

Clinical utility of circulating tumor-derived endothelial cells in lung cancer detection and prognosis prediction

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Abstract. Circulating tumor-derived endothelial cells (CTEC) have emerged as promising biomarkers reflecting tumor angiogenesis, disease progression and therapeutic response, although their clinical relevance in lung cancer remains under-explored. A total of 74 patients with lung cancer were enrolled in the present study, and both preoperative and postoperative peripheral blood samples were collected. A total of 20 healthy individuals were included as controls. CTEC (CD31⁺ aneuploid cells) and circulating tumor cells (CTC; CD31⁻ aneuploid cells) were simultaneously detected using an improved subtraction enrichment and immunostaining-fluorescence *in situ* hybridization technique. The overall positive detection rates of CTC and CTEC were 68.75 and 81.25%, respectively. CTEC accounted for a greater proportion of circulating rare cells in patients with lung cancer, and CTEC counts were significantly elevated in those with lymph node metastasis compared with CTC. Receiver operating characteristic curve analysis demonstrated that CTEC [area under the curve (AUC), 0.855] performed better than CTC (AUC, 0.673) in distinguishing

patients with lung cancer from healthy controls. Furthermore, a preoperative CTEC count ≥ 1 was identified as an independent risk factor for shorter progression-free survival. These findings suggested that CTEC may serve as reliable supplementary biomarkers for lung cancer screening, disease monitoring and prognosis prediction.

Introduction

Lung cancer remains the most prevalent malignancy worldwide (1-3). Despite advancements in early detection and therapeutic strategies, 70-75% of patients with lung cancer present with locally advanced or metastatic disease at the time of diagnosis, resulting in a generally poor overall prognosis (4-8). Postoperative recurrence and metastasis remain the primary causes of mortality in patients with lung cancer (1). Due to the biological heterogeneity and molecular complexity of lung tumors, traditional diagnostic methods, which mainly rely on imaging and histopathology, often fail to comprehensively capture tumor dynamics or accurately predict disease progression. As a result, significant challenges persist in early diagnosis, relapse prediction, prognostic evaluation and therapeutic monitoring in the clinical management of lung cancer.

Liquid biopsy has emerged as a promising, minimally invasive approach for real-time tumor surveillance. Among various circulating biomarkers, circulating tumor cells (CTC) have been widely investigated to determine their potential role in diagnosis, prognostication and treatment evaluation in lung cancer. Multiple studies have demonstrated that elevated CTC counts in the peripheral blood are markedly associated with disease burden, recurrence risk and shortened survival times (8-14). Persistent CTC positivity after surgery or systemic therapy is strongly associated with a higher risk of relapse and the presence of micrometastases (12-16). Furthermore, the persistence of CTC following neoadjuvant chemotherapy has been shown to predict early metastatic recurrence and reduced disease-free survival (17,18). For patients undergoing

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(neo) adjuvant or palliative chemotherapy, CTC enumeration has been extensively investigated as a dynamic biomarker of therapeutic response, with persistently elevated or increasing CTC levels after treatment initiation often indicating suboptimal treatment efficacy (15,19-34).

The clinical application of CTC detection remains technically challenging. A variety of enrichment and identification techniques based on polyploidy, cytokeratin expression or epithelial cell adhesion molecule positivity have been developed (24-26). However, these methods may inadvertently misclassify other rare cell types, such as circulating tumor-derived endothelial cells (CTEC), potentially leading to diagnostic ambiguity. CTEC are endothelial cells derived from the tumor vasculature, which are shed into the bloodstream during tumor progression and angiogenesis (26,27). Recent evidence suggests that CTEC, similar to CTC, may have diagnostic and prognostic value in multiple malignancies. Notably, Zhang *et al* (13) developed an enhanced subtraction enrichment and immunostaining-fluorescence *in situ* hybridization (SE-iFISH) method, enabling the simultaneous detection and characterization of aneuploid CTC and CTEC. Elevated CTEC counts have been recognized as a risk factor for adverse clinical outcomes in patients with non-small cell lung cancer (NSCLC) (1,7) and have also shown promise as a biomarker for monitoring the treatment response in estrogen receptor-positive lung cancer during neoadjuvant therapy (20).

Despite these findings, comprehensive studies elucidating the distinct cytogenetic and clinical characteristics of both CTEC and CTC in lung cancer remain limited. To address this gap, the present study utilized the SE-iFISH platform (Cytelligen, Inc.) to simultaneously detect aneuploid CTC and CTEC in peripheral blood samples. A total of 74 patients with lung cancer and 20 non-cancer controls were enrolled in the present study. The present study aimed to investigate the diagnostic performance and prognostic relevance of CTC and CTEC by analyzing their cytogenetic profiles in association with clinical and pathological factors, thereby offering novel insights into their potential clinical utility.

Materials and methods

Participants and sample collection. Patients with lung cancer were screened and evaluated by CTC testing between June 2018 and September 2020 at the Department of Thoracic Surgery of Hanzhong 3201 Hospital (Hangzhou, China). The inclusion criteria were as follows: i) Primary lung cancer confirmed by histology or cytology; and ii) Eastern Cooperative Oncology Group Performance Status ≤ 2 . The exclusion criteria were as follows: i) Prior malignancy in the past 5 years, except non-melanoma skin cancer; and ii) life expectancy ≤ 3 months. Peripheral blood samples were shipped to and processed at Biotechan Medical Laboratory within 24 h of collection. Clinical data, including age, body mass index, histological subtype, smoking status, sites of metastasis, treatment received and clinical outcome, were collected. All patients provided informed consent before sample and data collection. The present study was approved by the Ethics Committee of Hanzhong 3201 Hospital (approval no. 20180603A). The flowchart of the study design is shown in Fig. 1. The control subjects consisted of 20

healthy individuals recruited from the Physical Examination Center of 3201 Hospital during the same study period. To minimize selection bias, participants were selected based on a systematic random sampling strategy from a pool of eligible individuals who visited the center for routine health check-ups. Inclusion criteria were strictly applied to ensure a comparable baseline demographic profile. Participants were required to have: i) No history of malignant tumors, autoimmune diseases or active infections within the preceding 3 months; ii) normal hepatic and renal function tests; and iii) no history of benign pulmonary diseases. The sample size of 20 was determined based on the availability of eligible subjects during the recruitment period and to match the statistical power required for comparative analysis with the patient cohort, ensuring adequate representation of the background population.

SE. Peripheral blood (7.5 ml) was collected using an acid citrate dextrose anticoagulant tube (Becton, Dickinson and Company) and using a Human Circulating Rare Cell Subtraction Enrichment kit (cat. no. SE-001; Cytelligen, Inc.) according to the manufacturer's instructions. In brief, each tube of peripheral blood was centrifuged at 800 x g for 8 min at room temperature, followed by transfer of the lower layer of cells into centrifuge tubes that contained 3 ml of human CTC separation matrix. All probes (CEP8) and antibodies (anti-CD45, anti-CD31) used in the iFISH procedure were components of the above commercial kit. After centrifugation at 450 x g for 8 min at room temperature, the red blood cells were discarded. Subsequently, the buffy coat cells that included white blood cells (WBCs) and tumor cells were transferred to new tubes and incubated with an immunomagnetic particle-conjugated anti-CD45 antibody for 20 min. WBCs were then depleted by applying a magnetic bead sorting system and the magnetic beads-free solution was centrifuged at 450 x g for 8 min (4°C), followed by two rinses with human CTC buffer at room temperature. Finally, the cell pellet was resuspended and mixed with cell fixative solution and was coated on CTC slides (cat. no. CS-01; Cytelligen, Inc.) and dried overnight at 30°C, which were to be examined by iFISH.

iFISH. The iFISH process was carried out using the SE-iFISH Assay Kit (cat. no. IF-001; Cytelligen, Inc.) according to the manufacturer's updated protocol. For CTC and CTEC identification, the CTC slides were rinsed with saline-sodium citrate buffer for 10 min and then dehydrated in ethanol for 2 min. The monolayer cells coated on the CTC slides were hybridized with centromere probe 8 (CEP8; Cytelligen, Inc.) for 4 h at 37°C. The slides were then incubated with AlexaFluor® 594-conjugated anti-CD45 IgG (spectrum red; cat. no. A-21464; Invitrogen; Thermo Fisher Scientific, Inc.) and AlexaFluor® 488-conjugated anti-CD31 IgG (spectrum green; ca. no. A-21467; Invitrogen; Thermo Fisher Scientific, Inc.) for 2 h at room temperature in a dark room. Finally, the CTC slides were stained with DAPI (spectrum blue) and examined under a fluorescence microscope. DAPI+/CD45-/CD31-/aneuploid CEP8 cells were considered to be CTC, while DAPI+/CD45-/CD31+/aneuploid CEP8 cells were considered to be CTEC.

