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The Tumor-immune Index is Correlated With the Prognosis of Patients After Curative Resection for Nonsmall Cell Lung Cancer

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Abstract: We developed a novel tumor-immune index (TII) based on carcinoembryonic antigen levels, lymphocyte counts, and platelet counts, and explored its prognostic value in nonsmall cell lung cancer (NSCLC).

The prognostic value of the TII was evaluated based on a retrospective study of 205 patients with early NSCLC, who underwent resection in the whole year of 2006, and validated in another group of 228 patients enrolled in the next year of 2007.

The optimal cut-off point for the TII was 578×10^{-9} , and this value was used to stratify patients with NSCLC into low TII ($\leq 578 \times 10^{-9}$) and high TII ($> 578 \times 10^{-9}$) groups. Univariate and multivariate analyses revealed that high TII was an independent predictor for overall survival and recurrence-free survival in both the training and validation cohorts. The areas under the curve of the TII for survival and recurrence were significantly larger than those for tumor, node, metastasis (TNM) stage and carcinoembryonic antigen. In the subgroup analysis, the TII was also significantly correlated with overall survival ($P = 0.001$, $P = 0.009$, and $P = 0.007$ in the TNM I, II, and IIIa subgroups, respectively) and recurrence-free survival ($P < 0.001$, $P = 0.006$, and $P = 0.014$ in the TNM I, II, and IIIa subgroups, respectively). Similarly, for patients with N2-positive tumors, the overall survival and recurrence-free survival rates for patients in the high TII group were also significantly lower than the respective values for patients in the low TII group ($P = 0.026$ and $P = 0.007$, respectively).

The TII can be used to distinguish patients with similar pathologies and stages into high and low-risk categories based on the probability of recurrence according to a convenient blood-based test.

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Abbreviations: AUC = area under the curve, CEA = carcinoembryonic antigen, CI = confidence interval, COPD = chronic obstructive pulmonary diseases, HR = hazard ratio, NSCLC = nonsmall cell lung cancer, OS = overall survival, RFS = recurrence-free survival, TII = tumor-immune index, TNM = tumor node metastasis.

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INTRODUCTION

Lung cancer is one of the most common cancers worldwide, with nonsmall cell lung cancer (NSCLC) accounting for 80% of all diagnosed lung cancer cases.¹ The pathologic and anatomical extent of disease, as described by the tumor, node, metastasis (TNM) staging system, is one of the most important prognostic factors in NSCLC.² For patients with TNM stages I to IIIa of NSCLC, surgery is the main treatment. However, despite “curative” resection, nearly 30% to 70% of patients will die of recurrent disease depending on the tumor stage.^{3–5} These results suggest that another marker enabling accurate stratification of recurrence risk beyond that provided by TNM stage is necessary for more accurate prognostication. In this manner, it may be possible to stratify high-risk patients with stage I and II disease who may benefit from adjuvant chemotherapy, and high-risk patients with stage III disease who may need more careful consideration of surgery. Apart from this, for patients with stage IIIa NSCLC with mediastinal lymph node-positive (N2) disease, whether surgical resection is the best treatment has not yet been determined because of the poor outcomes.⁶ The added benefit of another marker will help clinicians identify the patients at greatest risk for recurrent disease and therefore determine the optimal treatment for this subset.

Many studies over the past 2 decades have found that carcinoembryonic antigen (CEA), as a tumor antigen, has an adjunctive role in the staging of lung cancer, and that elevated CEA levels are associated with poor prognosis in patients with resected NSCLC.^{7–12} As we know, as well as the characteristics of the tumor itself, tumor cell invasion into the peripheral blood and patients’ immune status also contribute to tumor recurrence and the reseeding of distant organs. It is well known that platelets can protect tumor cells from immune surveillance and promote tumor cell extravasation to metastatic sites.^{13–16} Lymphocytes play a crucial role in tumor defense by inducing cytotoxic cell death, and inhibiting tumor cell proliferation and migration, thereby dictating the host immune response to malignancy.¹⁷ However, an integrated index based on serum CEA levels and peripheral platelet and lymphocyte counts, which might better reflect the balance of tumor staging and the host immune status, has yet not been reported in lung cancer.

In this study, we developed a novel index, defined as the tumor-immune index (TII), based on CEA levels, and lymphocyte and platelet counts. The prognostic value of the TII in patients with resectable NSCLC was evaluated retrospectively.

MATERIALS AND METHODS

Patients

From 2006 to 2007, 568 adult patients with lung cancer underwent curative resection in our institute. After excluding patients who died in the perioperative period ($n = 13$), patients who received noncurative resection ($n = 29$), patients with

metastatic lung cancer (n = 25), patients without follow-up data (n = 12), patients with positive surgical margin (n = 8), patients with autoimmune diseases (n = 5), patients with infectious diseases (n = 10), and patients who underwent preoperative radiotherapy or chemotherapy for lung cancer (n = 33), the remaining 433 patients formed the analysis population in our study. We defined these patients who underwent the operation in the whole year of 2006 as a “training cohort” (n = 205) and patients who underwent the operation in the whole year of 2007 as a “validation cohort” (n = 228). In general, all the eligible patients had pathologically documented stage I, II, or III NSCLC, and had undergone complete surgical resection. The absence of previous chemotherapy, radiotherapy, and previous cancer was also verified for all patients.

