Original Article

Circulating tumor cells can predict the prognosis of patients with non-small cell lung cancer after resection: a retrospective study

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Background: The development of metastasis is the primary cause of death in patients with non-small cell lung cancer (NSCLC). However, identifying those NSCLC patients who will have loco-regional or distant disease recurrence after surgery is still challenging. Circulating tumor cells (CTCs) can accurately reflect the impact of micro-metastasis of tumor cells in circulating blood on patients' treatment and prognosis. The aim of the present study was to explore the value of preoperative CTC concentration in predicting postoperative metastasis and recurrence risk in patients with NSCLC.

Methods: This study enrolled 347 patients with stage I–IIIA NSCLC. The CTCs were isolated using folate receptor (FR) positivity from peripheral blood samples before surgery, and then enriched and analyzed. Patients were divided into two groups for retrospective survival analysis based on the geometric mean of CTC concentration. The primary study endpoint was recurrence-free survival. Spearman's correlation was used to evaluate the relationship between CTC concentration and clinical characteristics of NSCLC patients. A nomogram based on the multivariate Cox regression model was developed to predict recurrence and metastasis in the NSCLC patients. The performance of the nomogram was evaluated using the concordance index, calibration curve, and Hosmer-Lemeshow test.

Results: The median follow-up time was 38 months. Preoperative CTC concentration was not significantly related to tumor-node-metastasis staging ($P>0.05$) and was an independent prognostic factor for NSCLC patients [hazard ratio (HR), 5.489; 95% confidence interval (CI): 2.660–11.326, $P<0.001$]. The nomogram based on preoperative CTC concentration had a concordance index value of 0.82. Validation revealed that the nomogram possessed excellent predictive ability and calibration.

Conclusions: Preoperative CTC concentration is an independent and sensitive biomarker of prognosis in patients with NSCLC. Our nomogram based on preoperative CTC concentration is an effective and non-invasive tool for predicting the recurrence and metastasis of NSCLC.

Keywords: Circulating tumor cell concentration (CTC concentration); screening; recurrence and metastasis; non-small cell lung cancer (NSCLC); nomogram

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Introduction

Globally, lung cancer is the leading cause of cancer mortality in males and accounts for the second highest proportion of cancer-related deaths among females. There are approximately 1.8 million new cases of lung cancer and 1.6 million associated deaths each year (1). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. The development of metastasis is the primary cause of death in patients with NSCLC, and these patients have a 5-year survival rate of only 4% (2). Approximately 50% of patients with NSCLC who undergo surgery relapse within 5 years (3,4). A better understanding of the biology of early dissemination in NSCLC and strategies to identify the patients who are at high risk of relapse may help to inform novel approaches for adjuvant treatment to improve cure rates.

At present, the diagnosis and staging of lung cancer are mainly based on imaging or pathological examination results (5). It is difficult to accurately assess the impact of micro-metastasis of tumor cells in circulating blood on patients' treatment and prognosis; however, the detection of circulating tumor cells (CTCs) can assist with this (6). CTCs were first discovered 150 years ago, and in recent years, they have been considered to be the "foundations of metastasis" (7), although this has not been fully established in NSCLC. A study showed that CTCs enriched from the peripheral blood of patients with breast cancer, melanoma, NSCLC, and small cell lung cancer (SCLC) could form tumors in immune-compromised mice, confirming their tumorigenic potential (8-10). However, due to the scarcity of CTCs in the peripheral blood, their identification and enrichment have always been a technical obstacle, hindering the clinical application of CTC analysis. In recent years, several technological advances have been made in this field. For instance, our hospital has developed and validated an easy-to-use CTC enrichment and detection protocol based on the use of immunomagnetic beads to isolate and concentrate folate receptor (FR)-positive CTCs from peripheral blood. Furthermore, our previous study also proved that the detection of FR-positive CTCs can effectively improve the pathological diagnosis of pulmonary nodules (11), and had higher recovery efficiency than other CTCs detection techniques (12).

As CTCs carry all the genomic information of a patient's tumor burden, they have great clinical significance in the diagnosis, treatment, and detection of tumors (13-15). Existing studies have shown that CTC count is a powerful prognostic indicator in many cancers, including lung cancer (16). CTC number, measured with the Cell Search platform, is a prognostic test approved by the United States Food and Drug Administration in breast, colorectal, and prostate cancer but has been used also in lung cancer. In 2010, CTCs were included in the American Joint Committee on Cancer (AJCC) Cancer staging manual for breast cancer as a new indicator to complement pathological staging, namely for tumor cells detected in the blood but without clinical or radiographic evidence of distant metastasis, staging between M0 and M1. Previous studies (17-19) had investigated the predictive value of CTCs in patients with advanced NSCLC. However, the predictive effect of preoperative CTC concentration on the risk of metastasis and recurrence following lung cancer resection remains to be clarified. Therefore, the present study aimed to explore the role of preoperative CTC concentration in predicting the risk of postoperative metastasis and recurrence in patients with early NSCLC.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tlcr-21-149).