Identification of CTC and CTEC. CTC were identified based on the immunophenotypic profile CD31-/CD45-/DAPI+/CEP8 > 2 , whereas CTEC were defined as CD31+/

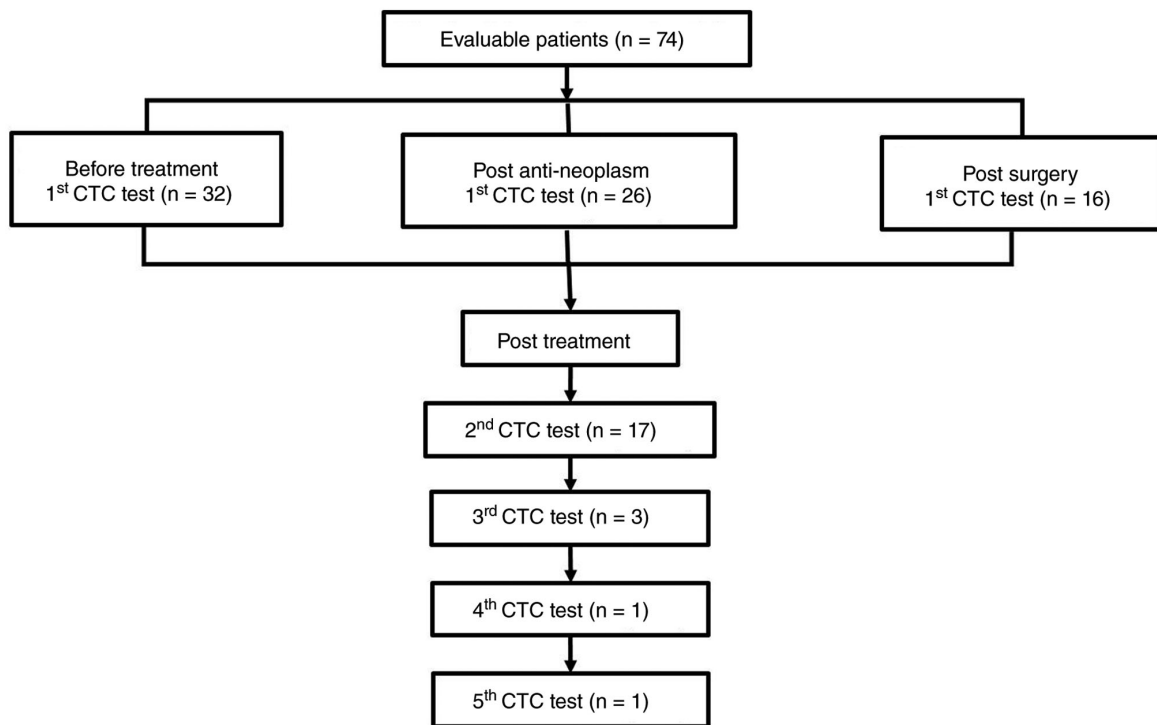


Figure 1. Flowchart. A total of 74 patients with lung cancer were enrolled. CTC, circulating tumor cells.

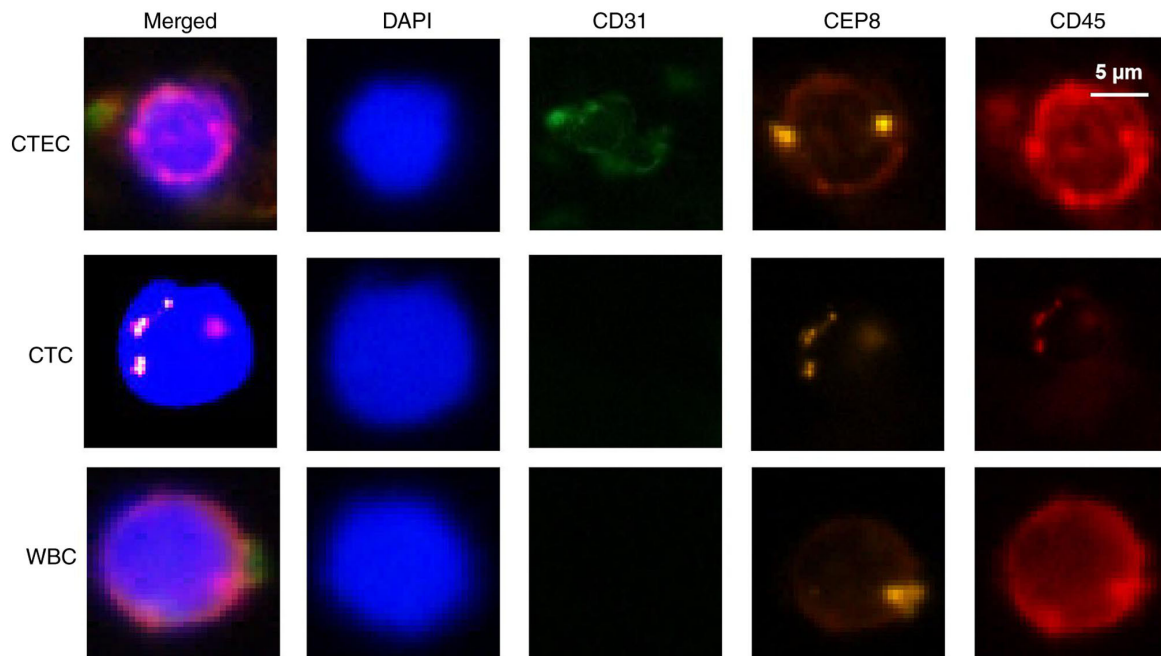


Figure 2. Identification of CTEC, CTC and WBC by subtraction enrichment and immunostaining-fluorescence *in situ* hybridization (scale bar, 5 μ m). CD45, red; CD31, green; CEP8, orange; DAPI, blue. The CTEC image (middle panel) has been replaced with a more representative cell exhibiting stronger CD31 (green) fluorescence. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells; WBC, white blood cells; CEP8, centromere probe 8.

CD45⁺/DAPI⁺/CEP8 >2 (Fig. 2). To eliminate potential interference from leukocytes, CD45⁺ cells characterized by the phenotype CD31⁺/CD45⁺/DAPI⁺/CEP8=2 were excluded during analysis (35).

Statistical analysis. Statistical analyses were performed using SPSS software (version 27.0; IBM Corp.). Continuous

variables with a normal distribution are presented as mean $\pm$ standard deviation (SD), while those with non-normal distribution are presented as the median (interquartile range or range). Categorical variables were compared using Fisher's exact test (for expected frequencies <5) or the Chi-square test. Comparisons of continuous variables between two independent groups were performed using the Mann-Whitney U-test,

while multiple-group comparisons were conducted using the Kruskal-Wallis H-test. Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method with log-rank tests. PFS was defined as the time from initial CTC sampling to disease progression (confirmed by RECIST 1.1 criteria) or all-cause death, whichever occurred first. OS was defined as the time from initial CTC sampling to all-cause death; patients without event occurrence were censored at the last follow-up date. Independent prognostic factors were identified via multivariate Cox proportional hazards regression models. Diagnostic performance was evaluated using receiver operating characteristic (ROC) curves and DeLong tests were applied to compare areas under the curve (AUCs). All tests were two-tailed and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics. A total of 74 patients diagnosed with lung cancer were enrolled in the present study and underwent CTC evaluation between June 2018 and September 2020 at the Department of Thoracic Surgery, Hanzhong 3201 Hospital (Hanzhong, China), along with 20 healthy controls. The clinical characteristics of these patients are summarized in Table I. The patients had a mean age of median age of 60.0 years (range: 53–67 years). Regarding sex distribution, the majority were male (70.3%). The majority of patients were diagnosed at stage IV (60.81%), with metastatic disease (59.5%) and adenocarcinoma histology (63.5%), and received platinum-based antineoplastic therapy (67.6%). Based on the timing of the first CTC sample collection, patients were categorized into three groups: Prior to any treatment ($n=32$), after surgery but before antineoplastic therapy ($n=16$) and after antineoplastic treatment ($n=26$). A total of 14 patients underwent two CTC tests, 2 patients underwent three CTC tests and 1 patient underwent five CTC tests. The average follow-up duration for these patients was 65.2 ± 6.6 months (range, 52.0–72.5 months). At the time of analysis, disease progression had occurred in 12 patients (70.59%) and 9 patients (52.94%) had died, resulting in a median PFS time of 17.5 months (95% CI, 8.35–26.65 months) and a median OS time of 20.0 months (95% CI, 10.98–29.02 months).

The demographic details of the control cohort are provided in Table SI. The control group had a mean age of 58.7 ± 10.2 years (median: 59.0 years, range: 32–76 years), including 12 males (60.0%) and 8 females (40.0%). No significant differences were observed in age (Mann-Whitney U-test, $P=0.621$) or sex distribution (Chi-square test, $P=0.968$) between the patient and control groups.