Clinicopathological Factors

Clinicopathological factors were selected on the basis of previous studies.^{18,19} The pathological stages of the patients were determined according to the international TNM classification system for lung cancer.²⁰ Chronic obstructive pulmonary disease was defined as follows: forced expiratory volume in 1 second (FEV1% predicted) 70%; and FEV1/forced vital capacity <70%.²¹ The cut-off value for CEA was set at the standard level (5 ng/mL) in this study, which was also used in many previous studies.^{10,22} Radiotherapy suggested that patients received planned postoperative adjuvant radiotherapy.

Follow-up

Clinical follow-up and computed tomography scans were performed at 3, 6, and 12 months, and then at yearly intervals. Recurrence was diagnosed and distinguished from second primary lung tumors by a multidisciplinary tumor board review of available imaging and pathology results. Recurrence was defined as local, regional, or distant recurrence. Survival was determined by contacting the patient or the treating physician, the latter of whom confirmed the date and cause of death for patients who died. Overall survival (OS) was defined as the time from surgery to death from any cause. Recurrence-free survival (RFS) was defined as the time from surgery to the earliest occurrence of relapse or death from any cause. Follow-up was completed on December 31, 2013. The median follow-up duration was 57 months (range 7–89 mo).

TII

The TII was defined as follows: $TII = C \times P/L$, where *C*, *P*, and *L* were the preoperative peripheral blood CEA level, platelet count, and lymphocyte count, respectively. Blood samples were obtained immediately before the surgery. After adjusting for TNM stage, a nonlinear relationship between the TII values and the risk of recurrence was observed (see Figure S1, Supplemental Content, <http://links.lww.com/MD/A537>, which illustrates the adjusted association between TII and the risk of NSCLC recurrence after curative resection). This suggested that using the TII as a continuous variable for the following analysis might be inappropriate. Thus, X-tile 3.6.1 software (Yale University, New Haven, CT) was used for bioinformatic analysis of the study data to determine the cut-off value of the TII for tumor recurrence.²³ Results from the X-Tile analysis revealed the optimal cut-off point for the TII in the training cohort was 578×10^{-9} (see Figure S2, Supplemental Content, <http://links.lww.com/MD/A537>, which illustrates the optimal cut-off value for the SII defined by X-tile). Subsequently, the TII scores were used stratify patients into the low TII ($\leq 578 \times 10^{-9}$) or high TII group ($> 578 \times 10^{-9}$) for the following analyses.

Ethics Statement

This was a retrospective study making use of data already collected. All data used in this study were routine clinical data collected in the process of diagnosis and treatment. The analysis procedure of data was done after anonymization. National legislation and the ethical committee of Shandong Cancer Hospital and Institute approved this retrospective study.

Statistical Analysis

Statistical analyses were performed with R (version 3.2.2, <http://www.R-project.org>). Continuous variables were summarized as mean \pm standard deviation and categorical variables were summarized as n (%). The relationship between the TII and the risk of NSCLC recurrence was explored using a smoothing plot (Figure S1, <http://links.lww.com/MD/A537>). Student *t* test and Pearson chi-square test or Fisher exact test were used to compare differences between the groups (Table 1,

TABLE 1. The Clinicopathologic Characteristics of Patients in the Training and Validation Cohorts

Characteristics	Training Cohort n = 205 (%)	Validation Cohort n = 228 (%)	P value
Age			0.804
≥65 y	158 (77.1)	178 (78.1)	
<65 y	47 (22.9)	50 (21.9)	
Sex			0.495
Male	160 (78.0)	184 (80.7)	
Female	45 (22.0)	44 (19.3)	
Comorbidities			0.939
Hypertension	34 (16.6)	43 (18.9)	
Diabetes	47 (22.9)	51 (22.4)	
COPD	25 (12.2)	26 (11.4)	
Smoking history			0.013*
Never smoking	27 (13.2)	51 (22.4)	
Ever smoking	178 (86.8)	177 (77.6)	
Weight loss			0.314
<5%	168 (82.0)	178 (78.1)	
≥5%	37 (18.0)	50 (21.9)	
LDH levels			0.527
<245 U/L	66 (32.2)	67 (29.4)	
≥245 U/L	139 (67.8)	161 (70.6)	
Serum CEA			0.250
<5 ng/mL	82 (40.0)	79 (34.6)	
≥5 ng/mL	123 (60.0)	149 (65.4)	
Type of surgery			0.529
Pneumonectomy	152 (74.1)	175 (76.8)	
Lobectomy	53 (25.9)	53 (23.2)	
TNM stage			0.110
Stage I	57 (27.8)	85 (37.3)	
Stage II	63 (30.7)	60 (26.3)	
Stage III	85 (41.5)	83 (36.4)	
Tumor histology			0.139
Squamous	112 (54.6)	102 (44.7)	
Adenocarcinoma	72 (35.1)	89 (39.0)	
Large cell	12 (5.9)	22 (9.6)	
Others	9 (4.4)	15 (6.6)	
Radiotherapy			0.822
Yes	171 (83.4)	192 (84.2)	
No	34 (16.6)	36 (15.8)	

CEA = carcinoembryonic antigen, COPD = chronic obstructive pulmonary disease, LDH = lactate dehydrogenase, TNM = tumor, node, metastasis.