Methods

Patients

Clinical and follow-up data of 28,767 patients who underwent surgical resection of NSCLC at Shanghai Pulmonary Hospital, Tongji University from March 2013 to December 2017 were retrospectively analyzed. The inclusion criteria for patients were as follows: (I) underwent a preoperative CTC test; (II) aged over 18 years; (III) no lung cancer or other malignant tumors in the previous 5 years. The exclusion criteria were: (I) patients who received chemotherapy, biological therapy, or immunotherapy before surgery; (II) patients who did not undergo a tumor marker test before surgery or an epidermal growth factor receptor (EGFR) test after surgery; (III) pregnant women; (IV) patients with severe heart disease, tuberculosis, or human immunodeficiency virus (HIV) infection; (V) patients who were lost follow-up. The study was approved by the institutional review board of Shanghai Pulmonary Hospital, Tongji University. The requirement for informed consent from each patient was waived due to the retrospective nature of this analysis. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

From March 2013 to December 2017, a total of 417 NSCLC patients who received resection in Shanghai Pulmonary Hospital, Tongji University, underwent preoperative CTC testing. Of them, 6 patients with stage IIIB, 32 patients who received preoperative chemotherapy, 17 patients who were lost to follow-up, 7 patients who did not undergo preoperative tumor marker testing, 4 patients who received targeted therapy, 2 patients with severe heart disease and 2 patients with tuberculosis were excluded. Therefore, a total of 347 NSCLC patients were finally enrolled in the study (Figure S1).

The primary endpoint of the study was recurrence-free survival (RFS), which is defined as the time from date of curative surgery to the time of recurrence or death. The follow-up period lasted from March 2013 to September 2020. The median follow-up of the 347 NSCLC patients was 38 months. The patients were divided into two groups according to the geometric mean of CTC concentration level [11 functional unit (FU) per 3 mL blood]: the CTC concentration ≥11 FU group and the CTC concentration <11 FU group, the method used to define the CTCs cut-off was recognized in the other published literatures (20).

Sample collection

An ethylene diamine tetraacetic acid (EDTA)-containing vacuum blood collection tube (purple cap, 6 mL; Becton, Dickinson and Company, NJ, USA) was used to collect 3 mL of peripheral venous blood from each patient within the 1 week before surgery. Samples of whole blood were temporarily stored at 4–10 ℃ and processed within 24 hours after collection. Each sample was assigned an identifier number, and any other labels were removed from the tube to ensure the sample analysis was conducted in a single-blinded fashion.

Sample processing

FR-positive CTCs were enriched and enumerated with the CytoploRare Kit (Genosaber Biotech, Shanghai, China), which is a commercially available CTC detection kit approved by the China Food and Drug Administration (CFDA). The detailed experimental procedure was performed according to the manufacturer's protocol.

Immunomagnetic beads

The immunomagnetic beads used were polystyrene beads coated with a cross-linked layer of hydrophilic polyether. The assembled anti-CD45/-CD14 immuno-magnetic beads (4.5 μm in diameter) were superparamagnetic and could enrich leukocytes, as CD45 is expressed on all human leukocytes while CD14 is expressed mainly on human monocytes.

After erythrocyte rupture, we used the immunomagnetic beads to remove the $CD45^+/CD14^+$ leukocytes, leaving a mixture of CTCs and residual white blood cells.

DNA probe of FR ligand-TaqMan probe

The FR was chosen as a specific marker of CTCs in blood samples. Through its binding to the FR located on the cell surface, folic acid, an essential vitamin, is endocytosed into cells. However, the FR is highly expressed in several types of tumor cells, including more than 78% of lung cancer cells. Furthermore, the FR can recognize live CTCs, and its abundance is unaffected by epithelial-to-mesenchymal transition.

A specific detection probe was designed to recognize cells positively expressing the FR and to quantitatively determine the cell number. It contained the folic-acid-targeting region of the FR, as well as an oligonucleotide for amplification by polymerase chain reaction (PCR) (5'-CT CAACTGGTGTCGTGGAGTCGGCAATTCAGTTG AGGGTTCTAA-3'). Samples without erythrocytes and leukocytes were incubated with detection probes (10 μL) for 40 minutes and eluted to remove the excess probe. Quantitative PCR (qPCR) was used to analyze the samples quantitatively on an ABI 7300 Real-Time PCR System (Applied Biosystems, USA). The primer sequences were as follows: forward, 5'-TATGATTATGAGGCATGA-3'; reverse, 5'-GGTGTCGTGGAGTCG-3'; TaqMan probe, 5'-FAM-CAGTTGAGGGTTC-MGB-3'. The level of FR-positive CTCs in each sample was calculated using the calibration curve generated by the standard reference materials provided with the kit (serially diluted oligonucleotides corresponding to 2.00–632.50 folate units (FU). The levels of FR-positive CTCs were measured in FU/3 mL of peripheral blood.

Statistical analysis

Continuous variables were expressed as means or medians if not normally distributed and compared by Kruskal-Wallis test. Categorical variables were presented as frequencies and percentages and compared using Spearman's χ^2 test.

Receiver operating characteristic (ROC) analysis was performed to determine the value of CTC concentration for predicting disease recurrence in NSCLC patients. The Kaplan-Meier method was used to estimate RFS in patients with early NSCLC, and comparisons were made using the log-rank test. Multivariable analyses with the Cox proportional-hazards model were used to estimate the simultaneous effects of prognostic factors on RFS. A nomogram was developed based on the multivariate Cox regression model using the R package. The calibration curve and Hosmer-Lemeshow test were used to evaluate the validity of the nomogram. All P values were two-sided with a significance level of 0.05. All statistical analyses were performed using SPSS version 23 (IBM, Chicago, IL, USA), PRISM software (version 8.0.1, GraphPad Software, La Jolla, CA, USA), and R language (version 1.3.1056).