Characteristics of CTC and CTEC. Among the entire cohort, 61 patients (82.43%) had detectable CTC and 60 patients (81.08%) were positive for CTEC at baseline (Fig. 3). The distribution of CTC and CTEC positivity across tumor stages is presented in Table II. No significant associations were observed between the counts of CTC or CTEC and tumor stage (Fisher's exact test; $P > 0.05$). Among the 60 patients with stage IV disease, 19 (32%) had ≥ 2 CTC per 7.5 ml of blood (range, 0–146) (Fig. 3). By contrast, only 2 (7%) of 27 patients with stage IIIB NSCLC had ≥ 2 CTC (range, 0–3), and no CTC

Table I. Clinical characteristics of all patients ($n=74$).

Characteristic	Parameter
Age, years	60.0 (42–78)
Sex	
Male	44 (59.46)
Female	30 (40.54)
BMI, kg/m ²	22.52 $\pm$ 3.20
Smoking habits	
Current smoker	25 (33.78)
Never smoked	23 (31.08)
Not documented	26 (35.14)
Tumor stage at diagnosis	
I	16 (21.62)
II	13 (17.56)
III	10 (13.51)
IV	45 (60.81)
Histological subtype	
SCLC	10 (13.51)
Metastatic disease	44 (59.5)
Adenocarcinoma	47 (63.51)
SCC	16 (21.62)
Other	1 (1.35)
Treatment received	
Radical surgery	12 (16.22)
Paclitaxel/cisplatin	14 (18.92)
Gemcitabine/cisplatin or nedaplatin	18 (24.32)
Etoposide/cisplatin	7 (9.46)
Pemetrexed/cisplatin or nedaplatin	7 (9.46)
Docetaxel/cisplatin or nedaplatin	4 (5.41)
Single-agent treatment	
Gemcitabine	1 (1.35)
EGFR-TKIs	6 (8.11)
Others	5 (6.75)
CTC test information	
Patients with 2 CTC tests	14
Patients with 3 CTC tests	2
Patients with 5 CTC tests	1
Follow-up duration, months	65.2 $\pm$ 6.6 (52.0–72.5)
Patients with disease progression	12 (70.59)
Survival data	
Deaths	9 (52.94)
Median PFS time, months	17.5 (95% CI, 8.35–26.65)
Median OS time, months	20.0 (95% CI, 10.98–29.02)

Values are expressed as the median (range), mean $\pm$ standard deviation or n (%). BMI, body mass index; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

were detected in patients with stage IIIA NSCLC. The presence of ≥ 2 CTC was significantly associated with liver and

Table II. Prevalence of CTC and CTEC among different tumor stages.

Stage	CTC			CTEC		
	Triploid	Multiploid	Total	Triploid	Multiploid	Total
I	1	1	2	0	1	2
II	1	1	3	0	1.5	2.5
III	1	1	2	0	1	2
IV	1	1	2	0	1	2

CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells.

Table III. Baseline CTC and CTEC numbers as per different sample collection time points and tumor stage.

Cell type number/group	Prior to any treatment (n=32)	Post anti-neoplasm (n=26)	Post surgery (n=16)	P-value
CTC number				0.481
Non-metastatic (n=33)	2 (0-9)	3 (1-10)	2 (0-11)	
Metastatic (n=41)	2 (0-11)	2 (0-10)	/	
CTEC number				0.391
Non-metastatic (n=33)	2 (0-60)	4 (1-34)	1.5 (0-139)	
Metastatic (n=41)	2 (0-45)	1 (0-20)	/	

P-values were calculated using the Kruskal-Wallis H-test to compare differences across time points within each cell type. /, data not available due to the absence of post-surgical follow-up samples in the metastatic cohort. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells.

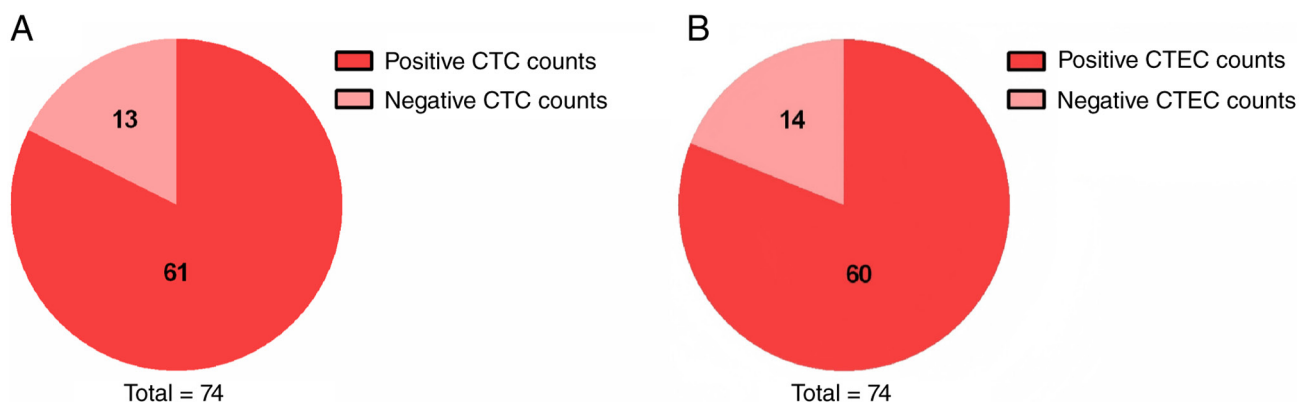


Figure 3. Proportions of patients with (A) CTC and (B) CTEC (>0 and >1) before treatment, after surgery and after chemotherapy. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells.

bone metastases compared with other metastatic sites (Fisher's exact test; $P=0.033$ and $P=0.014$, respectively). Furthermore, squamous histology was associated with lower CTC counts compared with other histological subtypes ($P=0.013$), a finding likely explained by the higher proportion of patients with stage III disease among patients with squamous cell carcinoma. No significant association was observed between performance status and CTC counts.

Table III shows that both CTC and CTEC counts remained relatively stable across sampling time-points and disease

stages. In non-metastatic patients, CTC counts exhibited minimal variation, ranging from a median of 2 (0-9) before treatment to 3 (1-10) after therapy, and returning to 2 (0-11) after surgery; a similar trend was observed in metastatic cases. In non-metastatic patients, the median CTEC counts increased slightly from 2 to 4, although the maximum value was decreased and the interquartile range was narrower. However, these changes, alongside those observed for CTC, did not achieve statistical significance (CTC, $P=0.481$; CTEC, $P=0.391$). Therefore, while numerical shifts were noted, the

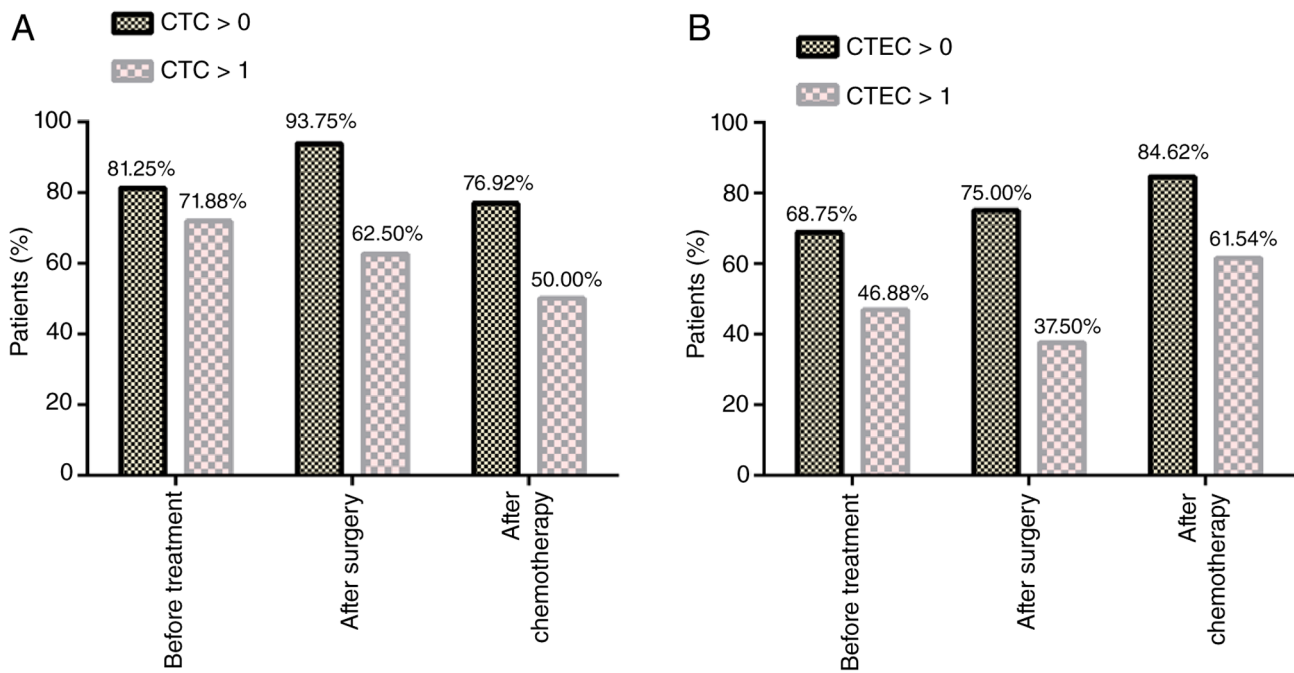


Figure 4. Dynamic changes in (A) CTC and (B) CTEC detection rates during the treatment course. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells.

present data did not establish a statistically significant or definitive therapy-related effect on CTEC or CTC counts in this cohort. The observed trends should be interpreted with caution due to the limited sample size at post-treatment time-points.