* Significant difference.

TABLE 2. Univariate Cox Regression Analyses of the TII With Clinicopathologic Characteristics (Training Cohort, n = 205 and Validation Cohort, n = 228)

Variables	OS		RFS	
	HR (95% CI)	P	HR (95% CI)	P
Training cohort				
Age, (≥65 vs <65)	1.2 (0.82, 1.8)	0.347	1.1 (0.78, 1.7)	0.495
Sex (female vs male)	0.93 (0.61, 1.4)	0.733	1.0 (0.68, 1.5)	0.912
Comorbidities (diabetes vs without)	1.0 (0.64, 1.5)	0.974	0.98 (0.63, 1.5)	0.912
Smoking history (ever vs never)	0.87 (0.52, 1.5)	0.600	0.85 (0.52, 1.4)	0.530
Weight loss (≥5% vs <5%)	0.8 (0.5, 1.3)	0.349	0.82 (0.53, 1.3)	0.400
LDH (≥245 vs <245)	0.89 (0.62, 1.3)	0.535	0.88 (0.62, 1.3)	0.497
CEA (≥5 vs <5)	1.2 (0.85, 1.7)	0.287	1.2 (0.86, 1.7)	0.267
Type of surgery (lobectomy vs pneumonectomy)	1.1 (0.74, 1.6)	0.649	1.1 (0.73, 1.6)	0.729
TNM stage (II vs I)	1.6 (1.0, 2.6)	0.054	1.5 (0.95, 2.4)	0.084
TNM stage (III vs I)	2.0 (1.2, 3.1)	0.004*	1.7 (1.1, 2.6)	0.015*
Tumor history (others vs squamous)	0.88 (0.62, 1.2)	0.461	0.83 (0.59, 1.2)	0.277
Radiotherapy (yes vs no)	0.8 (0.49, 1.3)	0.402	0.84 (0.51, 1.4)	0.756
TII group (>578 vs ≤578)	2.6 (1.8, 3.7)	<0.001*	2.3 (1.6, 3.3)	<0.001*
Validation cohort				
Age, (≥65 vs <65)	1.4 (1.0, 2.1)	0.056	1.4 (1.0, 2.1)	0.055
Sex (female vs male)	1.2 (0.79, 1.8)	0.427	1.1 (0.74, 1.7)	0.591
Comorbidities (diabetes vs without)	1.6 (1.1, 2.5)	0.019*	1.3 (0.86, 2.0)	0.210
Smoking history (ever vs never)	1.0 (0.69, 1.5)	0.894	1.1 (0.73, 1.6)	0.671
Weight loss (≥5% vs <5%)	1.2 (0.79, 1.7)	0.465	1.2 (0.79, 1.7)	0.468
LDH (≥245 vs <245)	1.1 (0.78, 1.6)	0.539	1.2 (0.84, 1.7)	0.312
CEA (≥5 vs <5)	1.8 (1.3, 2.6)	0.001*	1.8 (1.2, 2.5)	0.002*
Type of surgery (lobectomy vs pneumonectomy)	1.1 (0.75, 1.6)	0.640	1.1 (0.74, 1.6)	0.687
TNM stage (II vs I)	1.4 (0.9, 2.1)	0.155	1.5 (0.99, 2.3)	0.058
TNM stage (III vs I)	1.7 (1.2, 2.5)	0.007*	1.7 (1.2, 2.5)	0.006*
Tumor history (others vs squamous)	0.66 (0.48, 0.92)	0.013*	0.67 (0.48, 0.93)	0.016*
Radiotherapy (yes vs no)	1.3 (0.86, 2.0)	0.203	1.4 (0.91, 2.2)	0.122
TII group (>578 vs ≤578)	3.0 (2.2, 4.2)	<0.001*	2.6 (1.9, 3.6)	<0.001*

CI = confidence interval, COPD = chronic obstructive pulmonary diseases, HR = hazard ratio, LDH = lactate dehydrogenase, OS = overall survival, RFS = recurrence free survival, TII = tumor-immune index, TNM = tumor, node, metastasis.

* Significant difference.

Table S1, <http://links.lww.com/MD/A538>). Univariate and multivariate analyses were calculated using the Cox proportional-hazards regression model (Tables 2 and 3). Time-dependent receiver-operating characteristic curves were used to define sensitivity and specificity, and the differences in the area under the curve (AUC) were detected by using MedCalc version 13.0 (Fig. 1). OS and RFS were calculated using the Kaplan–Meier method, and the differences between the groups were assessed using the log-rank test (Figs. 2–4).

All data were double entered and then exported to tab-delimited text files. Probability values of less than 0.05 were considered statistically significant.

