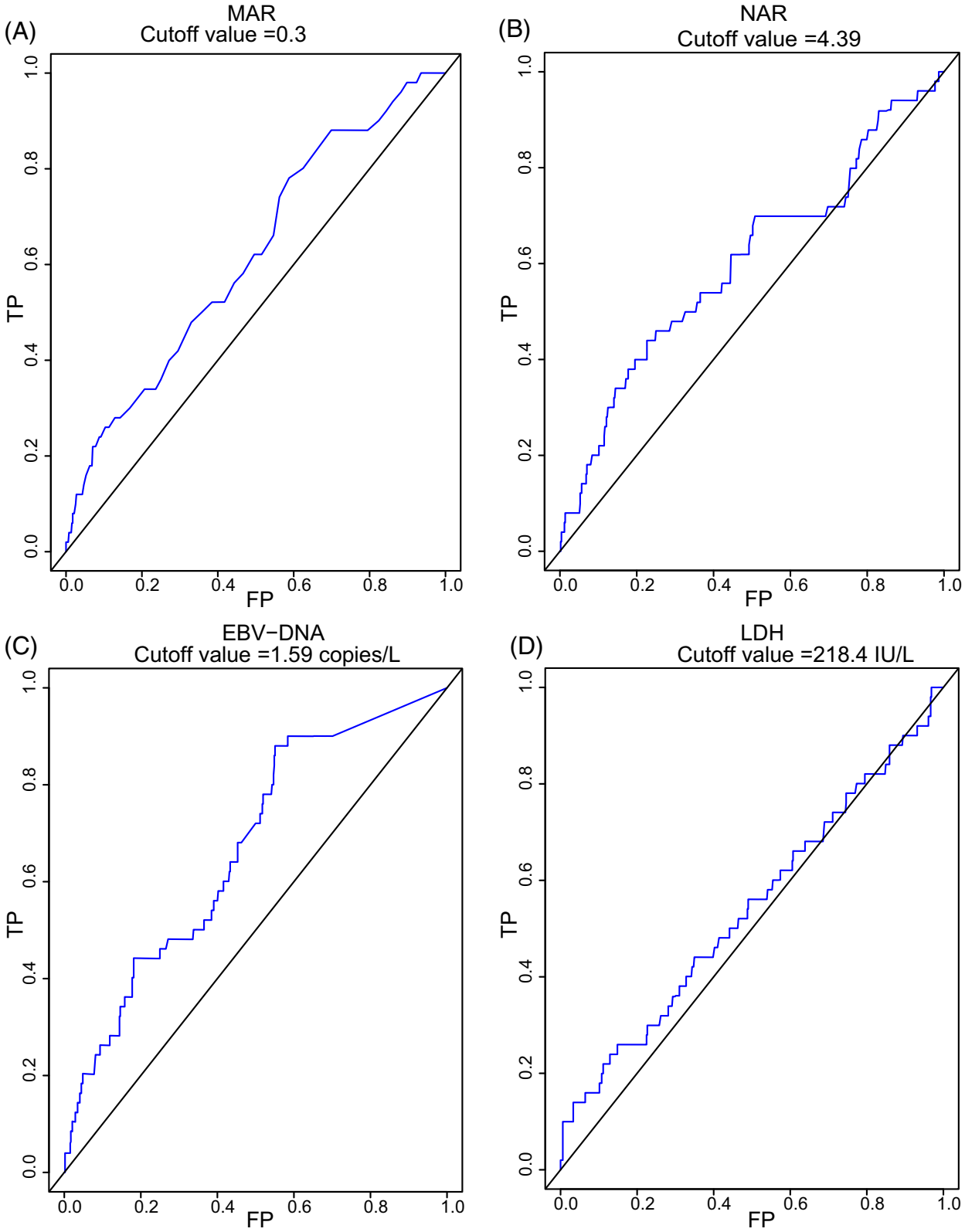

FIGURE 4 Time-dependent ROC curve analysis of the cut-off value for (A) monocyte to apolipoprotein A-I ratio (MAR), (B) neutrophil to apolipoprotein A1 ratio (NAR), (C) Epstein-Barr virus (EBV)-DNA copy numbers, and (D) serum lactate dehydrogenase (LDH) value for predicting 5-year overall survival. ROC, receiver operating curve