For the univariate CTC and CTEC analyses, patients were stratified into favorable (≤ 1 CTC or CTEC) and unfavorable (> 1 CTC or CTEC) prognostic groups based on baseline CTC and CTEC counts. As shown in Fig. 4A and B, the detection rates of CTC and CTEC displayed distinct during the course of treatment. The proportion of patients with detectable CTC (> 0) increased from 81.25% before surgery to 93.75% after surgery, but subsequently decreased following chemotherapy to 76.92%. A similar declining trend was observed in patients with CTC counts exceeding 1, dropping from 71.88% pre-surgery to 50.00% post-chemotherapy. By contrast, CTEC detection rates remained consistently high, with the proportion of patients exhibiting CTEC counts > 0 rising from 68.75% before treatment to 84.62% after chemotherapy. Notably, the proportion of patients with CTEC counts > 1 decreased following chemotherapy (from 46.88 to 37.50%), while the proportion with detectable CTEC (> 0) increased. This contrasting trend compared with CTC dynamics suggests differing patterns of change for CTEC and CTC during therapy, warranting further investigation in larger, dedicated longitudinal studies.

Comparative analysis of CTC and CTEC counts in stage I-IV lung cancer. Fig. 5A-I shows the stage-specific distribution of CTC and CTEC across lung cancer stages I-IV. Among patients with stage I lung cancer, CTC and CTEC were detectable in all patients, primarily ranging from 1 to 2 cells, with only a small subset exhibiting counts ≥ 5 cells, reflecting a limited circulating tumor burden in the early phase of the disease.

Patients with stage II lung cancer exhibited a similar but more centralized distribution, predominantly characterized by low single-cell counts, with a smaller proportion of patients exhibiting higher CTC and CTEC counts. In patients with stage III lung cancer, a clear rightward shift toward elevated cell counts was evident, with a greater proportion of patients exhibiting 3-5 CTC or CTEC, reflecting increased tumor cell dissemination and heightened biological activity. This trend became increasingly pronounced in patients with stage IV lung cancer, where high-count categories predominated, particularly for CTC, reflecting consistently elevated positivity across all detection thresholds. The expanded distribution of ≥ 3 and ≥ 5 cell clusters in advanced disease stages highlighted the systemic dissemination of tumor-associated cells during disease progression. Together, these quantitative and graphical results demonstrated a stepwise increase in CTC and CTEC from early-stage to metastatic disease, reinforcing their potential as minimally invasive biomarkers for monitoring tumor progression and enabling disease stratification.

Clinical screening value of CTC and CTEC in patients with lung cancer. The diagnostic significance of CTC and CTEC in lung cancer was subsequently evaluated. In healthy individuals, CTC were predominantly undetectable and CTEC were consistently absent, highlighting the rarity of aneuploid cells in the non-malignant bloodstream. By contrast, patients with lung cancer exhibited a marked increase in both cell populations, with CTC positivity rates reaching 81.08% (60/74) and CTEC positivity rates of 67.57% (50/74) (Fig. 6). Consistent with these qualitative differences, quantitative analyses demonstrated significantly higher levels of CTC and CTEC in the cancer cohort compared with controls (Mann-Whitney U-test; Fig. 6A and B; $P < 0.0001$). To evaluate their discriminatory capacity, ROC analyses were conducted. CTEC exhibited

Table IV. Sequential serum tumor markers, CTC counts and staging information for Patient 15 (69 years old, male) across five clinical evaluations.

Parameter	Day 0	Day 101	Day 242	Day 410	Day 493
CEA, ng/ml (normal range: <5 ng/ml)	3.9	3.1	33.2	7.5	6.9
CA19-9, U/ml (normal range: <37 U/ml)	4.3	23.1	24.2	12.9	13.9
NSE, ng/ml (normal range: <16.3 ng/ml)	3.2	3.9	4.9	8.9	0.7
CTC count	1	0	0	4	2
Neoplasm staging	T3N2M1	T3N2M1	T3N1M1	T3N1M1	T3N1M1

CTC, circulating tumor cell; NSE, neuron-specific enolase.

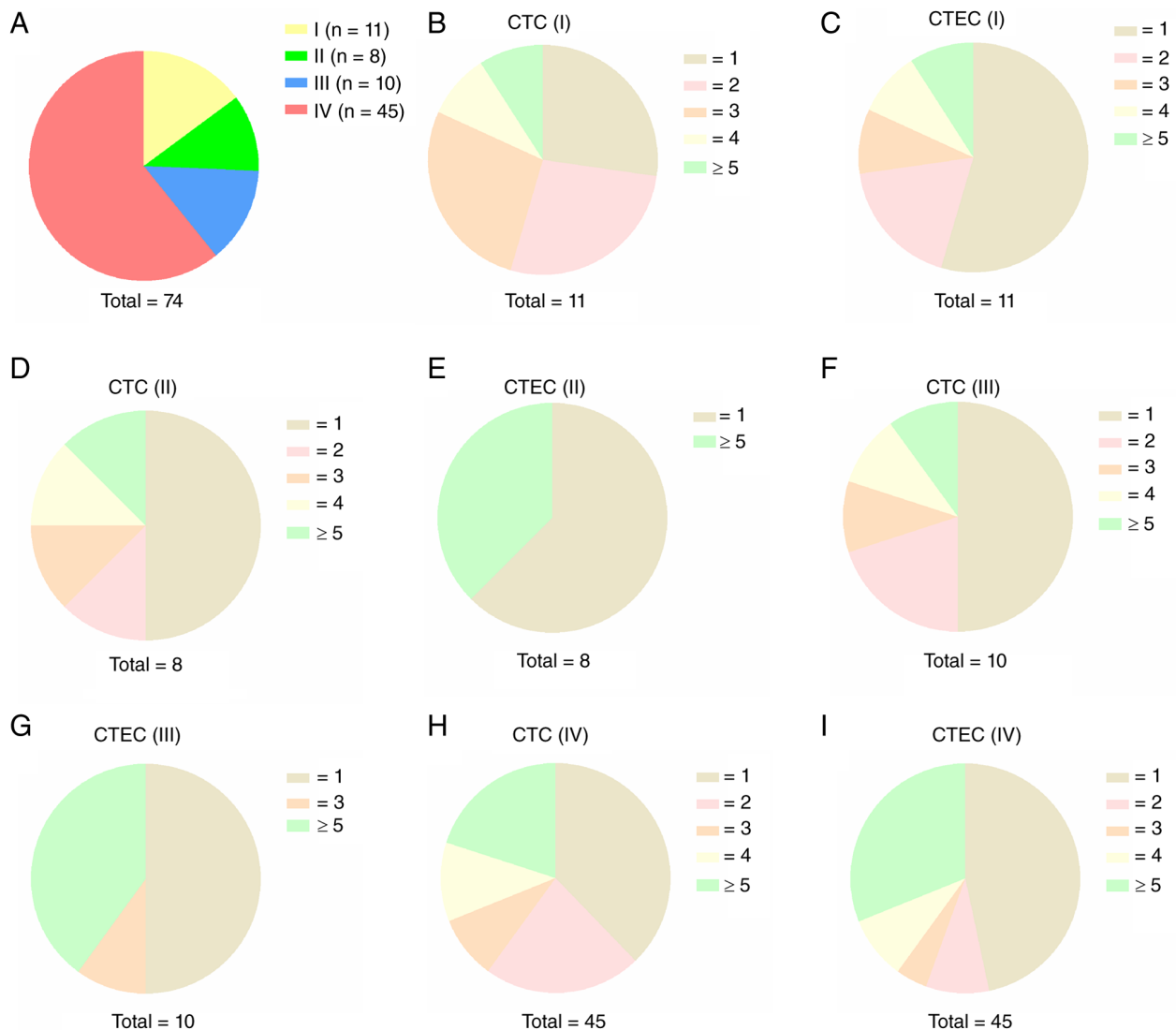


Figure 5. Stage-stratified distribution of CTC and CTEC in 74 patients with lung cancer. (A) Cross-stage overview. (B) Distribution of CTCs in Stage I patients (total n=11). (C) Distribution of CTECs in Stage I patients (total n=11). (D) Distribution of CTCs in Stage II patients (total n=8). (E) Distribution of CTECs in Stage II patients (Total=8). (F) Distribution of CTCs in Stage III patients (Total=10). (G) Distribution of CTECs in Stage III patients (total n=10). (H) Distribution of CTCs in Stage IV patients (total n=45) (I) Distribution of CTECs in Stage IV patients (total n=45). Values: 1, 2, 3, 4, ≥ 5 cells/7.5 ml peripheral blood. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells.

