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Increased CYFRA 21-1, CEA and NSE are Prognostic of Poor Outcome for Locally Advanced Squamous Cell Carcinoma in Lung: A Nomogram and Recursive Partitioning Risk Stratification Analysis[1,2,3]

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Abstract

OBJECTIVES: This study aimed to: (1) assess the prognostic significance of serum tumor markers in locally advanced squamous cell carcinoma in lung (LA-SCCL); (2) generate a nomogram to predict the overall survival (OS) and (3) identify a prognostic stratification to assist the therapeutic decision-making. *METHODS:* LA-SCCL patients receiving definitive radiotherapy and baseline tumor marker measurement were eligible for this retrospective study. Cox proportional hazards regression was used to determine independent factors associated with various survival indexes and a nomogram was created to estimate the 5-year OS probability for individual patient. The identified prognostic factors were recruited into a recursive partitioning analysis (RPA) for OS to stratify patients with distinct outcome. *RESULTS:* A total of 224 patients were eligible for analysis. Increased cytokeratin-19 fragment (CYFRA 21-1) was independently associated with inferior OS, progression free survival (PFS) and a borderline decreased local-regional progression free survival (LRPFS). Elevated carcino-embryonic antigen (CEA) served as an unfavorable determinant for OS and increased neuron-specific enolase (NSE) was predictive of poor distant metastasis free survival (DMFS). A nomogram integrating KPS, TNM stage, CEA and CYFRA 21-1 was created, resulting in a c-index of 0.62. RPA identified 4 prognostic classifications, with median OS of 27.6, 19.9, 17.3 and 10.9 months for low, intermediate, high and very-high risk groups, respectively. *CONCLUSIONS:* Baseline tumor marker panel including CYFRA 21-1, CEA and NSE can be prognostic of outcome for LA-SCCL receiving definitive radiotherapy. The RPA identified four prognostic subgroups, which could assist personalized therapy and clinical trial design in LA-SCCL.

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Introduction

Squamous cell carcinoma accounts for more than 50% of overall non-small cell lung cancer (NSCLC) in China [1, 2]. Though tremendous advancement in medicine therapy has been achieved for adenocarcinoma of lung cancer [3, 4], much less exciting news derive from squamous cell carcinoma (SCC). Under the scenario of lacking driven mutation-oriented treatment algorithm, an effective prognostic model could be of great help for the therapeutic decision making in patients with SCC.

There is no definite prognostic biomarker for NSCLC yet. Despite a large number of explorations or investigations on novel biomarkers detection, none has been extensively recognized. Tumor markers are

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easily obtained in serum and some has been successfully verified as a convenient complementary approach for disease diagnosis or post-therapy surveillances, such as AFP for liver cancer and PSA for prostate cancer. However, the prognostic significance of serum tumor markers for lung cancer is still controversial in spite of large number of studies. Moreover, the prognostic effect of tumor markers for lung cancer tended to vary with histology, stage as well as the specific therapeutic approaches [5–18], and hence, an investigation on the basis of a cohort with relatively homogeneous characteristics may gain more accurate assessment on the prognostic value of tumor markers.

In this retrospective study, we aimed to: [1] assess the performance of pre-therapy levels of carcino-embryonic antigen (CEA), carcinoma antigen 125 (CA125), squamous cell carcinoma antigen (SCC), cytokeratin-19 fragment (CYFRA 21-1) and neuron-specific enolase (NSE) in predicting long-term survival; [2] generate a nomogram to predict the 5-year overall survival (OS) and [3] identify a baseline prognostic stratification to assist the therapeutic decision-making in patients with locally advanced squamous cell carcinoma of lung (LA-SCCL).

Patients and Methods

Study Population Selection

The diagram for patient selection is shown in Figure 1. Out of the 946 patients receiving definitive radiotherapy (≥ 50Gy) in our LA-NSCLC database, 564 patients were histologically confirmed SCC.