RESULTS

Table 1 shows the clinicopathologic characteristics of the 433 study participants who received curative surgery. In the training cohort, 57, 63, and 85 patients had stage I, II, and III disease, respectively. Through the end of the study, 79 of the 205 patients had no evidence of NSCLC recurrence, whereas the remaining 126 patients had documented evidence of lung cancer recurrence with a median follow-up of 58.7 months (range 8–89 mo). In the validation cohort, 85, 60, and 83 patients had stage I, II, and III disease, respectively. Total

147 of 228 patients presented with tumor recurrence and 81 patients were still recurrence-free with a median follow-up of 55.6 months (range 11–83 mo). The clinicopathologic characteristics were similar between the 2 cohorts, except for smoking history. The validation cohort included more patients with smoking history than those in the training cohort.

The results of univariate analysis suggested that TNM stage and the TII were associated with both OS and RFS, whereas age, sex, comorbidities, smoking history, type of surgery, and radiotherapy had no prognostic significance for OS and RFS, in the training group (Table 2). In addition, we also found that patients with comorbidities, especially diabetes, were more likely to have higher TII values ($P = 0.008$, see Table S1, Supplemental Content, <http://links.lww.com/MD/A538>, which illustrates the correlation between TII and clinicopathologic characteristics). After adjusting for comorbidities and potential confounders found in the univariate analysis, a multivariate regression analysis was performed to estimate the independent prognostic factors for OS and RFS (Table 3). The results showed the TII was an independent prognostic factor for both OS (hazard ratio [HR] 3.5, 95% confidence interval [CI] 2.2–5.6, $P < 0.001$) and RFS (HR 3.1, 95% CI 1.9–4.9, $P < 0.001$). A lower TII was significantly associated with both higher OS

TABLE 3. Multivariate Cox Regression Analyses in the Training and Validation Cohorts

Variables	OS		DFS	
	HR (95% CI)	P	HR (95% CI)	P
Training cohort				
Age, (≥65 vs <65)	1.1 (0.72, 1.7)	0.667	0.98 (0.65, 1.5)	0.941
Comorbidities (diabetes vs without)	0.88 (0.55, 1.4)	0.574	0.87 (0.55, 1.4)	0.554
Smoking history (ever vs never)	0.96 (0.57, 1.6)	0.881	0.9 (0.54, 1.5)	0.703
CEA (≥5 vs <5)	0.7 (0.44, 1.1)	0.128	0.73 (0.46, 1.1)	0.169
Type of surgery (lobectomy vs pneumonectomy)	0.97 (0.65, 1.5)	0.881	0.94 (0.63, 1.4)	0.741
TNM stage (II vs I)	1.9 (1.1, 3.1)	0.015*	1.7 (1.1, 2.8)	0.030*
TNM stage (III vs I)	2.2 (1.4, 3.5)	0.001*	1.9 (1.2, 2.9)	0.006*
Tumor history (others vs squamous)	0.85 (0.59, 1.2)	0.390	0.8 (0.56, 1.1)	0.230
TII group (>578 vs ≤578)	3.5 (2.2, 5.6)	<0.001*	3.1 (1.9, 4.9)	<0.001*
Validation cohort				
Age (≥65 vs <65)	1.2 (0.84, 1.8)	0.279	1.3 (0.85, 1.9)	0.242
Comorbidities (diabetes vs without)	1.4 (0.9, 2.1)	0.143	1.1 (0.7, 1.6)	0.783
Smoking history (ever vs never)	1.1 (0.71, 1.6)	0.728	1.1 (0.74, 1.7)	0.623
CEA (≥5 vs <5)	1.1 (0.67, 1.7)	0.772	1.0 (0.65, 1.7)	0.871
Type of surgery (lobectomy vs pneumonectomy)	1.0 (0.68, 1.5)	0.956	1.0 (0.69, 1.5)	0.938
TNM stage (II vs I)	1.5 (0.96, 2.3)	0.078	1.6 (1.0, 2.4)	0.045*
TNM stage (III vs I)	1.8 (1.2, 2.6)	0.004*	1.7 (1.2, 2.5)	0.007*
Tumor history (others vs squamous)	0.65 (0.46, 0.9)	0.011*	0.68 (0.49, 0.95)	0.024*
TII group (>578 vs ≤578)	2.7 (1.8, 4.2)	<0.001*	2.4 (1.6, 3.8)	<0.001*

CI = confidence interval, COPD = chronic obstructive pulmonary diseases, HR = hazard ratio, LDH = lactate dehydrogenase, OS = overall survival, RFS = recurrence-free survival, TII = tumor-immune index, TNM = tumor, node, metastasis.

*Significant difference.