superior performance (AUC, 0.855) compared with CTC alone (AUC, 0.673). Notably, the combination of both biomarkers yielded a slightly higher AUC of 0.860 (Fig. 6C). However,

a formal comparison using the DeLong test indicated that this improvement was not statistically significant compared with using CTEC alone ($P=0.724$). Collectively, these results

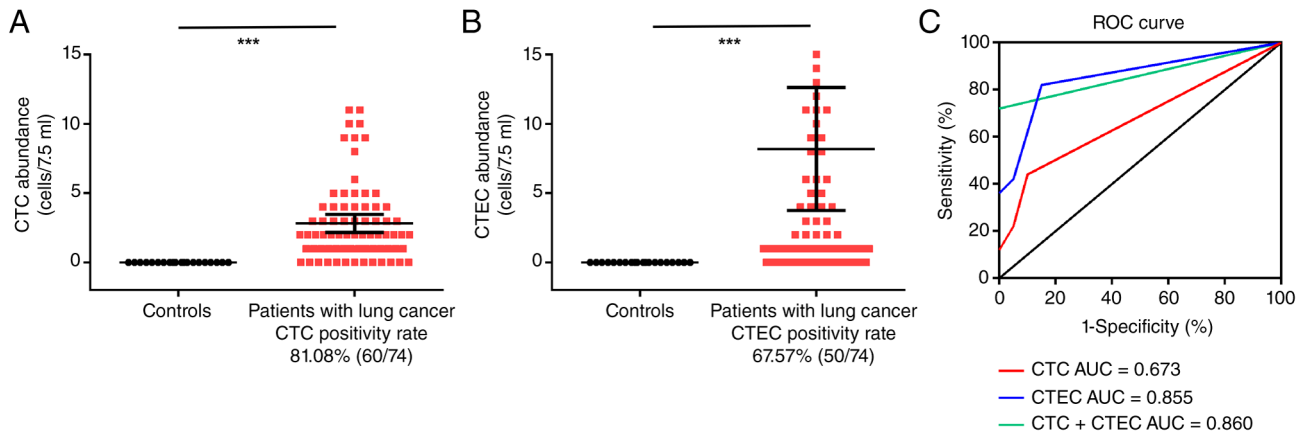


Figure 6. Comparison of (A) CTC and (B) CTEC abundance between controls and patients with lung cancer. (C) ROC curves of CTC, CTEC and their sum (CTC+CTEC) for distinguishing patients with lung cancer from healthy controls. The DeLong test showed no significant difference between the AUC of CTEC alone and the combination (CTC+CTEC) ($P=0.724$). *** $P<0.0001$. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells; ROC, receiver operating characteristic; AUC, area under the ROC curve.

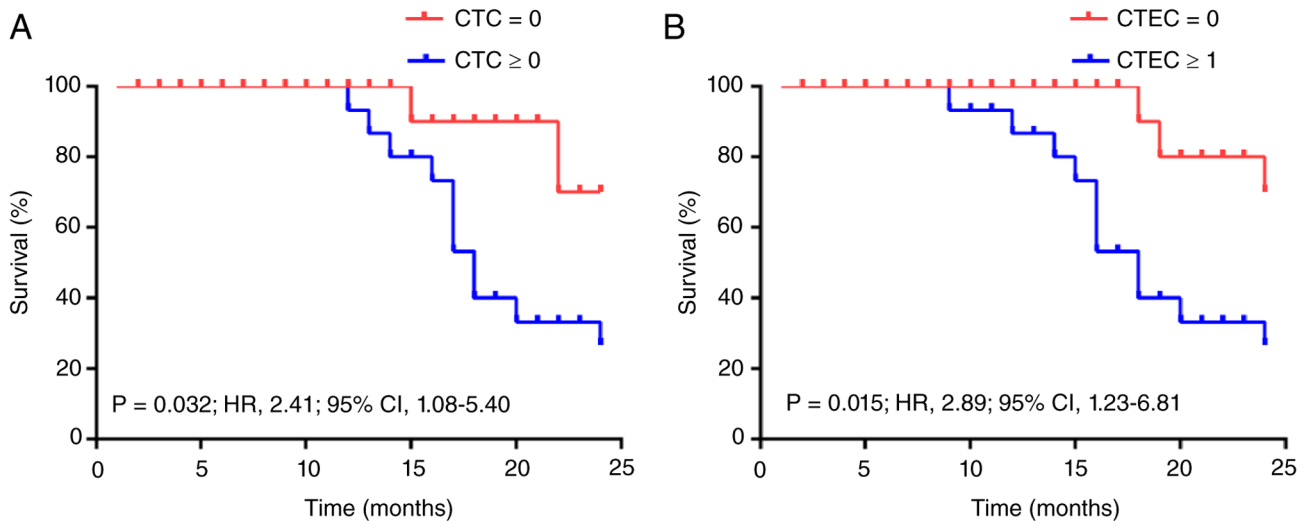


Figure 7. Kaplan-Meier survival curve analysis of progression-free survival in patients with lung cancer according to CTC and CTEC counts. (A) CTC=0 vs. CTC ≥ 1; (B) CTEC=0 vs. CTEC ≥ 1. CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells; HR, hazard ratio.

indicated that the enumeration of circulating aneuploid cells, particularly CTEC, offered a robust and minimally invasive approach for lung cancer detection.

Interpretation of biomarker variability and CTC stability in advanced-stage disease. Sequential clinical and laboratory data for a representative 69-year-old male patient with stage IV lung adenocarcinoma across five clinical evaluations are summarized in Table IV. Across the five sequential assessments of patient 15, a 69-year-old male with stage IV lung adenocarcinoma, serum CEA and CA19-9 levels exhibited notable fluctuations. Notably, this patient was selected as a representative case due to his long-term follow-up, confirmed lymph node metastasis (N1) and the pronounced variability observed in his tumor biomarkers, which provided a unique insight into the complex interplay between systemic inflammation and tumor burden. Peak values for these biomarkers were recorded at the third time-point (6 months post-surgery, coinciding with the initiation of adjuvant chemotherapy), with

CEA reaching 33.2 ng/ml (normal range: <5 ng/ml), CA19-9 reaching 24.2 U/ml (normal range: <37 U/ml) and NSE concurrently elevated to 4.9 ng/ml (normal range: <16.3 ng/ml).

Clinical prognostic significance of CTC and CTEC in patients with lung cancer. Based on the Kaplan-Meier curves (Fig. 7A and B), patients with detectable CTC or CTEC (≥1 cell) exhibited significantly reduced PFS rates compared with those with undetectable levels [log-rank test: For CTC, $P=0.032$; hazard ratio (HR), 2.41; 95% CI, 1.08-5.40; for CTEC, $P=0.015$; HR, 2.89; 95% CI, 1.23-6.81]. In both panels, the ≥1 cell groups demonstrated an earlier and steeper decline in survival probability, while the 0-cell groups maintained higher and more stable PFS throughout the 24-month observation period. These results suggested that even minimal detectable levels of CTC or CTEC were associated with substantially worse short-term disease control. An exploratory analysis combining both biomarkers (patients with both a CTC count of 0 and a CTEC count of 0 vs. all others) revealed a