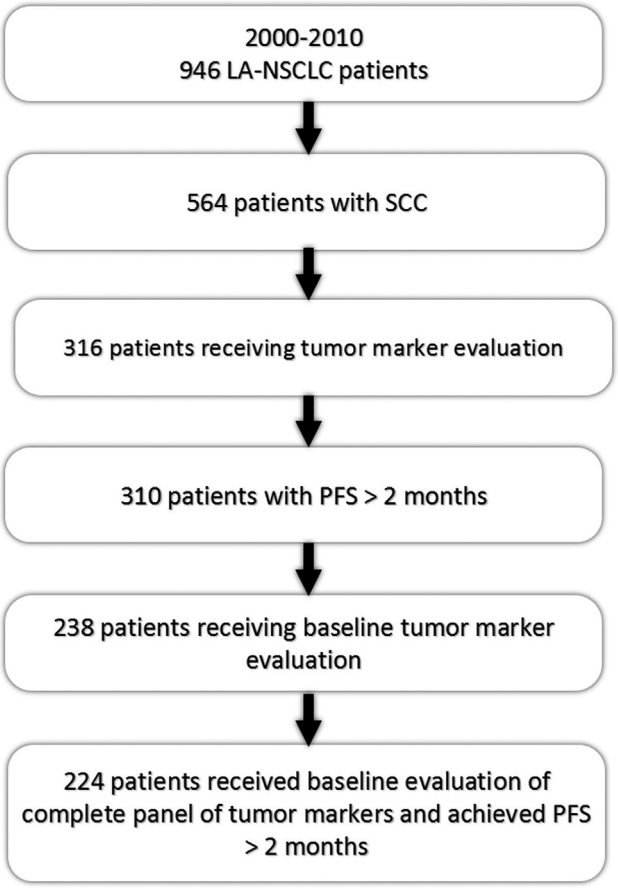


Figure 1. Diagram of patient selection and study design. LA-NSCLC: locally advanced non-small cell lung cancer; SCC: squamous cell carcinoma; PFS: progression free survival.

Three hundred and sixteen patients with SCC underwent tumor marker tests at least once in our hospital during the treatment and follow-up course. Excluding patients who progressed within 2 months after radiation start or had no pre-therapy examination of tumor marker, a total of 238 patients had baseline tumor maker tests and ultimately 224 patients with full panel of tumor marker data (CEA, CA125, SCC, CYFRA 21-1 and NSE) entered the final analysis.

Age, gender, baseline Karnofsky performance status (KPS), smoking status, history of weight loss, pathology, TNM staging, treatment modality, radiation technique, radiation dose and tumor markers results were retrospectively collected from the chart records. The dominant chemotherapy regimens concurrent with RT consisted of etoposide/cisplatin and paclitaxel/carboplatin. Sequential chemotherapy was mainly composed of platinum-based doublet agents regimen, such as NP (vinorelbine plus cisplatin), PC (paclitaxel plus carboplatin), GP (gemcitabine plus cisplatin) and pemetrexed plus cisplatin. This study was approved by the local institutional review board (IRB).

Tumor Marker Measurement

All patients received the full panel of tumor marker test including CEA, CA125, CYFRA21-1, SCC and NSE before the commencement of therapy. All assays were performed using commercial kits and following manufacturer's instruction blind to clinical information.

Statistical Consideration

Overall survival (OS), local regional progression free survival (LRPFS) and distant metastasis free survival (DMFS) were defined as the time from diagnosis until the first occurrence of specific event: death, local-regional recurrence or distant metastasis, respectively. Progression free survival (PFS) was defined as the duration between the cancer diagnosis and the date of any progression or cancer related death.

Continuous variables were presented as mean ± standard deviation and were compared using Mann–Whitney *U* test. Chi squared tests was adopted for categorical data comparison between groups. Kaplan–Meier method was used to estimate survival and follow-up time and log-rank test was performed to examine the significance of difference. To dichotomize the continuous values of tumor markers into categorical variables, receiver operating characteristic (ROC) analysis was rendered to identify the optimal cutoffs using the progression within 2 years after diagnosis as the event of interest. Cox proportional hazard regression model with backward step down selection was used to identify factors independently associated with survival indexes and to calculate hazard ratios (HRs) [19]. These analyses were all performed with SPSS version 16.0. All tests were two sided and a $P \leq .05$ was considered statistically significant.