TABLE 2 Comparing of prognostic parameters in patients with stage III

	5-year overall survival		
	AUC	95% CI	*P*-Value
NLR	0.535	0.492-0.576	Reference
NAR	0.614	0.573-0.655	$P = .041$*
EBV-DNA	0.655	0.614-0.694	$P = .023$*
LDH	0.559	0.517-0.600	$P = .640$
MAR	0.609	0.568-0.650	$P = .158$

Abbreviations: AUC, area under curve; CI, confidence interval; See Table 1 for other abbreviations.
*P <.05.

of NAR and EBV-DNA were all significantly higher than those of NLR ($P = .041$, and $P = .023$, respectively; Table 2).

3.3 | Survival analysis

As shown in Figure 5, in the univariate analysis, NAR value of >4.39 predicted poor 5-year OS, DMFS, RRFS, LRFS, and DFS ($P = .0001$, .0430, .0010, .2100, and .0056, respectively). A high level of NAR value was found to be an indicator of poor 5-year OS and 5-year RRFS in the multivariate analysis, however, that significance was not found for 5-year DMFS, LRFS, or DFS (Table 3). In the multivariate Cox regression analysis, NAR (hazard ratio [HR] = 1.88, 95% confidence interval [CI] 1.09-3.25), EBV-DNA (>1500 copies/mL vs ≤1500 copies/mL, HR = 4.21, 95% CI, 2.04-8.67), N stage (N2 vs N0 + N1: HR = 2.05; 95% CI, 1.20-3.50) and IC (HR = 0.40; 95% CI, 0.21-0.77) were all independent prognostic factors for 5-year OS. Meanwhile, EBV-DNA (>1500 copies/mL vs ≤1500 copies/mL, HR = 2.70, 95% CI, 1.41-5.17; HR = 1.92, 95% CI, 1.24-2.99, respectively), N stage (N2 vs N0 + N1: HR = 2.12; 95% CI, 1.21-3.73; HR = 1.77, 95% CI, 1.16-2.71, respectively), and IC (HR = 0.49; 95% CI, 0.26-0.93; HR = 0.58, 95% CI, 0.36-0.92, respectively) were found to be independent prognostic indicators of for 5-year DMFS and DFS. Furthermore, LDH level (HR = 2.04, 95% CI 1.14-3.62) was an independent predictor of 5-year DMFS. The Schoenfeld residual plot showed that no variable regularly changed over time in the 5-year OS multivariate Cox regression analysis (Figure 6).

4 | DISCUSSION

It is generally believed that N stage and EBV-DNA copy numbers are the important prognostic indicators for LA-NPC,[3] and our results also confirmed the robust prognostic power of both these indicators. In this study, we proposed a new peripheral hematological index as a survival indicator for stage III NPC, and a higher level of NAR was shown to be a risk factor for poor 5-year OS and RRFS, regardless of clinical characteristics and number of EBV-DNA copies. However, NAR did not show its prognostic value when predicting DFS probability. As described in our study, DFS defined as the time from treatment to first distant metastasis, regional failure, local failure, and death from any case. Considering the sum events of tumor metastasis and local recurrence were approximately 3.7 times than that of regional recurrence. The prognostic value of NAR for DFS was inevitably affected by the no significant prognostic value of NAR for DMFS or LRFS. In clinical diagnosis and treatment, DFS are more difficult to obtain than OS, and OS is generally recommended as a practical indicator to evaluate the prognosis of patients. To the best of our knowledge, this is the first time that the NAR index, which reflects the inflammation and lipid metabolism status of each patient with LA-NPC, has been studied as a prognostic factor for survival.