Results

Patient clinical characteristics

In total, 347 patients with NSCLC (6.9%, stage 0; 65.7%, stage IA; 11.5%, stage IB; 3.5%, stage IIA; 4.6%, stage IIB; 7.8%, stage IIIA) were included in this study. The median follow-up was 38 months. There were 170 patients in the CTC concentration ≥11 FU group and 177 patients in the CTC concentration <11 FU group. In the CTC concentration ≥11 FU group, there were 90 (52.9%) patients aged ≥60 years old, 76 (44.7%) males, and 21 (12.4%) smokers. There were no statistical differences between the 2 groups in terms of tumor-node-metastasis (TNM) stage or the presence of EGFR mutation (P>0.05). In the CTC concentration ≥11 FU group, 45 (26.5%) patients experienced disease recurrence, including 22 (12.9%) patients with extrapulmonary metastasis and 4 (2.4%) patients with multiple extrapulmonary metastases. In the CTC concentration <11 FU group, 9 (5.1%) patients suffered recurrence, including 3 (1.7%) patients with extrapulmonary metastasis; none of the patients had multiple metastases (*Table 1*).

Correlation between CTC concentration and clinical characteristics

To further explore the relationship between serum CTC concentration and clinical characteristics of NSCLC patients. Using Spearman's correlation, we found that CTC concentration was not significantly related with age (P=0.482), sex (P=0.516), smoking history (P=0.858), preoperative carcinoembryonic antigen (CEA) concentration (P=0.908), T stage (P=0.914), or EGFR mutation (P=0.083). CTC concentration was only associated with lymph node metastasis (P=0.049), disease recurrence (P<0.001), and metastatic site (P<0.001) in the NSCLC patients (*Figure 1A*).

The ROC curve analysis showed that preoperative CTC concentration had an area under the ROC curve (AUC) of 0.80 for predicting the recurrence of NSCLC (*Figure 1B*), while its AUC value for predicting lymph node metastasis was 0.63 (*Figure 1C*). The preoperative CTC concentration of the NSCLC patients was statistically related to the site of postoperative recurrence and metastasis (P<0.05). The CTC concentration of patients with recurrence in the lung was 15.99±5.69 FU. Meanwhile, the CTC concentration of patients with extrapulmonary metastasis and multiple metastases was 17.34±5.29 and 17.25±4.78 FU, respectively, which was significantly higher than that of NSCLC patients without recurrence and metastasis (CTC concentration: 11.24±5.12 FU) (*Figure 1D*,*E*, Table S1). These results suggest that CTC concentration could serve as a preoperative serum tumor biomarker of NSCLC metastasis.

Univariate and multivariate Cox regression for predicting the RFS of NSCLC patients

Univariate survival analysis was performed using the log-rank method, and a Cox regression model was used to analyze multiple survival-related factors. Both lymph node metastasis [hazard ratio (HR): 3.289; 95% confidence interval (CI): 1.781–6.073; P<0.001] and T staging (HR: 4.480; 95% CI: 1.600–12.546; P=0.004) were statistically significantly associated with RFS (*Table 2*). When the number of patients in the two groups was approximately equal, CTC concentration showed an excellent predictive effect on RFS (P<0.001). As shown in *Figure 2A*, patients with a CTC count of ≥11 FU had significantly poorer RFS than those with a CTC count of <11 FU. Further, CEA (HR: 2.286; 95% CI: 1.149–4.550; P=0.019) was also significantly associated with RFS, with a high CEA level being a detrimental factor for RFS (*Table 2*).

Multivariate analysis using Cox proportional hazards model showed that the independent influencing factors of RFS were serum CTC concentration (HR: 5.489; 95% CI: 2.660–11.326, P<0.001), T stage (HR: 7.180; 95% CI: 2.477–20.816, P<0.001), lymph node metastasis (HR: 2.169; 95% CI: 1.120–4.202, P=0.001), and sex