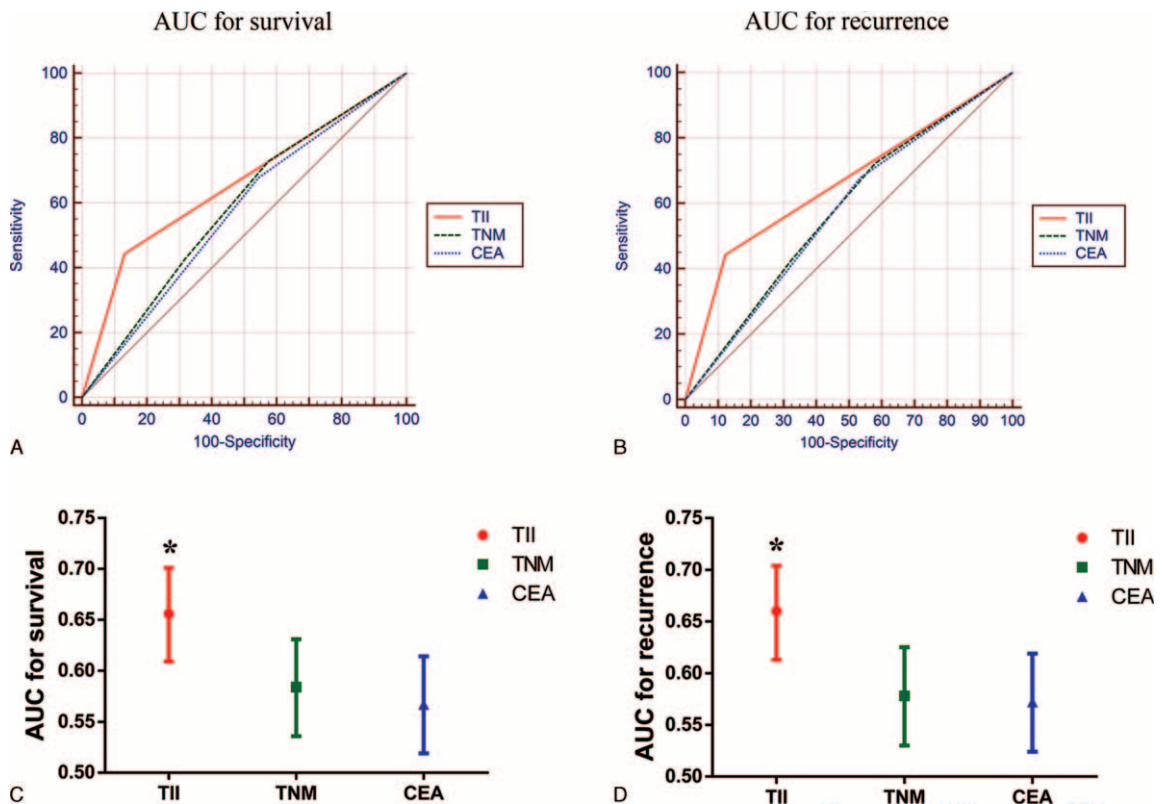


FIGURE 1. The discriminative ability of the TII and clinical indices was compared using the AUCs for survival and recurrence. (A) The AUC of TII, TNM, and CEA in predicting survival was 0.66 (95% CI 0.61–0.69), 0.59 (95% CI 0.54–0.63), and 0.56 (95% CI 0.52–0.61), respectively. (B) The AUC of CEA was significantly lower than that of TII in predicting tumor recurrence ($P=0.024$). (C) The AUC of TII, TNM, and CEA in predicting recurrence was 0.67 (95% CI 0.61–0.70), 0.58 (95% CI 0.53–0.63), and 0.57 (95% CI 0.52–0.63), respectively. (D) The AUC of CEA was significantly lower than that of TII in predicting survival ($P=0.016$). AUC = area under the curve, CEA = carcinoembryonic antigen, CI = confidence interval, TII = tumor-immune index, TNM = tumor, node, metastasis.

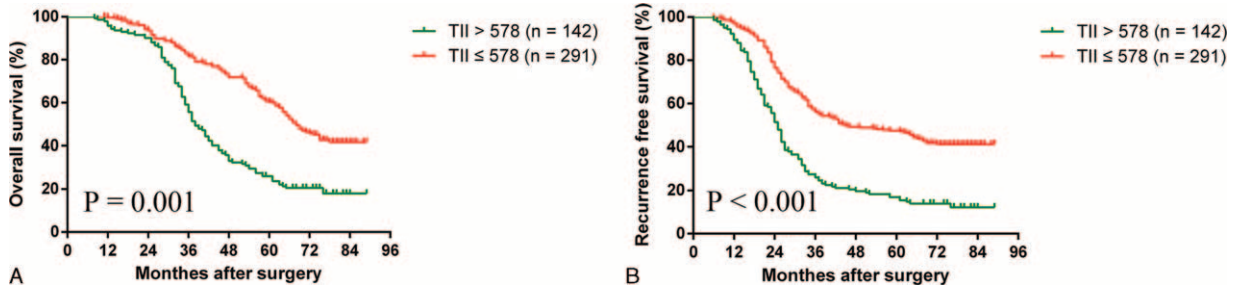


FIGURE 2. The Kaplan–Meier analysis of OS and RFS for the TII in total study population. (A) The OS rate in the low TII group was significantly higher compared with those in the high TII group ($P=0.001$). (B) The RFS rate in the low TII group was significantly higher compared with those in the high TII group ($P<0.001$). OS = overall survival, RFS = recurrence-free survival, TII = tumor-immune index, TNM = tumor, node, metastasis.