Neutrophils play an important role in innate immunity.[33] Generally, neutrophils represent the largest proportion of the peripheral polymorphonuclear granulocytes (PMN), that are recruited by the tumor, potentially promoting tumor progression by releasing matrix metalloprotein 9 (MMP-9) and neutrophil elastase.[34-37] Trellakis et al also showed that PMN counts in head and neck squamous cell carcinoma (HNSCC) patients were obviously higher than those in healthy volunteers, while the peripheral lymphocyte counts were almost the same between the two group.[38] Moreover, the median and high level of infiltrating PMN in tumor tissue was associated with a poor 5-year survival rate in locally advanced HNSCC. Previous studies have consistently reported that increased neutrophil count before treatment was associated with a poor prognosis in NPC patients.[39] Sumner et al also reported that higher neutrophils levels predicted poor survival in patients with oropharyngeal and laryngeal cancers.[40] Thus, we believe that for patients with HNSCC, the neutrophil count could be a prognostic factor in predicting survival.

HDL-C/apoA-I regulates the circulating lipid metabolism, and increased apoA-I level inhibit the infinite proliferation and migration potential of tumors.[41] A recent study showed that high levels of apoA-I could be a protective factor for 5-year OS in patients with LA-NPC.[11] The potential independent prognostic effect of NAR could be explained by the adhesion between neutrophils, endothelial cells, and tumor cells promoting the migration and invasion of tumor cells.[42-46] In this process, the neutrophils activates and over-expresses CD11b and intercellular adhesion molecule-1 (ICAM-1) in the membrane, and the neutrophils would adhere to endothelial cells and tumor cells via the Mac-1/intercellular adhesion molecule-1 (ICAM-1) pair. However, the activated-expression of CD11b and ICAM-1 on the neutrophil membrane and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells would be reduced by apoA-I.[16,47] Therefore, lower circulating apoA-I would not inhibit the neutrophils or neutrophils-circulating tumor cells clusters moving and invading areas rich in chronic inflammatory factors (such as the tumor site), thereby resulting in an increased number of infiltrating neutrophils in tumor tissue. This then reduces the survival rate. Additionally, the recruited CD11b positive cells in hypoxia areas of the tumor have been reported to stimulate the lymphangiogenesis and lymph node metastasis in a glossectomy orthotopic mouse model of tongue cancer.[48]