Table 1 Baseline clinical characteristics of 347 NSCLC patients

Characteristic	Subcategories	CTC concentration (FU), n (%)			P
		Total (n=347)	<11 (n=177)	≥11 (n=170)	
Age (years)	<60	170 (49.0)	90 (50.8)	80 (47.1)	0.520
	≥60	177 (51.0)	87 (49.2)	90 (52.9)	
Sex	Female	198 (57.1)	104 (58.8)	94 (55.3)	0.511
	Male	149 (42.9)	73 (41.2)	76 (44.7)	
Smoking	No	303 (87.3)	154 (87.0)	149 (87.6)	0.873
	Yes	44 (12.7)	23 (13.0)	21 (12.4)	
TNM stage	0	24 (6.9)	10 (5.6)	14 (8.2)	0.341
	IA	228 (65.7)	124 (70.1)	104 (61.2)	
	IB	40 (11.5)	21 (11.9)	19 (11.2)	
	IIA	12 (3.5)	6 (3.4)	6 (3.5)	
	IIB	16 (4.6)	7 (4.0)	9 (5.3)	
	IIIA	27 (7.8)	9 (5.1)	18 (10.6)	
T stage	Tis	24 (6.9)	10 (5.6)	14 (8.2)	0.681
	T1	250 (72.0)	132 (74.6)	118 (69.4)	
	T2	66 (19.0)	32 (18.1)	34 (20.0)	
	T3	7 (2.0)	3 (1.7)	4 (2.4)	
N stage	N0	311 (89.6)	164 (92.7)	147 (86.5)	0.142
	N1	9 (2.6)	4 (2.3)	5 (2.9)	
	N2	27 (7.8)	9 (5.1)	18 (10.6)	
EGFR mutation	Negative	149 (42.9)	84 (47.5)	65 (38.2)	0.104
	Positive	198 (57.1)	93 (52.5)	105 (61.8)	
Follow-up time (months), median (interquartile range)	–	38 [35–46]	38 [36–46]	38 [35–45]	0.974
Recurrence	No	293 (84.4)	168 (94.9)	125 (73.5)	0.000*
	Yes	54 (15.6)	9 (5.1)	45 (26.5)	
Location of metastasis	No metastasis	293 (84.4)	168 (94.9)	125 (73.5)	0.000*
	Lung	25 (7.2)	6 (3.4)	19 (11.2)	
	Brain	19 (5.5)	2 (1.1)	17 (10.0)	
	Bone	5 (1.4)	1 (0.6)	4 (2.4)	
	Liver	1 (0.3)	0 (0)	1 (0.6)	
	Extrapulmonary	25 (7.2)	3 (1.7)	22 (12.9)	
	Multiple	4 (1.2)	0 (0)	4 (2.4)	
CEA (ng/mL)	<8.55	317 (91.4)	162 (91.5)	155 (91.2)	1.000
	≥8.55	30 (8.6)	15 (8.5)	15 (8.8)	

*, P<0.05. NSCLC, non-small cell lung cancer; CTC, circulating tumor cell; FU, functional unit; CEA, carcinoembryonic antigen.