Based on the factors identified by Cox proportional hazards regression model, a nomogram was created to calculate individual's probability of OS and a recursive partitioning analysis (RPA) was utilized to develop a prognostic sub-stratification by using the packages of "rms" and "rpart" in R version 3.2.2 (http://www.r-project.org/). In the nomogram, each patient was assigned a series of scores corresponding to all involved variables and the final sum of the scores was projected to the relevant 5-year survival probability. The predictive performance of the nomogram was measured by concordance index (c-index), which quantifies the level of concordance between nomogram-predicted and the actual chance of having the event of interest. The value of c-index ranges from 0.5 to 1. The higher the c-index, the more accurate is the prediction. Calibration

curves were plotted by comparing predicted probabilities from the nomogram versus observed Kaplan–Meier estimates of survival probability. Bootstraps with 1000 resamples were applied to these activities [20]. In RPA, variables independently correlated with OS were examined by the Kaplan–Meier method for the best stratification. A minimum number of 20 patients in a node were required to enable the further splitting. Afterwards, the preliminary risk strata were evaluated by plotting Kaplan–Meier curves in terms of OS. The log-rank test was further performed to determine whether there was sufficient divergence across terminal node populations and whether any two groups were similar enough in survival to be merged [21].

Results

Patients' Characteristics and Optimal Cutoffs Identification for Tumor Markers

The general characteristics of 224 patients are shown in Table 1. The median age was 62, with 19% of patients elder than 70. Ninety-two percent of patients were male and 87% with baseline KPS ≥ 80. Forty percent of patients carried stage IIIA disease and 60% with stage IIIB disease. Intensity modulated radiotherapy (IMRT) was the predominant technique for the radiation delivery. Approximately half of study patients received concurrent chemoradiotherapy, one forth received sequential chemoradiotherapy and the remaining underwent RT alone. The upper limits of reference concentration recommended by manufactures of CEA, CA125, SCC, CYFRA 21-1 and NSE were 5.0 ng/ml, 35.0 U/ml, 1.5 ng/ml, 3.3 ng/ml and 18.0 ng/ml, respectively. The median values of radiation dose, CEA, CA125, SCC, CYFRA 21-1 and NSE were 60Gy, 3.33 ng/ml, 20.21 U/ml, 1.6 ng/ml, 6.5 ng/ml and 13.88 ng/ml respectively. Using the 2-year progression as the event of interest, ROC analysis identified that optimal cutoff points for CEA, CA125, SCC, CYFRA 21-1 and NSE were 5.3 ng/ml, 17.1 U/ml, 2.5 ng/ml, 5.2 ng/ml and 19.5 ng/ml, respectively. Considering the notable difference between the upper limit of reference concentration and ROC identified optimal threshold of CA125, we selected the larger value of 35.0 U/ml as the cutoff point for CA125 in the following analysis.

Univariate Analysis of Tumor Markers for Survival Indexes

The median follow-up time was 63.8 months for overall patients. The median OS, LRPFS, DMFS and PFS were 22.1, 22.1, 49.5 and 12.4 months, with the 5-year rate of 18%, 33%, 46% and 19%, respectively. As shown in Table 2, patients with CYFRA 21-1 ≤ 5.2 ng/ml had significantly better OS (median: 27.1 vs. 20.6 months, $P = .033$), LRPFS (median: 30.8 vs. 16.4 months, $P = .040$) and PFS (median: 20.4 vs. 10.9 months, $P = .015$), as well as a DMFS benefit with a trend approaching significance (median: not reached vs. 44.2, $P = .082$). Survival curves of CYFRA 21-1 identified groups are shown in Figure 2. Similarly, we found patients with NSE ≤ 19.5 ng/ml presented with statistically favorable OS, DMFS or PFS compared with counterpart whereas no LRPFS difference was observed. Univariate analysis also identified significant superior OS in the subgroup of patients with CEA ≤ 5.3 ng/ml or SCC ≤ 2.5 ng/ml. There was no difference with respect to LRPFS, DMFS or PFS between patients with different level of CEA, CA125 or SCC.