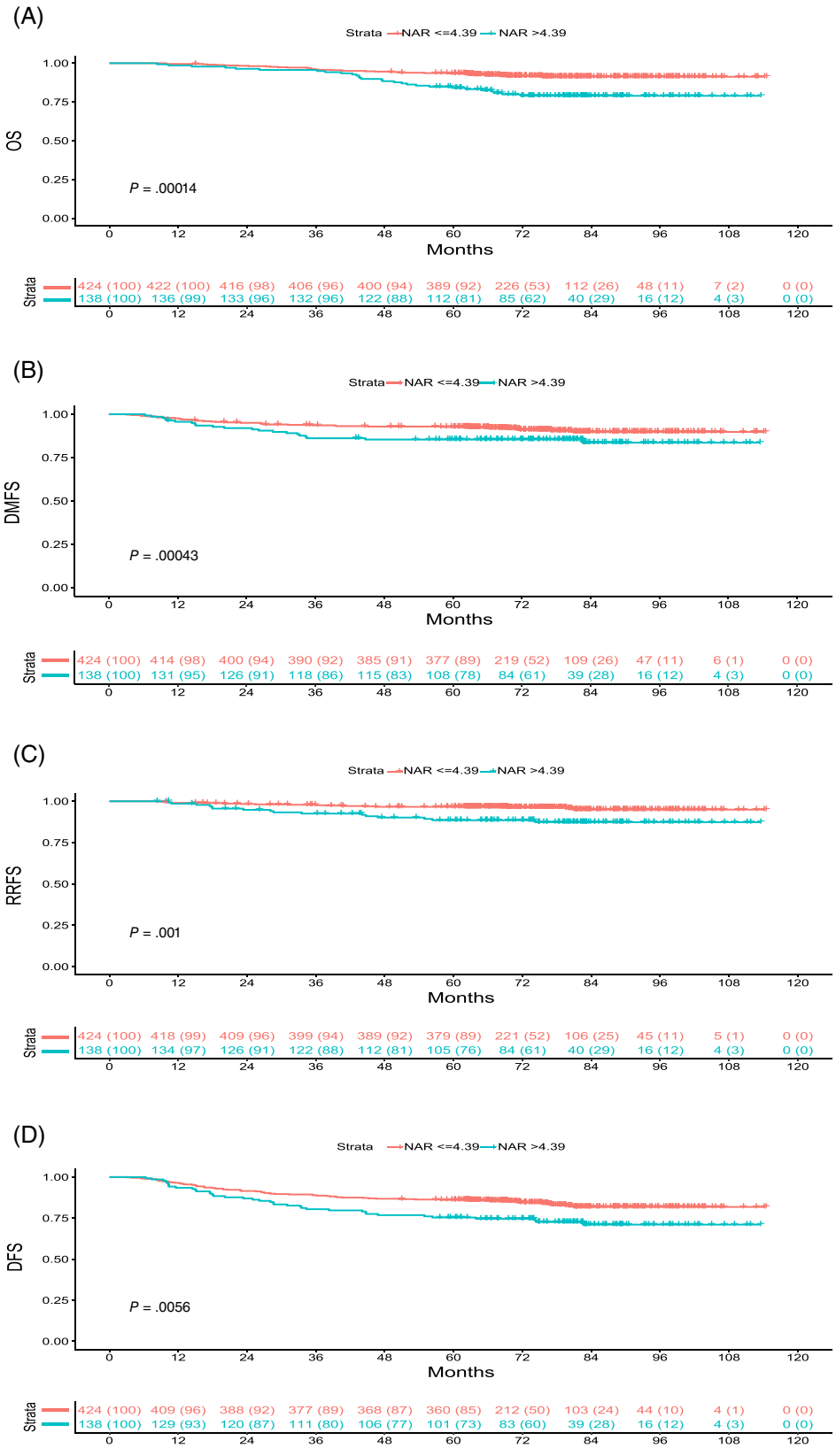

FIGURE 5 (A) 5-year overall survival curve stratified by dichotomous neutrophil to apolipoprotein A-I ratio (NAR), (B) 5-year distant metastasis curve stratified by NAR, (C) 5-year regional recurrence-free curve stratified by NAR, (D) 5-year disease-free survival curve stratified by NAR. P-values were calculated by a log-rank test. DFS, disease-free survival; DMFS, distant metastasis-free survival; OS, overall survival; RRFS, regional recurrence-free survival

Similarly, our study also showed that the patients with an elevated NAR value pretreatment exhibited a high risk of regional recurrence. Moreover, peripheral neutrophils-circulating tumor cells clusters have been reported to promote tumor progression in breast cancer.[49] The key cell-to-cell junction protein was VCAM-1 in this study, while the important protein for the cluster was ICAM-1 as reported by another