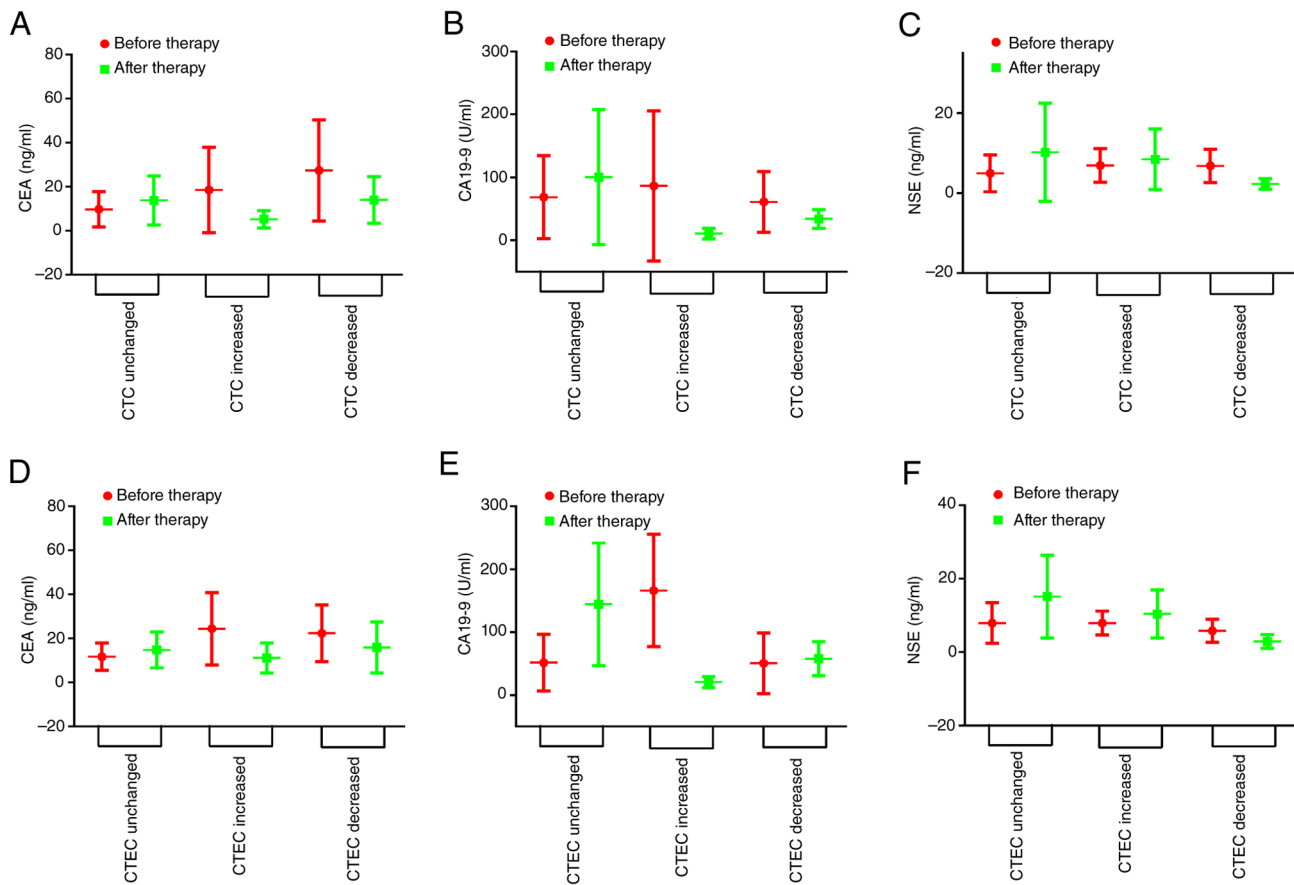


Figure 8. Comparison of tumor marker levels before and after therapy across subgroups stratified by CTC and CTEC dynamics. (A) CEA levels in the CTC dynamic subgroups (unchanged, increased, decreased). (B) CA19-9 levels in the CTC dynamic subgroups. (C) NSE levels in the CTC dynamic subgroups. (D) CEA levels in the CTEC dynamic subgroups. (E) CA19-9 levels in the CTEC dynamic subgroups. (F) NSE levels in the CTEC dynamic subgroups. Due to the limited sample sizes in the dynamic subgroups (CTC-decreased: n=8; CTC-unchanged: n=5; CTC-increased: n=3; CTEC-decreased: n=9; CTEC-unchanged: n=4; CTEC-increased: n=3), formal statistical comparisons were not performed, and values are presented as mean $\pm$ SD with one additional decimal place for enhanced precision. CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; NSE, neuron-specific enolase; CTC, circulating tumor cells; CTEC, circulating tumor-derived endothelial cells.

trend towards an even shorter PFS time in the dual-positive group, although this was not statistically significant ($P=0.058$; HR, 2.65; 95% CI, 0.97-7.25), potentially due to limited patient numbers in the double-negative subgroup.

Dynamic changes of serum tumor biomarkers across CTC response categories. Fig. 8 illustrates the dynamic changes in serum levels of CEA, CA19-9 and NSE before and after treatment across patient subgroups stratified by CTC and CTEC kinetics (unchanged, increased or decreased). Due to the limited sample size in each dynamic category and the non-normally distributed nature of the biomarker data (as indicated by the variability shown in Fig. 8), non-parametric statistical analyses were performed. Specifically, for intra-group comparisons (pre-vs. post-treatment), the Wilcoxon signed-rank test was utilized; for inter-group comparisons (between different kinetic subgroups), the Mann-Whitney U-test was applied. Overall, fluctuations in tumor biomarker levels were differentially associated with circulating cell dynamics. In the CTC kinetic subgroups (Fig. 8A-C), a Wilcoxon signed-rank test revealed no statistically significant within-group changes in CEA, CA19-9, or NSE levels from pre- to post-treatment (all $P>0.05$). However, visually, patients with decreasing CTC

counts tended to show concomitant declines in CEA and CA19-9 levels. In the CTEC kinetic subgroups (Fig. 8D-F), the 'CTEC decreased' subgroup exhibited a marked reduction in all three biomarkers. Although formal testing was limited by the small sample size (note: some subgroups have $n<5$), the directionality of change suggested a strong treatment effect. It should be noted that while Fisher's exact test is typically recommended for categorical data with $n<5$, the present analysis primarily focused on continuous variables (biomarker levels), for which non-parametric tests are more appropriate even in small samples. The 'CTEC increased' subgroup showed a slight decrease in median CEA and CA19-9 levels, with NSE levels remaining stable. To further quantify the relationship between cell dynamics and biomarker reduction, a supplementary analysis was conducted. When comparing the magnitude of change ($\Delta = \text{Post-treatment} - \text{Pre-treatment}$) between groups, patients with a reduction in CTEC counts displayed significantly greater decreases in CEA and CA19-9 levels compared to those with stable or increased CTEC counts (Mann-Whitney U test, $P<0.05$ for CEA and CA19-9). These findings suggested that CTEC dynamics may more sensitively reflect therapy-induced biological responses compared with CTC enumeration. The parallel decline of CTEC and serum

tumor marker levels highlights the potential of CTEC as a surrogate indicator for treatment efficacy.

Discussion

The current prospective study presents a comprehensive assessment of CTEC and CTC in patients with lung cancer, focusing on their detection efficiency, diagnostic utility and prognostic significance through the application of the SE-iFISH platform. The findings consistently demonstrated that CTEC offered greater biological and clinical value than CTC, indicating that tumor-derived endothelial components may represent an underrecognized but critical aspect of liquid biopsy in lung cancer.

Previous studies have preliminarily explored the utility of CTCs and CTECs in lung cancer management. Zhu *et al* (34) found that specific CTEC subtypes correlated with chemotherapeutic response and prognosis in small cell lung cancer, though this study was restricted to SCLC populations and did not cover non-small cell lung cancer. Zhang *et al* (36) reported that combined detection of aneuploid CTEC and CTC improved diagnostic accuracy for stage I-II NSCLC to >90%, yet failed to explore the dynamic kinetics of these biomarkers or their association with treatment response. Zhang *et al* (13) demonstrated that elevated aneuploid CTEC subtypes were independently associated with shortened OS in advanced lung cancer, but did not validate the predictive value of CTEC for PFS or systematically compare the diagnostic performance of CTEC and CTC. Compared with these prior works, the present study has four distinct novelties: First, a cohort spanning all stages (I-IV) and major pathological subtypes (both SCLC and NSCLC) was enrolled, and for the first time it was confirmed that CTEC has significantly better detection stability than CTC during treatment, with less fluctuation in positivity rate, making it a more suitable dynamic monitoring biomarker. Second, a preoperative CTEC count ≥ 1 was identified as an independent risk factor for shorter PFS in lung cancer, a threshold with superior predictive efficacy compared with traditional CTC cut-offs. Third, the present study found for the first time, to the best of our knowledge, that CTEC dynamics were more strongly correlated with treatment-induced changes in serum tumor markers (CEA, CA19-9) than CTC enumeration, supporting CTEC as a more sensitive surrogate for therapeutic response. Fourth, baseline CTEC/CTC data were established from healthy controls, verifying that single CTEC testing achieved an AUC of 0.855 for lung cancer screening, which increased to 0.860 when combined with CTC, providing robust evidence for liquid biopsy-based lung cancer screening strategies.

One of the most striking findings of the present study was the consistently higher detection rate and relative abundance of CTEC compared with CTC in peripheral blood. This observation suggested that tumor-derived endothelial cells contributed more markedly to the population of circulating tumor-related rare cells than classical epithelial tumor cells in lung cancer. Mechanistically, this phenomenon was consistent with previous findings demonstrating that tumor-derived endothelial cells can arise through two interconnected processes: The ‘cancerization’ of resident endothelial cells in the tumor stroma and the hypoxia-driven ‘endothelialization’ of

carcinoma cells mediated by additional microenvironmental stresses (35). The SE-iFISH technique enabled the identification of aneuploid CD31⁺/CD45⁻ cells, providing evidence that these endothelial-like malignant cells actively circulate in peripheral blood. Their presence reflects ongoing neovascularization and endothelial plasticity, which are key biological features associated with tumor growth, nutrient supply and metastatic spread. Therefore, the predominance of CTEC may represent not merely a quantitative phenomenon but also a qualitative indicator of tumor aggressiveness.