Multivariate Analysis for Survival Indexes

With regard to multivariate analyses, independent factors associated with OS, LRPFS, DMFS, and PFS are listed in Supplemental Table 1. Unsurprisingly, tumor stage demonstrated significant association with OS, DMFS and PFS, with obvious superiority in patients carrying IIIA diseases, whereas such beneficial effect was not observed for LRPFS. Higher baseline CYFRA 21-1 (> 5.2 ng/ml) remained independently associated with poorer OS (HR = 1.379, 95% CI: 1.018–1.869, $P = .038$), PFS (HR = 1.473, 95% CI: 1.068–2.032, $P = .018$) and also presented a borderline correlation with LRPFS (HR = 1.439, 95% CI: 0.986–2.098, $P = .059$). However, no association was identified between CYFRA 21-1 and DMFS after adjusting for confounders. In addition to tumor stage and CYFRA 21-1, better KPS (HR = 0.514, 95%CI: 0.342–0.773, $P = .001$) and lower baseline CEA (HR = 1.477,

Table 1. General Characteristics of the Study Population

Factors		Patient Number (%)	Factors		Patient Number (%)
Age (year)	Median (Range)	62 (26, 84)	RT dose (Gy)	Median (Range)	60 (50, 73.75)
	≤ 70	181 (81)		<60	57 (25)
	>70	43 (19)		≥ 60	167 (75)
Gender	Male	206 (92)	CEA (ng/ml)	Median (Range)	3.33 (0.39, 932.4)
	Female	18 (8)		≤ 5.3	169 (75)
Weight loss	No	157 (70)		>5.3	55 (25)
	Yes	67 (30)	CA 125 (U/ml)	Median (Range)	20.21 (1.89, 705.2)
KPS	≥ 80	194 (87)		≤ 35.0	163 (73)
	< 80	30 (13)		> 35.0	61 (27)
Smoking	No	25 (11)	SCC (ng/ml)	Median (Range)	1.60 (0.1, 133.0)
	Yes	199 (89)		≤ 2.5	148 (66)
Stage	IIIA	90 (40)		>2.5	76 (34)
	IIIB	134 (60)	CYFRA 21-1 (ng/ml)	Median (Range)	6.50 (0.75, 69.6)
RT technique	2DRT	19 (8)		≤ 5.2	84 (38)
	3DCRT	37 (17)		> 5.2	140 (62)
	IMRT	168 (75)			
Treatment modality	RT alone	58 (26)	NSE (ng/ml)	Median (Range)	13.88 (1.85, 81.33)
	Sequential CRT	60 (27)		≤ 19.5	185 (83)
	Concurrent CRT	106 (47)		> 19.5	39 (17)

KPS: Karnofsky performance status; 2DRT: two-dimensional radiotherapy; 3DCRT: three-dimensional conformal radiotherapy; IMRT: intensity modulated radiotherapy; CRT: chemotherapy and radiotherapy; RT: radiotherapy; CEA: carcino-embryonic antigen; CA125: carcinoma antigen 125; SCC: squamous cell carcinoma antigen; CYFRA 21-1: cytokeratin-19 fragment; NSE: neuron-specific enolase.

Table 2. Effect of Tumor Markers on Survival Indexes

Factors		OS			LRPFS			DMFS			PFS		
		HR	95%CI	*P*	HR	95%CI	*P*	HR	95%CI	*P*	HR	95%CI	*P*
CEA (ng/ml)	>5.3 vs. ≤ 5.3	1.430	1.029, 1.988	0.033	1.335	0.890, 2.002	0.163	1.104	0.675, 1.804	0.693	1.317	0.929, 1.867	0.122
CA 125 (U/ml)	> 35.0 vs. ≤ 35.0	1.170	0.843, 1.623	0.349	1.076	0.719, 1.612	0.722	1.175	0.745, 1.854	0.487	1.079	0.768, 1.515	0.660
SCC (ng/ml)	>2.5 vs. ≤ 2.5	1.429	1.055, 1.936	0.021	1.288	0.885, 1.876	0.186	1.392	0.909, 2.132	0.128	1.280	0.932, 1.757	0.127
CYFRA 21-1 (ng/ml)	> 5.2 vs. ≤ 5.2	1.390	1.027, 1.881	0.033	1.482	1.018, 2.157	0.040	1.476	0.951, 2.291	0.082	1.490	1.082, 2.052	0.015
NSE (ng/ml)	> 19.5 vs. ≤ 19.5	1.446	1.006, 2.078	0.046	1.020	0.630, 1.650	0.936	1.833	1.128, 2.979	0.014	1.486	1.018, 2.170	0.040

OS: overall survival; LRPFS: local regional progression free survival; DMFS: distant metastasis free survival; PFS: progression free survival; HR: hazard ratio; CI: confidence interval; CEA: carcino-embryonic antigen; CA125: carcinoma antigen 125; SCC: squamous cell carcinoma antigen; CYFRA 21-1: cytokeratin-19 fragment; NSE: neuron-specific enolase.