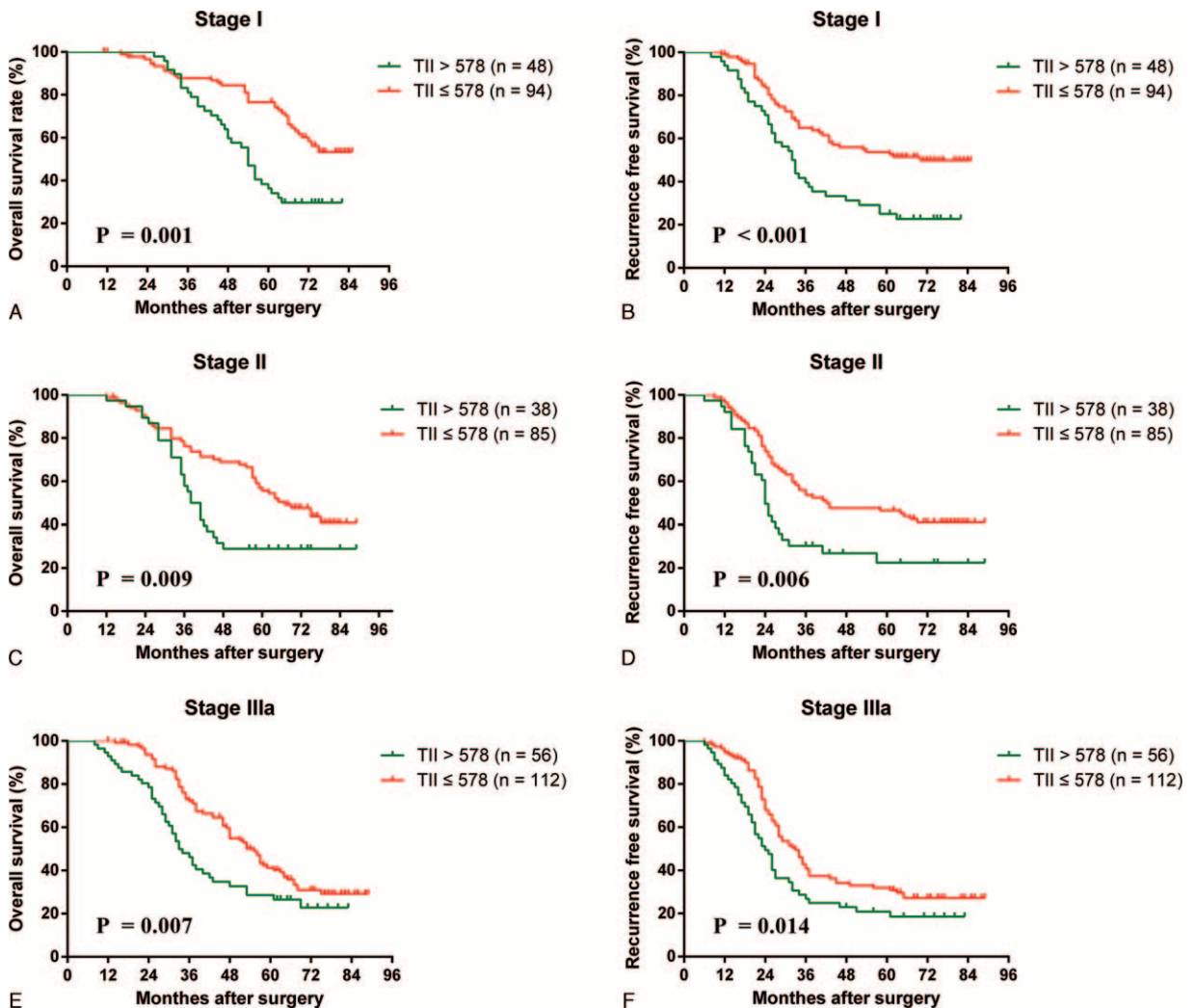


FIGURE 3. The Kaplan–Meier analysis of OS and RFS for the TII in different TNM stages. Both the OS and RFS in the low TII group were significantly higher compared with those in the high TII group in patients with stage I (A, $P=0.001$; B, $P<0.001$), stage II (C, $P=0.009$; D, $P=0.006$), and IIIa (E, $P=0.007$; F, $P=0.014$). OS = overall survival, RFS = recurrence-free survival, TII = tumor-immune index, TNM = tumor, node, metastasis.