TABLE 3 The multivariate analysis for the full cohort

Outcome	Variable	Group	Multivariate analysis HR (95% CI)	*P*-Value
OS	NAR	>4.39 vs ≤4.39	1.88 (1.09-3.25)	.024*
	EBV-DNA (copies/mL)	>1500 vs ≤1500	4.21 (2.04-8.67)	<.001**
	MAR	>0.3 vs ≤0.3	1.48 (0.77-2.86)	.236
	LDH (IU/L)	>218.4 vs ≤218.4	1.46 (0.81-2.60)	.206
	N stage	N2 vs N0-1	2.05 (1.20-3.50)	.009**
	T stage	T3 vs T1-2	2.55 (0.97-6.72)	.060
	IC	Yes vs No	0.40 (0.21-0.77)	.006**
	Age	>45 vs ≤45	0.80 (0.45-1.40)	.427
	Gender	Female vs male	0.86 (0.47-1.55)	.614
DMFS	NAR	>4.39 vs ≤ 4.39	1.31 (0.73-2.33)	.364
	EBV-DNA (copies/mL)	>1500 vs ≤1500	2.70 (1.41-5.17)	.003**
	MAR	>0.3 vs ≤0.3	1.26 (0.67-2.40)	.467
	LDH (IU/L)	>218.4 vs ≤218.4	2.04 (1.14-3.62)	.016*
	N stage	N2 vs N0-1	2.12 (1.21-3.73)	.009**
	T stage	T3 vs T1-2	1.85 (0.78-4.39)	.164
	IC	Yes vs No	0.49 (0.26-0.93)	.028*
	Age	>45 vs ≤45	0.73 (0.41-1.29)	.278
	Gender	Female vs male	0.55 (0.28-1.10)	.091
RRFS	NAR	>4.39 vs ≤4.39	3.13 (1.42-6.91)	.005**
	EBV-DNA (copies/mL)	>1500 vs ≤1500	1.05 (0.50-2.20)	.900
	MAR	>0.3 vs ≤0.3	0.90 (0.39-2.12)	.815
	LDH (IU/L)	>218.4 vs ≤218.4	0.48 (0.14-1.61)	.233
	N stage	N2 vs N0-1	2.89 (1.35-6.19)	.006**
	T stage	T3 vs T1-2	1.19 (0.42-3.38)	.750
	IC	Yes vs No	0.93 (0.42-2.03)	.853
	Age	>45 vs ≤45	0.73 (0.33-1.59)	.427
	Gender	Female vs male	1.86 (0.90-3.84)	.095
LRFS	NAR	>4.39 vs ≤4.39	1.78 (0.83-3.82)	.139
	EBV-DNA (copies/mL)	>1500 vs ≤1500	1.38 (0.68-2.79)	.376
	MAR	>0.3 vs ≤0.3	1.66 (0.76-3.59)	.202
	LDH (IU/L)	>218.4 vs ≤218.4	0.78 (0.27-2.23)	.636
	N stage	N2 vs N0-1	0.85 (0.36-1.99)	.708
	T stage	T3 vs T1-2	1.24 (0.32-4.85)	.757
	IC	Yes vs no	0.48 (0.20-1.18)	.112
	Age	>45 vs ≤45	2.03 (1.04-4.00)	.039*
	Gender	Female vs male	2.35 (1.18-4.66)	.015*
DFS	NAR	>4.39 vs ≤4.39	1.42 (0.93-2.19)	.107
	EBV-DNA (copies/mL)	>1500 vs ≤1500	1.92 (1.24-2.99)	.004**
	MAR	>0.3 vs ≤0.3	1.44 (0.91-2.30)	.119
	LDH (IU/L)	>218.4 vs ≤218.4	1.55 (0.98-2.46)	.064
	N stage	N2 vs N0-1	1.77 (1.16-2.71)	.009**
	T stage	T3 vs T1-2	1.61 (0.83-3.13)	.158
	IC	Yes vs no	0.58 (0.36-0.92)	.021*
	Age	>45 vs ≤45	0.84 (0.56-1.28)	.421
	Gender	Female vs male	1.16 (0.75-1.78)	.507

*P <.05.
**P <.01.
Abbreviations: DFS, disease-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio; IC, induction chemotherapy; LRFS, local recurrence-free survival; OS, overall survival; RRFS, regional recurrence-free survival; See Tables 1 and 2 for other abbreviations.