The clinical associations observed in the present study further reinforce the biological significance of CTEC. Elevated CTEC counts were closely associated with lymph node metastasis and advanced tumor stage, both of which are well-established adverse prognostic indicators in lung cancer (34,35). The fact that CTC counts did not show similar associations underscores the potential of CTEC as a more sensitive indicator of vascular invasion and metastatic propensity. From a mechanistic standpoint, this distinction is plausible: While CTC are primarily reflective of direct tumor cell shedding into the circulation, CTEC are likely to originate from the disrupted vasculature surrounding tumor nests or from intratumoral endothelial transdifferentiation, thereby providing a more immediate measure of angiogenic activity and vascular permeability. These processes are critical to the metastatic cascade, enabling tumor cells to intravasate through structurally abnormal vessels and to establish secondary foci (34,35). The strong link between elevation of CTEC counts and nodal metastasis observed in the present study therefore highlighted their potential utility as a surrogate marker for invasive and angiogenic behavior in lung cancer.

The diagnostic analyses in the present study further supported the clinical utility of CTEC. ROC curve analysis showed that CTEC exhibited higher AUC values than CTC in differentiating patients with lung cancer from healthy individuals. Furthermore, combination of both cell types resulted in the highest discriminative accuracy, indicating their complementary roles in diagnosis. Conceptually, CTC capture the dimension of tumor cell dissemination, whereas CTEC reflect vascular remodeling and endothelial dysfunction. Integrating both metrics provides a more comprehensive ‘liquid biopsy’ profile of tumor biology by encompassing both cellular components and microenvironmental features of malignancy. This dual-parameter approach may improve diagnostic accuracy in clinical settings where lung cancer is suspected, potentially aiding differentiation from healthy individuals. Future studies focusing specifically on early-stage (stage I/II) patients and including benign pulmonary disease controls are needed to evaluate the true potential of CTEC/CTC for early detection.

The prognostic implications of CTEC observed in the present study are equally noteworthy. Elevated pre-treatment CTEC counts were independently associated with shorter PFS times, whereas CTC counts lacked comparable predictive value. These findings indicated that CTEC may enable the stratification of patients into distinct risk categories before treatment initiation, thereby providing clinically relevant information for individualized management strategies. The potential application of CTEC as a real-time monitoring tool is highly promising: Longitudinal tracking of CTEC counts may enable early detection of the therapeutic response or

impending relapse, facilitating timely clinical interventions. The biological plausibility of these findings is supported by evidence that aneuploid CTEC may reflect aggressive tumor behavior associated with persistent vascular remodeling, hypoxia-induced endothelial transdifferentiation, and recurrent intravasation and extravasation events (37,38). These dynamic processes are central to tumor evolution and therapy resistance, suggesting that CTEC function as circulating indicators of microenvironmental activity rather than simply reflecting passive cellular shedding.

A plausible explanation for the superior detectability and stability of CTEC compared with CTC lies in their distinct origin and physiological properties (37,38). Unlike epithelial tumor cells, which are prone to rapid apoptosis or immune-mediated clearance upon entering the bloodstream, CTEC, by virtue of their endothelial-like characteristics, may exhibit enhanced resilience and prolonged survival within the circulatory system. Their partial retention of endothelial surface markers may enable immune evasion and transient integration into the vasculature, allowing CTEC to persist sufficiently long for reliable detection by SE-iFISH. Additionally, the level of aneuploidy observed in CTEC could enhance their proliferative and migratory capabilities, thereby further differentiating them from conventional CTC populations. These biological differences highlight the potential of CTEC as more consistent and reproducible biomarkers across various disease stages. The strong link between elevation of CTEC counts and nodal metastasis observed in the present study may highlight their potential utility as a surrogate marker for invasive and angiogenic behavior in lung cancer. While CTEC are traditionally linked to blood vessel angiogenesis, emerging evidence suggests that tumor-associated endothelial cells can also contribute to lymphangiogenesis (the formation of new lymphatic vessels), which is a critical step in lymphatic metastasis. CTEC shed from the tumor vasculature may thus reflect a pro-metastatic microenvironment conducive to both hematogenous and lymphatic spread. Their detection could signify active vascular remodeling that facilitates tumor cell entry into both circulation systems.

Several methodological and interpretive limitations warrant consideration. Although the study cohort was prospectively collected, it was of moderate size and limited to a single institution, potentially restricting the generalizability of the findings. While the follow-up duration was adequate for assessing PFS, it was insufficient to fully evaluate OS. Additionally, although pre- and post-treatment samples were analyzed, the temporal dynamics of CTEC and CTC fluctuations beyond the immediate treatment period remain poorly characterized. Expanding future studies to include multi-center cohorts with extended longitudinal sampling would enhance statistical power and provide clearer insights into the temporal kinetics of these biomarkers. An additional critical avenue of research involves the functional characterization of CTEC themselves. Detailed phenotyping, such as evaluation of angiogenic or immunomodulatory marker expression [for example, VEGFR2, programmed-death ligand 1 (PD-L1) or mesenchymal markers], could reveal mechanistic associations between CTEC biology and therapeutic resistance. Notably, previous studies have demonstrated that PD-L1-positive aneuploid CTEC are linked to reduced responses to immune

checkpoint inhibitors in NSCLC (1,21,22), highlighting their potential as predictive biomarkers for immunotherapy efficacy.

In light of these findings, the integration of CTEC assessment into comprehensive liquid biopsy frameworks for lung cancer management is strongly supported. Beyond their utility as diagnostic or prognostic biomarkers, CTEC offer potential for dynamic risk stratification across the disease continuum. For example, serial monitoring of CTEC levels during systemic therapy may allow early detection of non-responders, while persistent elevation or recurrence of CTEC after treatment could indicate minimal residual disease or predict imminent relapse. The interplay between CTEC kinetics and therapeutic interventions, particularly anti-angiogenic or immunomodulatory regimens, requires systematic investigation. These insights could inform the development of adaptive treatment algorithms in which real-time biomarker dynamics guide timely therapeutic adjustments. A primary limitation of the present study was its single-center design and moderate sample size, particularly for longitudinal analyses. The observed dynamics of CTEC and CTC during therapy, while suggestive, require validation in larger, multi-center prospective cohorts with standardized sampling timepoints to confirm reproducibility and establish their definitive role in therapeutic monitoring.

In summary, the present study reinforced the notion that CTEC are not merely byproducts of vascular disruption but dynamic indicators of tumor angiogenesis, invasiveness and progression. Their increased prevalence, stronger association with metastatic burden, higher diagnostic accuracy and independent prognostic significance collectively highlight their potential as clinically actionable biomarkers in lung cancer. Future large-scale, multi-institutional studies incorporating longitudinal sampling and molecular profiling of CTEC are expected to validate these findings and establish standardized clinical protocols for their use. Through such efforts, CTEC analysis may transition from a promising research tool to an essential component of precision oncology in the management of lung cancer. Additionally, although efforts were made to ensure a representative sample, the control group was recruited from a single medical center's physical examination center. This may have introduced a selection bias towards individuals who prioritize regular health check-ups, potentially limiting the generalizability of the findings to the broader population.

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Availability of data and materials

The data generated in the current study may be requested from the corresponding author.