95%CI: 1.054–2.068, *P* = .023) also served as favorable predictors for OS. Besides CYFRA 21-1, therapeutic modality of concurrent chemotherapy appeared to confer an additional LRPFS advantage beyond that achieved with RT alone or sequential CRT. In terms of DMFS, we also identified a statistical significance for higher risk of distant metastasis in patients with baseline NSE> 19.5 ng/ml (HR = 1.753, 95% CI: 1.077–2.853, *P* = .024). With respect to PFS, superior result was observed among patients with stage IIIA disease, better performance status as well as the lower level of baseline CYFRA 21-1.

Nomogram Development for OS and Internal Validation

On the basis of the multivariate analysis, the final nomogram integrating KPS, overall TNM stage, CEA and CYFRA 21-1 is presented in Figure 3*A*. Bootstrap validation revealed that the c-index for OS prediction was 0.62 (95% CI, 0.58–0.66), indicating a moderate discrimination. The calibration curves for the probabilities of 1-y, 3-y and 5-y OS showed a good agreement between the nomogram predicted and the actually observed OS (Figure 3*B*).

RPA Classification for Prognostic Stratification

On the basis of four independent prognostic variables including KPS, sub-stage, CEA and CYFRA 21-1, a decision tree was established through RPA, resulting in a 5-class stratification (Figure 4*A*). Node 1 included patients with stage IIIA disease; node 2 patients had stage IIIB disease, with KPS ≥ 80, baseline CEA ≤ 5.3 ng/ml and CYFRA 21-1 ≤ 5.2 ng/ml; node 3 patients carried stage IIIB disease, with KPS ≥ 80, baseline CEA ≤ 5.3 ng/ml but CYFRA 21-1 > 5.2 ng/ml; patients at node 4 had stage IIIB disease, KPS ≥ 80 and baseline CEA> 5.3 ng/ml; and patients at node 5 had IIIB disease and KPS< 80. The median OS for five terminal nodes was 27.2, 28.4, 19.9, 17.3 and 10.9 months, respectively. Given the similar survival trend, node 1 and node 2 were collapsed into a single class (median OS = 27.6 months) and an obvious divergence of OS curves across four classes was observed,