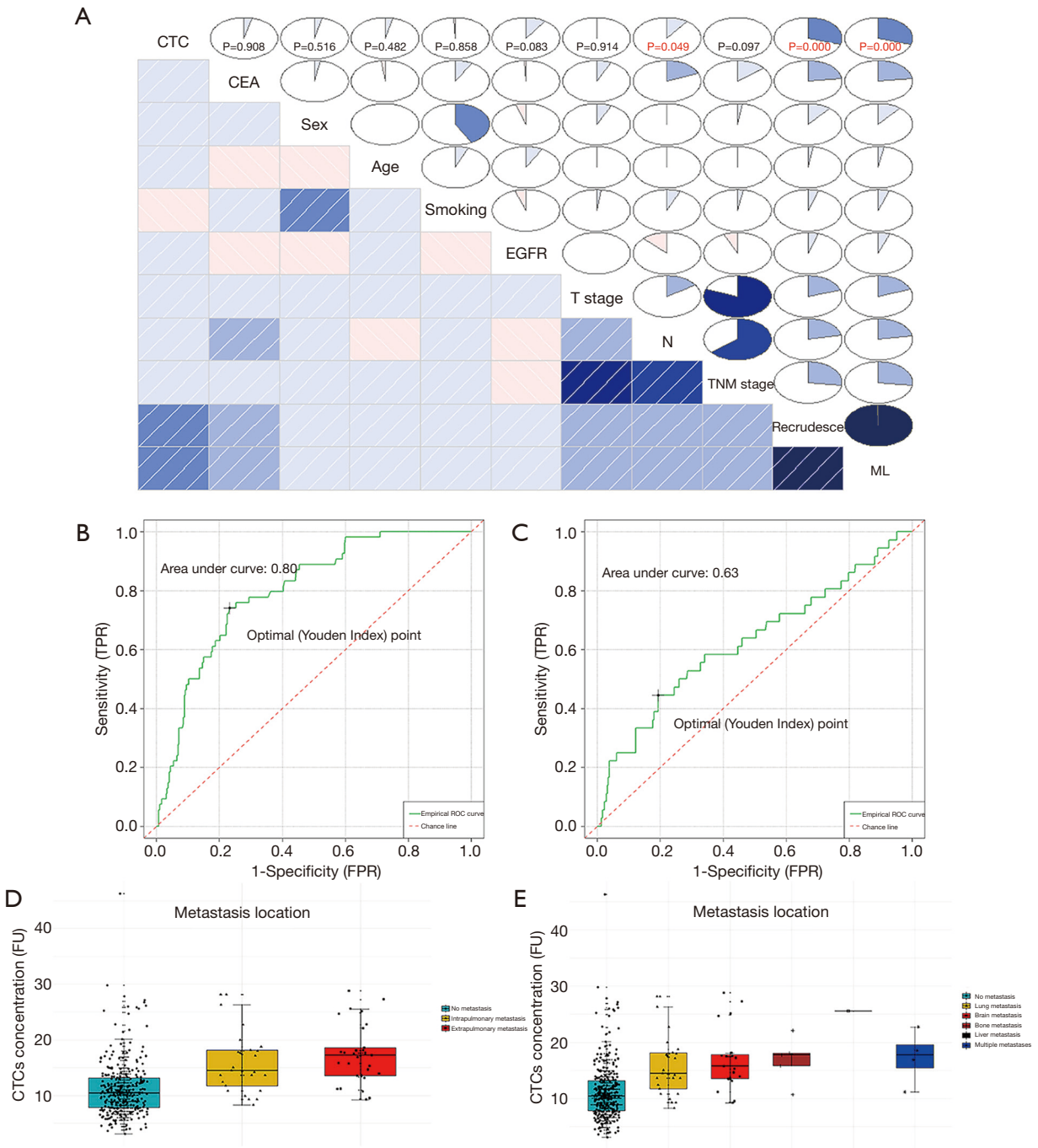

Figure 1 Correlations between CTC concentration and clinicopathological characteristics of NSCLC patients. (A) Spearman's correlation was used to analyze the correlation between the CTC concentration and the clinicopathological characteristics of 347 NSCLC patients; (B) ROC curve analysis was used to predict disease recurrence in 347 NSCLC patients, and the AUC was 0.80; (C) ROC curve analysis was used to predict lymph node metastasis in 347 NSCLC patients, and the AUC was 0.63; (D) CTC concentration in NSCLC patients with intrapulmonary metastasis, with extrapulmonary metastasis, and without metastasis; (E) CTC concentration in NSCLC patients with lung metastasis, brain metastasis, bone metastasis, liver metastasis, and multiple distant metastases and in those without metastasis. CTC, circulating tumor cell; NSCLC, non-small cell lung cancer; ROC, receiver operating characteristic; AUC, area under the ROC curve; ML, "multiple locations" metastasis.

Table 2 Univariate and multivariate Cox regression analysis predicting recurrence and metastasis in NSCLC patients (n=347)

Characteristic	Univariable analysis			Multivariable analysis		
	HR	95% CI	P	HR	95% CI	P
CTCs (FU) (<11 *vs.* ≥11)	5.690	2.780–11.647	0.000	5.489	2.660–11.326	0.000
T stage (Tis/T1/T2 *vs.* T3)	4.480	1.600–12.546	0.004	7.180	2.477–20.816	0.000
Lymph node metastasis (N0 *vs.* N1/N2)	3.289	1.781–6.073	0.000	2.169	1.120–4.202	0.001
CEA (ng/mL) (<8.55* *vs.* ≥8.55*)	2.286	1.149–4.550	0.019	1.860	0.919–3.764	0.084
Age (years) (<60 *vs.* ≥60)	1.157	0.677–1.977	0.594	–	–	–
Sex (female *vs.* male)	1.742	1.018–2.979	0.043	1.929	1.113–3.342	0.019
Smoking history (N *vs.* Y)	1.330	0.649–2.727	0.436	–	–	–
EGFR mutation (N *vs.* Y)	1.370	0.787–2.384	0.265	–	–	–

*, the cutoff-value of CEA as 8.55 ng/mL was according to the dichotomized value (as shown in Figure S2). NSCLC, non-small cell lung cancer; CTC, circulating tumor cell; FU, functional unit; HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen.