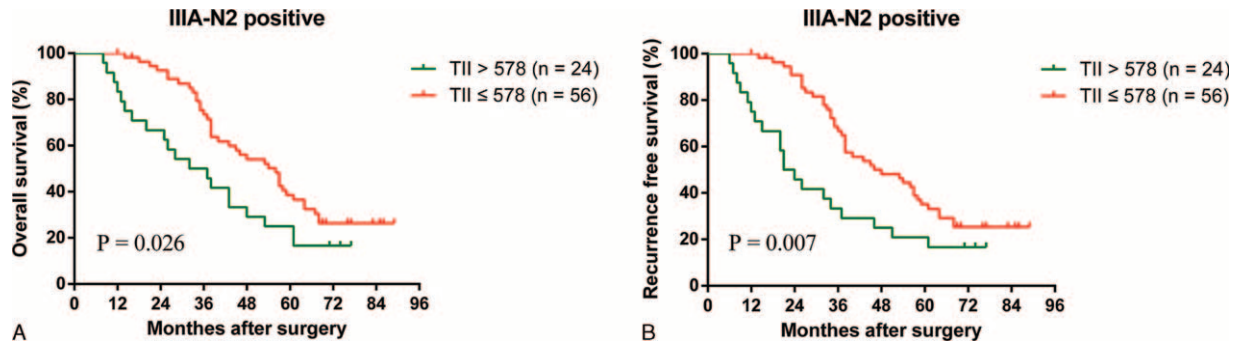


FIGURE 4. The Kaplan–Meier analysis of OS and RFS for the TII in patients with N2-positive. (A) The OS in the low TII group was significantly higher compared with those in the high TII group in patients with N2-positive ($P=0.026$). (B) The RFS in the low TII group was significantly higher compared with those in the high TII group in patients with N2-positive ($P=0.007$). OS=overall survival, RFS=recurrence-free survival, TII=tumor-immune index.

and RFS rates. The prognostic value of the TII was further confirmed in another independent validation cohort of 228 patients. These results from univariate and multivariate analyses were similar to those obtained from the previous training cohort. The high TII remained decreased both OS (HR 2.7, 95% CI 1.8–4.2, $P<0.001$) and RFS (HR 2.4, 95% CI 1.6–3.8, $P<0.001$) rates (Table 3). In addition, TNM stage and tumor history also had prognostic significance in predicting both OS and RFS in the validation cohort.

The discriminative ability of the TII and clinical indices was compared using the AUCs for recurrence and survival (Fig. 1). TII was considered as an indicator of survival, with AUC of 0.66 (95% CI 0.61–0.69), and the optimal cut-off point was 578×10^9 , with a sensitivity of 43.3% and a specificity of 86.0% (Fig. 1A). In addition, TII was also considered as an indicator of recurrence, with AUC of 0.67 (95% CI 0.61–0.70). The optimal cut-off point had a sensitivity of 42.8% and a specificity of 86.6% (Fig. 1C). TNM yielded the AUC values of 0.59 (95% CI 0.54–0.63) and 0.58 (95% CI 0.53–0.63) in discriminating survival and recurrence, whereas CEA yield the AUC values of 0.56 (95% CI 0.52–0.61) and 0.57 (95% CI 0.52–0.62). Among these markers, TII was the strongest predictor of survival (Fig. 1B) and recurrence (Fig. 1D).

In all the studied patients, the OS in the high TII group was significantly lower than that in the low TII group (Fig. 2A; $P=0.001$). The cumulative 1, 3, and 5-year RFS rates were 89.4%, 26.1%, and 16.9%, respectively, in the high TII group, and 96.9%, 56.1%, and 47.5%, respectively, in the low TII group. Similarly, the high TII group also had a lower RFS rate than the low TII group (Fig. 2B; $P<0.001$). The 1, 3, and 5-year OS rates were 95.7%, 55.6%, and 26.0%, respectively, in the high TII group, and 99.6%, 81.6%, and 60.4%, respectively, in the low TII group.

We investigated the prognostic significance of the TII in the different TNM stage subgroups in greater detail. The results showed the TII was significantly correlated with RFS ($P=0.001$, $P=0.009$, and $P=0.007$ in the TNM I, II, and IIIa subgroups, respectively; Figure 3A, C, and E) and OS ($P<0.001$, $P=0.006$, and $P=0.014$ in the TNM I, II, and IIIa subgroups, respectively; Figure 3B, D, and F).

Exploratory subgroup analyses were conducted to evaluate the consistency of the results in patients with N2-positive lesions. Through the end of follow-up, patients in the high TII group had an OS rate of 16.7%, compared with 26.4% for patients in the low TII group. The KaplanMeier curve identified

significance between the 2 groups ($P=0.026$; Figure 4A). The RFS rates were 16.7% and 25.3% in the high and low TII groups, respectively, at the end of follow-up. Similarly, the RFS rate in the high TII group was also significantly lower than that in the low TII group ($P=0.007$; Figure 4B).

DISCUSSION

Several studies revealed the prognostic significance of tumor-associated antigen or immune-related biomarkers in peripheral blood in postsurgery patients with lung cancer.^{7–17,24,25} In the present study, a novel tumor immune-based prognostic index (TII) was constructed based on CEA levels, and lymphocyte and platelet counts; then it was proved to be an independent predictor of recurrence and survival for patients with early-stage NSCLC after surgery. On the one hand, the predictive ability of the TII was found to be as strong as that of TNM stage for total patients. On the other hand, it was also possible to distinguish patients with similar TNM stages into high and low-risk categories based on the probability of recurrence according to a convenient blood-based test. Thus, there is potential for the TII to be used as a marker for tumor recurrence and treatment response surveillance or combined with TNM stage to provide more accurate guidance of postoperative adjuvant therapy in patients with NSCLC.