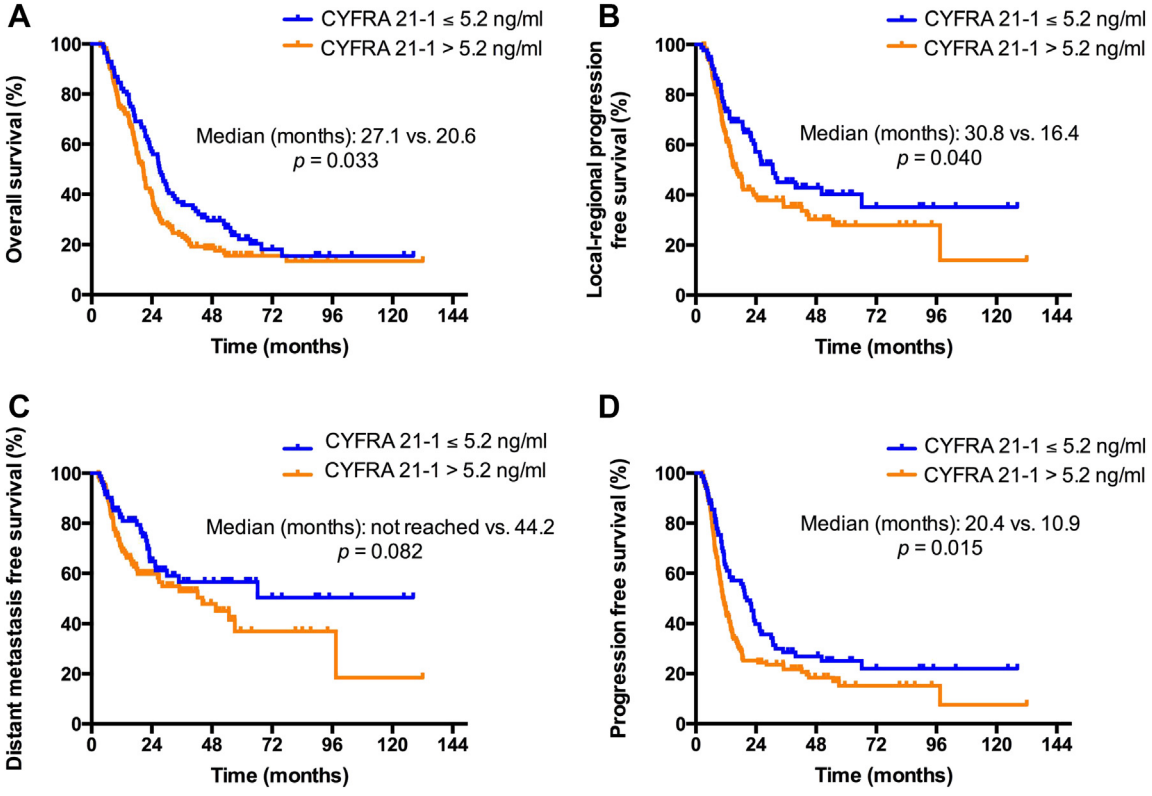


Figure 2. Kaplan–Meier estimates of (A) overall survival, (B) local-regional progression free survival, (C) distant metastasis free survival and (D) progression free survival between patients with low- and high-level of baseline cytokeratin-19 fragment (CYFRA 21-1).

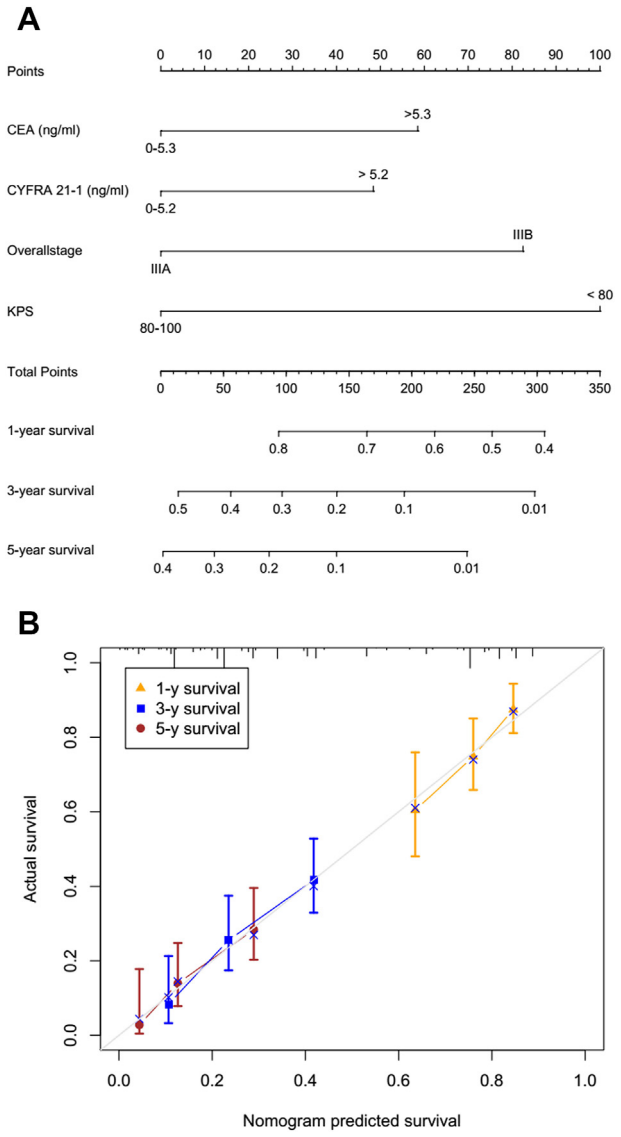


Figure 3. (A) Nomogram predicting 1-year, 3-year and 5-year overall survival for locally advanced squamous cell carcinoma. In the nomogram, each variable value is assigned a score, and the final sum of the scores is projected to the corresponding probability of survival; (B) Calibration plots for nomogram-predicted 1-y, 3-y and 5-y overall survival (x-axis) as compared to Kaplan–Meier OS estimates (y-axis) for internal validation. A plot along the 45-degree line would indicate a perfectly accurate nomogram prediction model.

displaying a 3-y OS rate of 38%, 25%, 8% and 0 for low, intermediate, high and very-high risk classes, respectively ($P < .0001$) (Figure 4*B*).