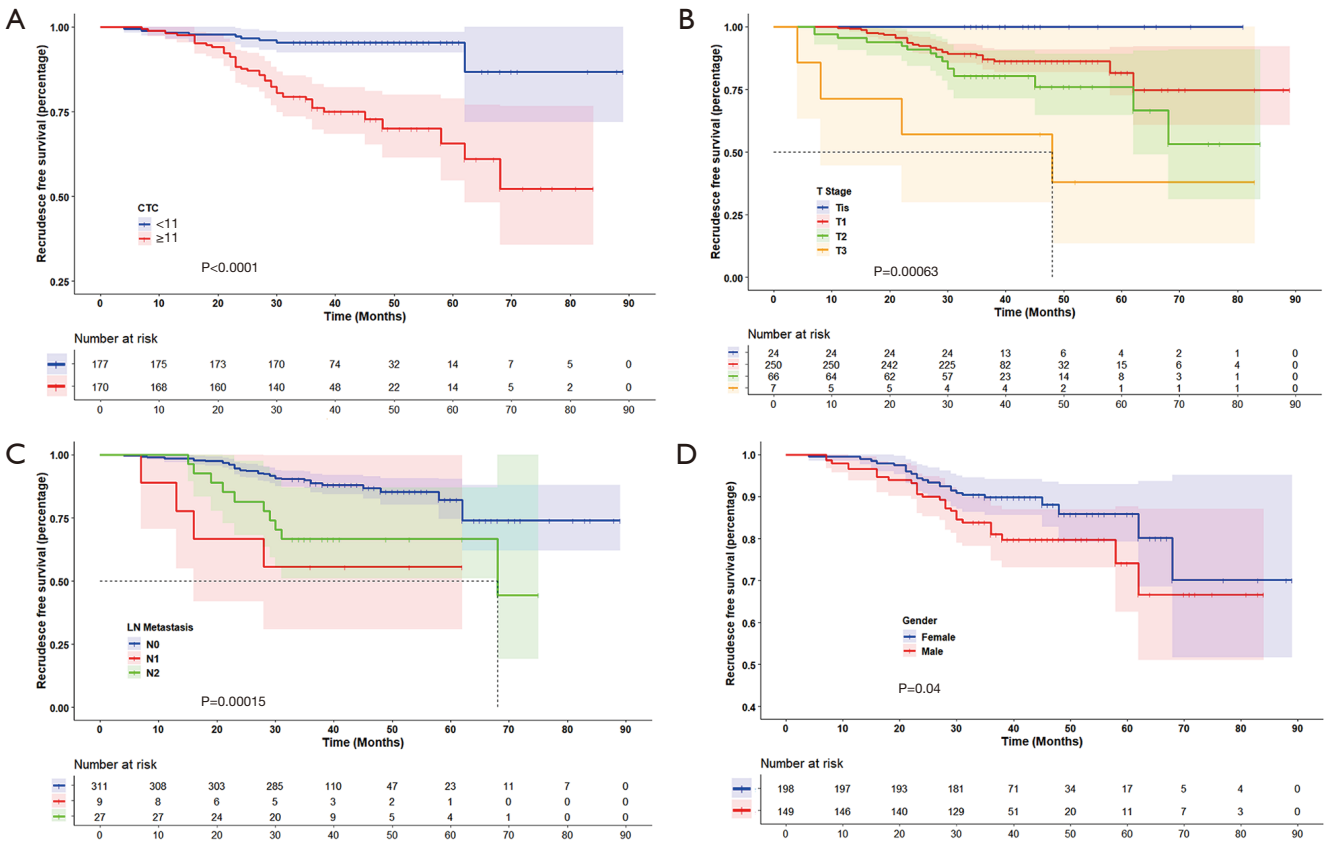

Figure 2 CTC concentration as an independent predictor of RFS. (A) Kaplan-Meier curves showing RFS in 347 NSCLC patients stratified as CTC concentration high or low (≥11 FU or <11 FU per 3 mL blood, respectively). (B) Kaplan-Meier curves showing RFS in 347 NSCLC patients stratified by T stage (Tis, T1, T2, and T3). (C) Kaplan-Meier curves showing RFS in 347 NSCLC patients stratified by lymph node metastasis (N0, N1, and N2). (D) Kaplan-Meier curves showing RFS in 347 NSCLC patients stratified by sex. The number of patients at risk for each time point is indicated below the time point. CTC, circulating tumor cell; RFS, recurrence-free survival; NSCLC, non-small cell lung cancer; FU, functional unit.

(HR: 1.929; 95% CI: 1.113–3.342, P=0.019), showing that preoperative CTC concentration and tumor size were independent prognostic factors for early-stage NSCLC patients. A high preoperative CTC concentration was considered a risk factor for poor prognosis in NSCLC. The risk of recurrence/metastasis in the group with high CTC concentration was 5.489 times higher than that in the group with low CTC concentration. Subsequently, using the Kaplan-Meier method and the log-rank test, we found that CTC concentration had the best predictive value for RFS in the NSCLC patients (*Figure 2*). Therefore, preoperative CTC concentration could represent a useful biomarker to predict clinical outcomes of early-stage NSCLC patients.

Nomogram and calibration curves

A nomogram (*Figure 3A*) to predict NSCLC recurrence was established based on the results of the multivariate Cox regression. The factors included in the prediction model were serum CTC concentration, T stage, lymph node metastasis, and sex, all of which can be assessed preoperatively. The concordance index value of the prediction model was 0.82. Validation revealed that the nomogram exhibited excellent predictive ability and calibration, suggesting its clinical utility in the preoperative screening of NSCLC patients (*Figure 3B*,*C*,*D*,*E*).

Discussion

The study of CTCs can be traced back to 1896, when Thomas Ashworth put forward this statement of CTCs' existence (21). Over the next 100 years, the development of CTC enrichment technology was slow (22,23), and it was not until 1998 that Racila proposed separation of leukocyte using immunomagnetic beads (12), and CTCs were not used in clinical applications. Researchers' efforts to explore the clinical application of CTCs have not ceased. To date, CTCs have been used as a liquid biopsy for tumor molecular pathology, to predict tumor prognosis, and to monitor treatment effects, among other uses. CTC concentration is relatively low in the general population (24,25). Further, Hofman *et al.* demonstrated the identification of CTCs in resectable NSCLC using isolation by size of epithelial tumor cell (ISET) technology according to cytopathological criteria of malignancy and the absence of non-hematological cells in the blood of healthy controls (26). Krebs *et al.* found that CTCs count, measured using a semiautomated epithelial cell adhesion molecule-based immunomagnetic technique, was higher in NSCLC patients with stage IV compared to patients with stage III; they also showed that CTCs might to provide prognostic information and or early indication of response to chemotherapy (27). Hofman *et al.* evaluated the prognostic relevance of CTCs detected both by ISET and CellSearch (CS) in 210 patients undergoing radical surgery for NSCLC. The authors reported a significantly shorter disease-free survival (DFS) in patients with preoperative detectable CTCs and suggested that ISET and CS should be considered as complementary methods (28). Recently, researchers of the M.D. Anderson Cancer Center (Texas, USA) demonstrated that lysates of Micro Cavity Array (MCA)-enriched CTCs collected from the peripheral blood of 38 patients with stage III NSCLC are amenable to molecular characterization and that MCA-enriched CTCs represent an independent prognostic marker (29).

The present study demonstrated a significant association between CTC concentration and lymph node metastasis, suggesting that CTC concentration is a vital prognostic feature for patients with NSCLC. Furthermore, NSCLC patients with a high CTC concentration (≥11 FU) displayed a poorer RFS rate than those with a low CTC concentration (<11 FU). To exclude the interaction of various factors associated with prognosis, multivariate RFS analysis was performed, and only sex, CTC concentration, T stage, and lymph node metastasis were revealed to act as independent prognostic factors for NSCLC patients. Based on the RFS of the NSCLC patients, we established a risk scoring system to predict the recurrence risk in early NSCLC over time. The concordance index value was 0.82. The NSCLC patients in the present study showed no significant differences in CTC concentration in terms of stage, age, sex, or EGFR mutation status (all P>0.05). In fact, preoperative CTC concentration was only found to be significantly associated with lymph node metastasis (P <0.05). The preoperative CTC concentration of NSCLC patients with extrapulmonary metastasis was considerably higher than that of patients with intrapulmonary recurrence, and NSCLC patients with multiple distant metastases had the highest preoperative CTC concentration. At the same time, we also found that preoperative CTC concentration had a good predictive value for predicting postoperative recurrence and metastasis of NSCLC, with an AUC value of 0.80. Based on the fact that preoperative CTC concentration had no statistical relationship with T stage but demonstrated high prognostic value, it could potentially