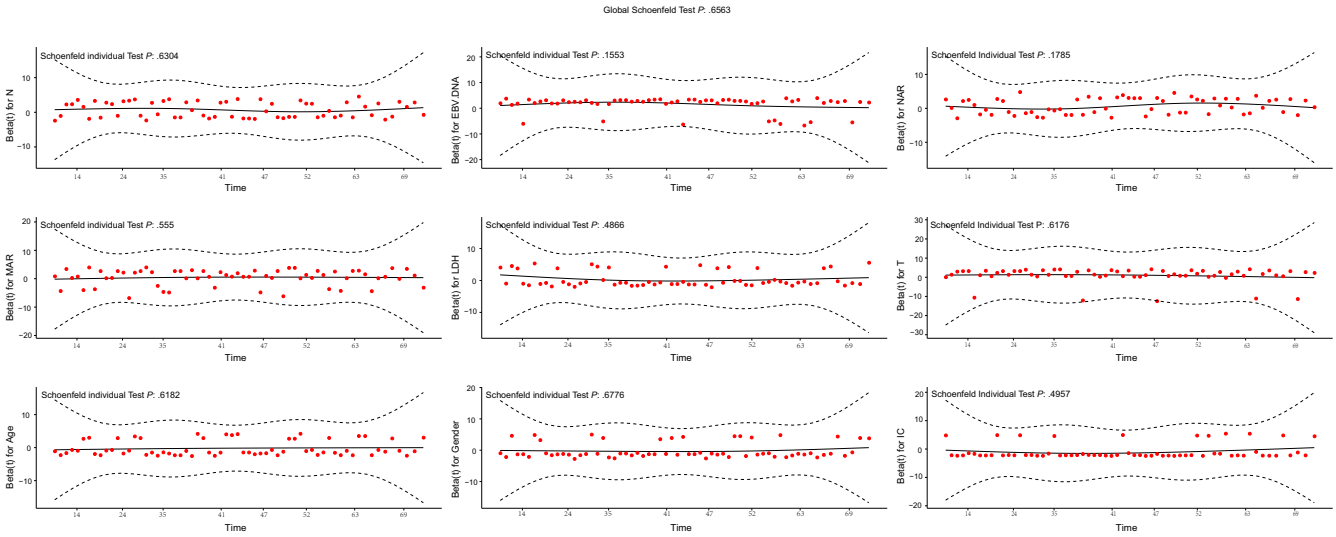

FIGURE 6 Standardized Schoenfeld residual plot related to 5-year overall survival was generated for each selected variable. The solid horizontal lines were basically parallel to the *X*-axis in each panel

study.[44] Considering the heterogeneous expression of adhesion molecules between different tumors, the important cell-to-cell junction protein pair between peripheral neutrophils and circulating tumor cells needs to be identified in patients with NPC.

In this study, the NHR index was not selected after LASSO regression analysis. However, the effect of HDL-C on survival outcomes in patients with NPC provided conflicting results among different studies. Liu et al have reported that increased HDL-C was an independent poor prognostic factor for patients with NPC.[50] In contrast, Yao et al. showed that higher HDL-C value was a protective factor for LA-NPC.[13] As apoA-I is the main component among the lipoproteins of plasma HDL-C, Chang et al showed that apoA-I and not HDL-C was an independent risk factor for 5-year OS and DM in patients with LA-NPC.[11] Meanwhile, apoA-I and HDL-C exerted their effect on CD11b expression through ABCA1 receptor and scavenger receptor B1 (SR-B1), respectively.[41] In addition, a previous study reported that apoA-I showed a faster inhibition rate than HDL-C for CD11b expression on the neutrophil membrane.[16] In summary, apoA-I and HDL-C may have different model of regulating the biological activities of neutrophils. HDL-C/apoA-I could also regulate inflammation status by regulating monocyte/macrophage subtypes and may be involved in converting tumor-associated macrophages from type II to type I.[51] However, CD11b activation promotes macrophage polarization rather than macrophage recruitment at tumor sites.[52] Therefore, although the MAR index was selected after performing LASSO regression, MAR was not shown to be an independent indicator for survival in the multivariate analysis.

Our study also had several limitations. This was a retrospective study with a small sample size and limited outcome events; hence, selection bias was inevitable. The significance of the NAR index on survival prognosis in patients with LA-NPC needs to be confirmed in a large-scale prospective cohort study. Moreover, as cytokines levels reflect the severity of inflammation status in each patient, the lack of cytokine measurement, such as serum interleukin-8, interleukin-2, transforming growth factor-β, C-C motif chemokine ligand 5, and programmed death-1, was another limitation of this study.

5 | CONCLUSION

In this study, the NAR index showed a high correlation with OS and regional control rate. Unlike other blood biomarkers, such as plasma EBV-DNA and circulating tumor cells, the NAR indicator is less costly, easier to obtain without complicated calculation, and is also convenient for dynamic monitoring. Further research is necessary to explore whether the kinetic value of NAR can be a response factor for immunotherapy treatment.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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