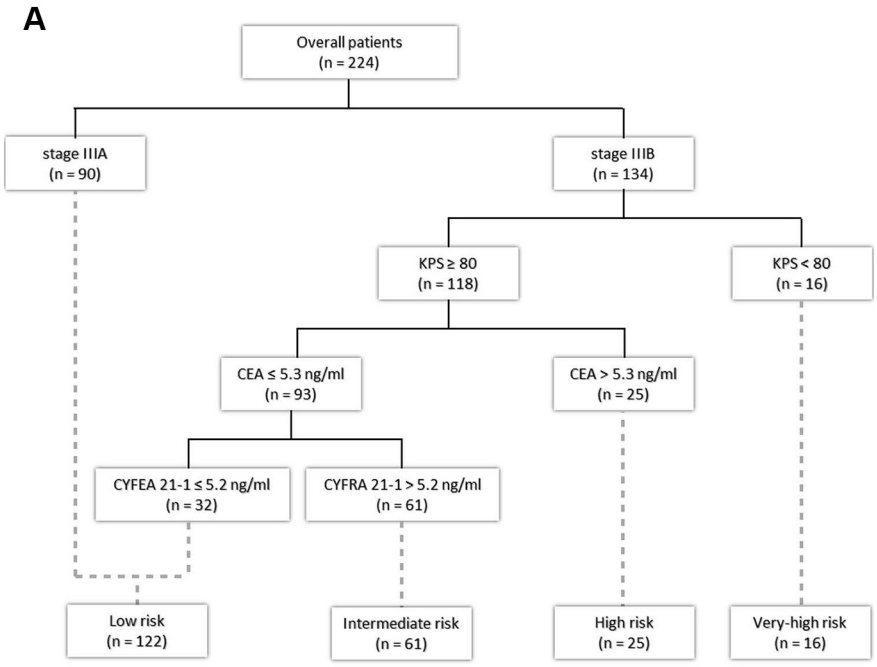
Discussion

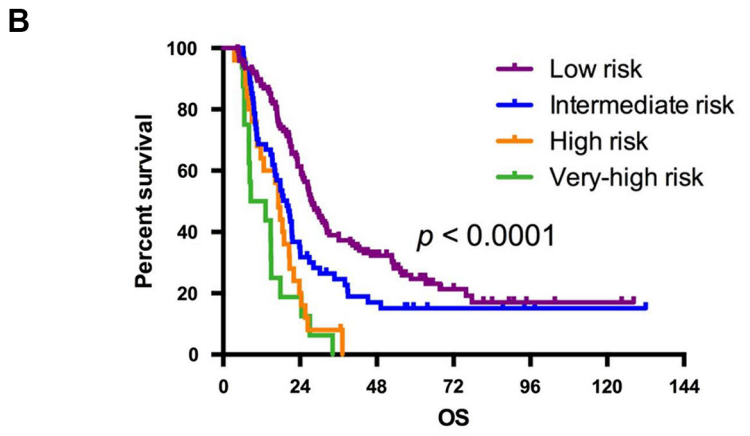
In this retrospective study focusing on inoperable LA-SCCL receiving definitive radiotherapy, we identified a prognostic tumor marker signature including CEA, CYFRA 21-1 and NSE. Taking all independent prognostic variables into consideration, we generated a nomogram to quantitatively estimate individual OS probability. Furthermore, by integrating these prognostic determinants, RPA identified four classes of LA-SCCL with distinct survival outcome, which may assist the therapeutic decision-making in clinical practice and trial design.

Classical prognostic factors such as stage, KPS and weight loss have been widely recognized in NSCLC [22, 23]. However, the prognostic significance of serum tumor markers for lung cancer is still controversial and none has been recommended in routine clinical practice [5–18]. Moreover, the prognostic effect of tumor markers was apt to vary with histology and stage as well as the specific therapeutic approaches, reinforcing the complexity of judgment on prognostic value of tumor markers. In terms of inoperable locally-advanced NSCLC, though diverse results were reported with respect to the prognostic value of multiple serum tumor markers, CYFRAL 21–1 appeared to be most frequently reported as a prognostic determinant [5–7, 11–18]. Considering the potential histology and stage specificity of the prognostic role of tumor markers, we selected patients with stage III squamous carcinoma receiving definitive radiotherapy as the study population to attenuate the potential confounding effects. In the present study, increased CYFRA 21-1 and CEA were consistently found to be unfavorable indicators for overall survival. Besides OS, we also analyzed the effect of tumor markers on local-regional, distant and overall progression. Once again, CYFRA 21-1 demonstrated significant association with overall PFS as well as a trend approaching statistical significance with regard to LRPFS. In terms of DMFS, elevated NSE presented as an independent negative determinant. These results leverage the establishment of a tumor markers based signature, allowing for the prediction of not only survival but also the pattern of failure.