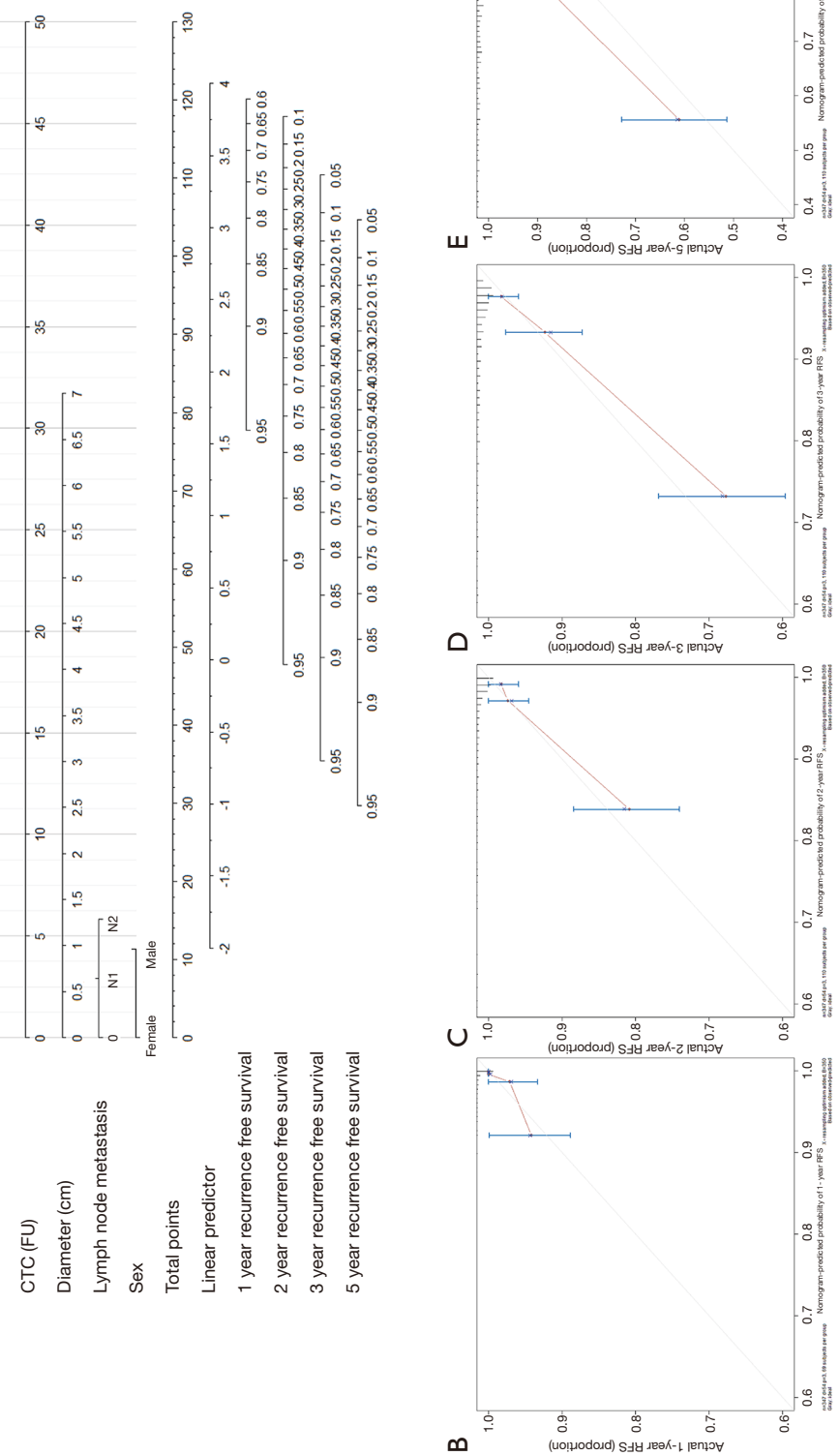

Figure 3 Nomogram and calibration curves. (A) A nomogram to predict recurrence and metastasis of NSCLC was drawn based on the multivariate Cox regression model. (B) Calibration curve of nomogram for 1-year RFS. (C) Calibration curve of the nomogram for 2-year RFS. (D) Calibration curve of the nomogram for 3-year RFS. (E) Calibration curve of the nomogram for 5-year RFS. RFS, recurrence-free survival; NSCLC, non-small cell lung cancer.

serve as an independent and sensitive preoperative screening method for NSCLC patients.