Authors' contributions

XL, JY and BC conceived and designed the study, and acquired the clinical data. ZZ and JZ performed the SE-iFISH experiments, processed the liquid biopsy samples and conducted the primary data analysis. MZ and ZW supervised the project, secured funding and provided critical resources and administrative support. MZ, XL and ZZ drafted the manuscript. XL, JY, BC, ZZ, JZ, MZ and ZW participated in the interpretation of the data and critically revised the manuscript for important intellectual content. MZ and ZW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of 3201 Hospital (approval no. 20180603A). Written informed consent to participate in the study was obtained from each subject prior to sample collection and biomarker analysis.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Herbst RS, Morgensztern D and Boshoff C: The biology and management of non-small cell lung cancer. *Nature* 553: 446-454, 2018.
- Wan Z, Zhu Z, Wang P, Xu X, Ma T, Li H, Li L, Qian F and Gu W: Targeting focal adhesion kinase in lung diseases: Current progress and future directions. *Biomolecules* 15: 1233, 2025.
- Mangang KN and Rao GSNK: Lung cancer biomarkers. *Clin Chim Acta* 580: 120707, 2026.
- Thai AA, Solomon BJ, Sequist LV, Gainor JF and Heist RS: Lung cancer. *Lancet* 398: 535-554, 2021.
- Campos-Carrillo A, Weitzel JN, Sahoo P, Rockne R, Mokhnatkin JV, Murtaza M, Gray SW, Goetz L, Goel A, Schork N and Slavin TP: Circulating tumor DNA as an early cancer detection tool. *Pharmacol Ther* 207: 107458, 2020.
- Wu F, Fan J, He Y, Xiong A, Yu J, Li Y, Zhang Y, Zhao W, Zhou F, Li W, *et al*: Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. *Nat Commun* 12: 2540, 2021.
- Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csósz T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, *et al*: Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 375: 1823-1833, 2016.
- Brosnan EM, Weickhardt AJ, Lu X, Maxon DA, Barón AE, Chonchol M and Camidge DR: Drug-induced reduction in estimated glomerular filtration rate in patients with ALK-positive non-small cell lung cancer treated with the ALK inhibitor crizotinib. *Cancer* 120: 664-674, 2014.
- Lin PP: Integrated EpCAM-independent subtraction enrichment and iFISH strategies to detect and classify disseminated and circulating tumors cells. *Clin Transl Med* 4: 38, 2015.
- Lin PP, Gires O, Wang DD, Li L and Wang H: Comprehensive in situ co-detection of aneuploid circulating endothelial and tumor cells. *Sci Rep* 7: 9789, 2017.
- Lin PP: Aneuploid circulating tumor-derived endothelial cell (CTEC): A novel versatile player in tumor neovascularization and cancer metastasis. *Cells* 9: 1539, 2020.
- Lei Y, Sun N, Zhang G, Liu C, Lu Z, Huang J, Zhang C, Zang R, Che Y, Mao S, *et al*: Combined detection of aneuploid circulating tumor-derived endothelial cells and circulating tumor cells may improve diagnosis of early stage non-small-cell lung cancer. *Clin Transl Med* 10: e128, 2020.
- Zhang J, Hong Y, Wang L, Hu W, Tian G, Wu D, Wang Y, Dai L, Zhang Z, Yang Y and Fang J: Aneuploid subtypes of circulating tumor cells and circulating tumor-derived endothelial cells predict the overall survival of advanced lung cancer. *Front Oncol* 13: 829054, 2023.
- Wang Y, Liu Y, Zhang L, Tong L, Gao Y, Hu F, Lin PP, Li B and Zhang T: Vimentin expression in circulating tumor cells (CTCs) associated with liver metastases predicts poor progression-free survival in patients with advanced lung cancer. *J Cancer Res Clin Oncol* 145: 2911-2920, 2019.
- Wang Y, Liu Y, Zhang Z, Lu B, Gao Y, Tong L, Hu M, Lin PP, Li B and Zhang T: Post-therapeutic circulating tumor cell-associated white blood cell clusters predict poor survival in patients with advanced driver gene-negative non-small cell lung cancer. *BMC Cancer* 23: 578, 2023.
- Yu JJ, Xiao W, Dong SL, Liang HF, Zhang ZW, Zhang BX, Huang ZY, Chen YF, Zhang WG, Luo HP, *et al*: Effect of surgical liver resection on circulating tumor cells in patients with hepatocellular carcinoma. *BMC Cancer* 18: 835, 2018.
- Wang Y, Zhang L, Tan J, Zhang Z, Liu Y, Hu X, Lu B, Gao Y, Tong L, Liu Z, *et al*: Longitudinal detection of subcategorized CD44v6⁺ CTCs and circulating tumor endothelial cells (CTECs) enables novel clinical stratification and improves prognostic prediction of small cell lung cancer: A prospective, multi-center study. *Cancer Lett* 571: 216337, 2023.
- Rahbari NN, Schölch S, Bork U, Kahlert C, Schneider M, Rahbari M, Büchler MW, Weitz J and Reissfelder C: Prognostic value of circulating endothelial cells in metastatic colorectal cancer. *Oncotarget* 8: 37491-37501, 2017.
- Zhong X, Zhang H, Zhu Y, Liang Y, Yuan Z, Li J, Li J, Li X, Jia Y, He T, *et al*: Circulating tumor cells in cancer patients: Developments and clinical applications for immunotherapy. *Mol Cancer* 19: 15, 2020.
- Han T, Zhang J, Xiao D, Yang B, Chen L, Zhai C, Ding F, Xu Y, Zhao X and Zhao J: Circulating tumor-derived endothelial Cells: An effective biomarker for breast cancer screening and prognosis prediction. *J Oncol* 2022: 5247423, 2022.
- Liu Y, Yuan D, Ye W, Lv T and Song Y: Prognostic value of circulating endothelial cells in non-small cell lung cancer patients: A systematic review and meta-analysis. *Transl Lung Cancer Res* 4: 610-618, 2015.
- Zhang J, Wang HT and Li BG: Prognostic significance of circulating tumor cells in small-cell lung cancer patients: A meta-analysis. *Asian Pac J Cancer Prev* 15: 8429-8433, 2014.
- Bhakdi SC, Suriyaphol P, Thaicharoen P, Grote STK, Komoltri C, Chaiyaprasithi B and Charnkaew K: Accuracy of tumour-associated circulating endothelial cells as a screening biomarker for clinically significant prostate cancer. *Cancers (Basel)* 11: 1064, 2019.
- Xing C, Li Y, Ding C, Wang S, Zhang H, Chen L, Li P and Dai M: CD44⁺ circulating tumor endothelial cells indicate poor prognosis in pancreatic ductal adenocarcinoma after radical surgery: A pilot study. *Cancer Manag Res* 13: 4417-4431, 2021.
- Yuan DM, Zhang Q, Lv YL, Ma XQ, Zhang Y, Liu HB and Song Y: Predictive and prognostic significance of circulating endothelial cells in advanced non-small cell lung cancer patients. *Tumour Biol* 36: 9031-9037, 2015.
- Jiang Z, Zhou J, Li L, Liao S, He J, Zhou S and Zhou Y: Pericytes in the tumor microenvironment. *Cancer Lett* 556: 216074, 2023.
- Yang C, Bork U, Schölch S, Kulu Y, Kaderali L, Bolstorff UL, Kahlert C, Weitz J, Rahbari NN and Reissfelder C: Postoperative course and prognostic value of circulating angiogenic cytokines after pancreatic cancer resection. *Oncotarget* 8: 72315-72323, 2017.
- Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y and Zhou J: Circulating tumor cells: Biology and clinical significance. *Signal Transduct Target Ther* 6: 404, 2021.
- Ke H, Kao S, van Zandwijk N, Rasko JEJ and Yeo D: Circulating tumor cell detection may offer earlier diagnosis in patients suspected of asbestos-related lung cancer. *Lung Cancer* 192: 107829, 2024.

30. Dai CS, Mishra A, Edd J, Toner M, Maheswaran S and Haber DA: Circulating tumor cells: Blood-based detection, molecular biology, and clinical applications. *Cancer Cell* 43: 1399-1422, 2025.
31. Felden J, Schulze K, Krech T, Ewald F, Nashan B, Pantel K, Lohse AW, Riethdorf S and Wege H: Circulating tumor cells as liquid biomarker for high HCC recurrence risk after curative liver resection. *Oncotarget* 8: 89978-89987, 2017.
32. Pereira-Veiga T, Schneegans S, Pantel K and Wikman H: Circulating tumor cell-blood cell crosstalk: Biology and clinical relevance. *Cell Rep* 40: 111298, 2022.
33. Gootjes EC, Kraan J, Buffart TE, Bakkerus L, Zonderhuis BM, Verhoef C, Verheul HMW and Sleijfer AS: CD276-positive circulating endothelial cells do not predict response to systemic therapy in advanced colorectal cancer. *Cells* 9: 124, 2020.
34. Zhu HH, Liu YT, Feng Y, Zhang LN, Zhang TM, Dong GL, Xing PY, Wang HY, Shi YK and Hu XS: Circulating tumor cells (CTCs)/circulating tumor endothelial cells (CTECs) and their subtypes in small cell lung cancer: Predictors for response and prognosis. *Thorac Cancer* 12: 2749-2757, 2021.
35. Luo S, Ou Y, Zheng T, Jiang H, Wu Y, Zhao J and Zhang Z: optimal strategy for colorectal cancer patients' diagnosis based on circulating tumor cells and circulating tumor endothelial cells by subtraction enrichment and immunostaining-fluorescence in situ hybridization combining with CEA and CA19-9. *J Oncol* 2021: 1517488, 2021.
36. Zhang L, Liu Z, Zhang Y, Wu Z, Liu Y and Wang Z: The predictive value and clinical application of measuring circulating endothelial progenitor cells in lung adenocarcinoma. *J Cardiothorac Surg* 21: 352, 2026.
37. Zhang L, Zhang X, Liu Y, Zhang T, Wang Z, Gu M, Li Y, Wang DD, Li W and Lin PP: PD-L1⁺ aneuploid circulating tumor endothelial cells (CTECs) exhibit resistance to the checkpoint blockade immunotherapy in advanced NSCLC patients. *Cancer Lett* 469: 355-366, 2020.
38. Naito T, Tanaka F, Ono A, Yoneda K, Takahashi T, Murakami H, Nakamura Y, Tsuya A, Kenmotsu H, Shukuya T, *et al*: Prognostic impact of circulating tumor cells in patients with small cell lung cancer. *J Thorac Oncol* 7: 512-519, 2012.



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