Another notable merit of the present study was the introduction of specific cutoffs derived from ROC analysis to dichotomize the tumor markers. It is well known that the regular reference levels of tumor markers initially arise from the need of differentiation diagnosis between patients and healthy population, whereas their predicting abilities for prognosis may not be sufficiently reliable. On the basis of the previously reported PFS on LA-NSCLC [24–26] and our own PFS data in the present study, we adopted ROC analysis with progression within 2 years as the event of interest to determine the optimal threshold. This method facilitated improvement on the dichotomization of patients with distinct outcomes, allowing for an increased sensitivity of the prediction.

Prognostic modeling is playing an increasingly important role in the disease management for NSCLC. Nomogram has been successfully built and validated for predicting OS in resectable NSCLC [27]. Regarding unresected NSCLC treated with chemoradiotherapy, two nomograms have been developed by the same group of authors from Netherlands [28, 29], including one focusing on stage III NSCLC. In the nomogram for stage III NSCLC, the final model consisted of gender, WHO performance status, T stage, GTV, number of positive lymph node stations, overall treatment time and EQD2, resulting in a C-index of 0.62 [29]. In our study, besides the general demographics, disease characteristics and treatment information, serum tumor marker levels were also incorporated into the candidate variables for model building. On the basis of Cox regression multivariate analysis, we ultimately plotted a nomogram composed of KPS, overall stage, CEA and CYFRA 21-1, resulting in a moderate discrimination and a comparable c-index with the Netherlands study. The prognostic performance of our nomogram would be improved through several ways, such as prospective data collection to reduce selection bias, larger number of study patient involvement and more intact variable selection to diminish overfitting, as well as external validation to assess the generalizability of the model.





	MS (mon)	1-y OS (%)	3-y OS (%)	5-y OS (%)
Low risk	27.6	87	38	25
Intermediate risk	19.9	69	25	15
High risk	17.3	60	8	0
Very-high risk	10.9	50	0	0

Figure 4. (A) Prognostic stratification (low-, intermediate-, high- and very high-risk groups) of locally advanced squamous cell carcinoma determined by Recursive partitioning analysis (RPA); (B) overall survival curves and survival data for four classes stratified by RPA.

In squamous cell lung cancer, there is a paucity of genetic alteration guided therapeutic decision-making algorithm and TNM staging remains the dominant decisive element during the process of treatment strategy identification. In the regular clinical scenario, stage III SCC patients would consistently receive standard chemo-radiotherapy [30, 31]. Nevertheless, the observed survival of stage III SCCL was actually quite diverse [1, 24–26, 32–34]. RPA is a statistical method to create a decision tree by indefinitely splitting study population into sub-populations until achieving the optimum sensitivity or specificity. In the current study, RPA analysis identified four classes of LA-SCCL with distinct outcome, allowing for a potential of altered and personalized management for patients in different prognostic category. In addition, this classification would also facilitate more rational clinical trial design concerning SCCL.

We admit the existence of limitations in our study. First, the retrospective nature of the study would inevitably introduce selection and recall biases. Second, due to lack of external data, we could only assess the efficacy of models based on internal validation results. Third, the during- and post-therapy tumor marker data were incomplete and therefore only baseline data were included into the analysis, impeding the exploration of the effect of dynamic change on outcome.

In conclusion, this retrospective study identified a tumor marker panel of CEA, CYFRA 21-1 and NSE, which were independently prognostic of outcome in LA-SCCL. A nomogram integrating KPS, overall TNM staging, CEA and CYFRA 21-1 has been generated for estimation of individual patient-level probability of overall survival and warrants further external validation. Moreover, RPA developed a four-class stratification for the prognosis prediction of LA-SCCL, meriting further evaluation in a larger population. This RPA classification would assist multidisciplinary treatment decision-making and clinical trial design for SCCL.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2018.05.008.

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