Studies have shown that CTCs have good prospects of application in various tumors, such as breast, colon, prostate, and lung cancers (27,30-34), with an increase in CTC count indicating a poor prognosis. Importantly, CTCs can be used as a straightforward method of examination to monitor the effects of surgery, radiotherapy, and chemotherapy (35,36). In our study, multivariate analysis using the Cox proportional-hazards model showed that sex, CTC concentration, T stage, and lymph node metastasis were independent factors of RFS in early lung cancer. In this multivariate regression model, CTCs contributed the most to the prediction, followed by tumor size. Consistent with the findings of previous studies, in this study, CTC concentration demonstrated significant predictive power for the prognosis of patients with early lung cancer undergoing surgery. Therefore, based on the RFS of NSCLC patients, we established a risk scoring system to predict the risk of recurrence in NSCLC patients over time. Sex, preoperative serum CTC concentration, T stage, and lymph node metastasis can be evaluated in patients preoperatively. The concordance index value of the prediction model was 0.82, showing good predictive value. Therefore, our nomogram may be of great significance in the preoperative screening of NSCLC patients. This model could significantly reduce the false positive rate, avoid the overdiagnosis and overtreatment of NSCLC patients, and be used to identify the subgroup of NSCLC patients requiring aggressive treatment.

There are some notable points and limitations in this study. Firstly, this is a retrospective study, and the data were collected from a single center. Further research on multi-center cohorts and different populations is warranted. Secondly, based on this study, we found that CTC concentration can predict the prognosis of NSCLC patients treated with surgery, with a high CTC count indicating a poor prognosis. This means that preoperative CTC monitoring is necessary, and patients with a high CTC concentration should have more aggressive preoperative or posterior treatment plans. However, postoperative CTC concentration has not yet been confirmed as a predictive or surrogate marker for RFS in the post-treatment setting, and we do not yet know how CTCs will impact clinical decision-making for a given systemic therapy. It is clear that ongoing randomized studies with prospectively embedded CTC-based validation studies are needed to determine the surrogate value of CTCs for overall survival (OS) before CTCs can be used for definitive clinical decision making.

Conclusions

Our results showed preoperative CTC concentration to be an independent and sensitive prognostic biomarker of NSCLC and to be closely related to the RFS of NSCLC patients. A CTC concentration ≥11 FU was associated with a higher risk of disease recurrence. This study established a nomogram to predict NSCLC recurrence in NSCLC patients, which can be used for preoperative screening of early NSCLC patients and provide a theoretical basis for personalized clinical decision-making for patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the institutional review board of Shanghai Pulmonary Hospital, Tongji University. The requirement for informed consent from each patient was waived due to the retrospective nature of this analysis. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 Relationship between CTC concentration and metastatic sites in NSCLC patients (n=347)

Characteristic	CTC concentration (FU)
All patients	12.09±5.54
Metastatic site	
No metastasis	11.24±5.12
Lung	15.99±5.69
Brain	17.02±5.67
Bone	16.89±4.13
Liver	25.56
Multiple	17.25±4.78
Intrapulmonary	15.99±5.69
Extrapulmonary	17.34±5.29

CTC, circulating tumor cell; NSCLC, non-small cell lung cancer; FU, functional unit.

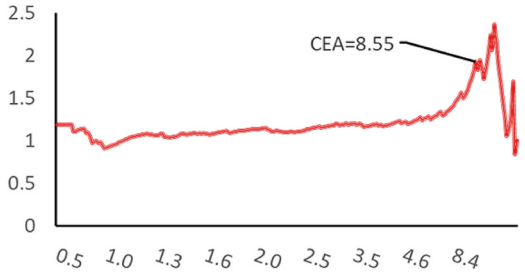

Figure S2 The cutoff-value of CEA according to the dichotomized value. The patients were divided into two group according to their dichotomized CEA value, and the patients were divided into two groups according to the dichotomized value. The log-rank method was used to detect the statistical significance of survival. According to the number of patients and statistical power, the optimal CEA cutoff value was selected. Based on this curve, we found that CEA did not increase significantly in patients with early NSCLC, and CEA was high to a certain extent before it impacted survival, resulting in imbalance between the two groups. NSCLC, non-small cell lung cancer; CEA, carcinoembryonic antigen.

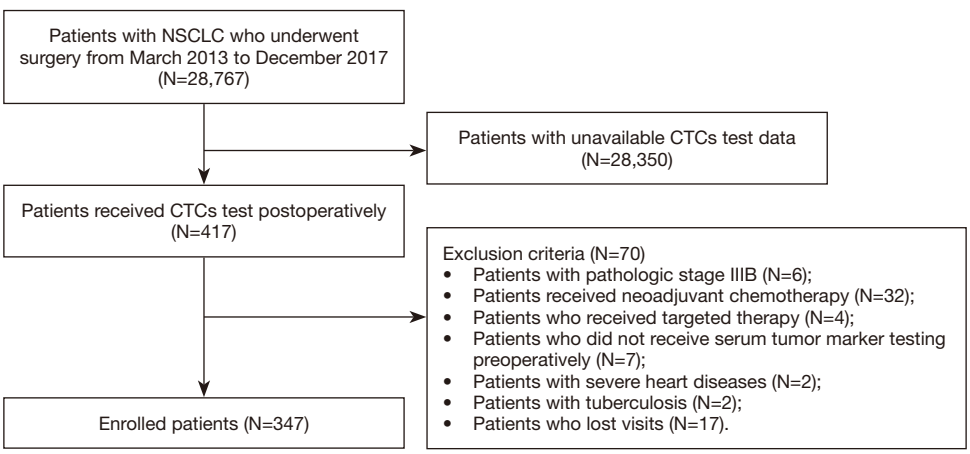

Figure S1 Flowchart for patient selection in this study. NSCLC, non-small cell lung cancer; CTC, circulating tumor cell.

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