As an integrated index based on peripheral CEA levels and lymphocyte and platelets counts, the predictive value of the TII for tumor recurrence and metastasis might be explained by the function of the 3 biomarkers. Many previous studies showed the prognostic value of CEA in serum/plasma in early-stage NSCLC.^{22,24} In addition, some studies also evaluated the use of consecutive measurements of serum CEA during treatment and follow-up. They observed increases in the serum CEA level to be significant as prognostic factors for early recurrence,⁷ progression,⁹ or progression-free survival.²⁶ It should also be noted that a limited number of studies also reported no association between serum CEA levels and the prognosis of NSCLC.^{27–29} Likewise, we also found that CEA was not an independent prognostic factor for either RFS or OS in this study. These different conclusions cast doubt on the use of CEA itself as a strong enough indicator to guide treatment decisions, although it does provide prognostic information as a tumor antigen. Lymphocytes play crucial roles in surveillance and destroying metastatic embolic cells.^{17,30,31} The lungs have the largest concentration of natural killer cells of any peripheral organ.³² Previously published data suggested that inhibition of

natural killer cell-mediated immunity might increase the likelihood of successful tumor metastasis.^{33,34} Evidence is also emerging that platelets can facilitate tumor cell survival within the vasculature (immune evasion), which enables tumor cell survival and proliferation within target tissues of metastasis.^{35–39} In the clinic, thrombocytosis (high platelet count) was reported to be associated with poor prognosis in many cancers, including lung cancer.^{40,41} To overcome the limits of using CEA alone, we integrated lymphocyte and platelet counts as cancer immunomodulation factors to develop the TII index.

Results from our study paralleled the well established association between tumor cells and the host immune system. As we know, cancer immunotherapy was recently selected as the breakthrough of the year in 2013 by editors of the journal *Science*. It focused on the immune microenvironment as well as the tumor itself. In light of therapeutic cancer vaccines, cell-to-cell interactions triggered by tumor antigens and resulting in proper activation of the immune system have been considered for predicting clinical responses.⁴² Interestingly, the TII also could reflect the ability of the host immune system to survey and eliminate detached metastatic tumor cells and thus play important roles in the outcomes of patients who underwent surgical resection for early-stage NSCLC. This suggests that patients with NSCLC who have a higher TII might benefit more from targeted immunotherapy after surgery.

Currently, TNM classification is still one of the most important prognostic factors in NSCLC. However, such classification struggles to explain why some people do not experience relapse despite having the same TNM stage as other patients. In the clinic, another marker that enables accurate stratification of recurrence risk beyond that provided by TNM stage is necessary for more accurate prognostication. We found the TII could effectively predict patients' RFS and OS in different TNM subgroups. For patients with N2-positive NSCLC in particular, the optimal management strategy remains controversial.² Surgical resection has been favored for these patients in some centers, whereas other centers assumed that surgical resection was not indicated for these patients because of the high recurrence rate and the low OS after the operation.⁶ The results of our study demonstrated the prognostic significance of the TII remained strong in patients with N2-positive lesions. If this finding can be further verified, it may largely affect our treatment decisions for patients with N2-positive NSCLC, thus effectively improving their long-term survival.

Recent findings for hepatocellular carcinoma recurrence illustrated that immune-related factors in peripheral blood might be related to higher circulating tumor cell (CTC) counts.⁴³ Previous studies of lung cancer also reached a positive conclusion toward the role of CTCs in the prognostic prediction of recurrence after curative resection.⁴⁴ Therefore, the finding that patients with higher TII values had poorer prognosis could be reasonably explained by the following mechanisms regarding CTC. Patients with higher CEA levels have greater numbers of CTCs invading the peripheral blood, platelets can protect CTCs from different potential damage, and lymphocytes then eliminate CTCs to prevent tumor metastasis and recurrence. However, these specific underlying mechanisms need further experimental confirmation.

There are a few limitations of this study. As noted in previous studies,^{43,45} we also used lymphocyte and platelet counts to represent protumor and antitumor powers. But this quantitative method might ignore the disparity of their capabilities between different patients. In addition, we have to admit that CEA and platelets may affect tumor biology through

nonimmunological mechanisms, like induce epithelial–mesenchymal transition in tumor cells.¹⁴ Nevertheless, the endogenous immunological response during the natural course of cancer constitutes the concept of cancer immunomodulation and has been accepted by many researchers.⁴⁶ Actually, emerging evidence suggests that efficiently stimulating endogenous anticancer immunity is a prerequisite for the successful outcome of conventional cancer therapies.⁴⁷ Because of the limitations of retrospective studies, it is difficult to find out these mechanisms underlying the association between the TII and tumor recurrence. Future investigations are needed to elucidate this by clarifying the immunological and nonimmunological mechanisms among peripheral lymphocytes, platelets, and vascular invasion.

Taken together, our data suggested TII can be used to distinguish patients with similar TNM stages into high and low-risk categories based on the probability of recurrence according to a convenient blood-based test. To the best of our knowledge, this is the first study to show the prognostic value of the TII for patients with early-stage NSCLC after surgery. Because the TII has the advantages of simplicity, convenience, and reproducibility, this approach merits further investigations exploring its potential applications in preventing NSCLC recurrence.

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DTL and BBL conceived and designed the experiments. BBL, RF, and JL performed the experiments. SGL and BBL analyzed the data. HBG contributed reagents/materials/analysis tools. DTL and BBL wrote the article.

All the authors read and approved the final manuscript.

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