Original Article

Circulating tumor cells are associated with lung cancer subtypes: a large-scale retrospective study

Lei Zhu[1#^], Erji Gao[1#], Yusuke Tomita[2], An Li[3*], Bo Tao[1*]

[1]Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China; [2]Department of Respiratory Medicine, Kumamoto University Hospital, Kumamoto, Japan; [3]Department of Oncology, The First Affiliated Hospital of Gannan Medical University, Ganzhou, China

Contributions: (I) Conception and design: L Zhu, E Gao, A Li; (II) Administrative support: B Tao; (III) Provision of study materials or patients: B Tao; (IV) Collection and assembly of data: L Zhu, A Li; (V) Data analysis and interpretation: B Tao, A Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

[*]These authors contributed equally to this work as co-corresponding authors.

Correspondence to: An Li, PhD. Department of Oncology, The First Affiliated Hospital of Gannan Medical University, 23 Qingnian Road, Ganzhou 341000, China. Email: lian8860562@163.com; Bo Tao, PhD. Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University School of Medicine, 507 Zhengmin Road, Shanghai 200433, China. Email: tbo0820@163.com.

Background: Circulating tumor cells (CTCs) provide a unique resource to decipher cell molecular properties of lung cancer. However, the clinicopathologic and radiological features associated with CTCs in different lung cancer subtypes remain poorly characterized. The aim of this study was to explore the clinicopathological and radiological features of CTCs in different lung cancer subtypes.

Methods: The CTC data were obtained using the CellSearch Circulating Tumor Cell Kit. CTCs were detected in 5,128 surgical patients with lung adenocarcinoma (LUAD), 2,226 with lung squamous cell carcinoma (LUSC), 248 with small cell lung cancer (SCLC), 99 with large cell carcinoma, and 70 with metastatic carcinomas. A Pearson correlation analysis was conducted to analyze the patients' clinical information, radiological features, and molecular characteristics, and logistic regression was used to examine the correlations between these factors and CTCs.

Results: For LUAD, the presence of tumor lobation, air bronchogram, and the epidermal growth factor receptor (EGFR) mutation were significantly associated with CTC levels. While the multivariable logistic regression analysis indicated that CD68 and P40 expression were independent factors associated with CTCs. For LUSC, tumor size, tumor spiculation, pleural indentation, air bronchogram, the expression levels of CK8/18, GPA33, and leucocyte common antigen (LCA) were significantly associated with CTC levels. The multivariable logistic regression analysis indicated that tumor size, pleural indentation, and air bronchogram were independent factors affecting CTCs. For SCLC, no factors were found to be significantly associated with CTC levels. For large cell carcinoma, tumor lobation and spiculation were significantly associated with CTC levels. For metastatic lung cancers, the presence of the positive lymphoid node was the only factor significantly associated with CTC levels.

Conclusions: We conducted a comprehensive analysis of the tumor properties, radiological features, and genomic characteristics that are significantly associated with CTCs in different lung cancer subtypes. This study helps elucidate the formation mechanism and relevant major regulation molecules of CTCs.

Keywords: Circulating tumor cells (CTCs); lung cancer; subtypes

^ ORCID: 0000-0001-5133-7840.

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Introduction

Circulating tumor cells (CTCs) represent a distinct metastatic precursor of tumor initiation, and have potential value in monitoring malignancy progression (1). High CTC levels lead to an increased tumor burden, a low sensitivity to therapy, and a worse prognosis (2,3). Recent studies have shown that CTC-based classification could be used to reveal the tumor genomic panorama and differentiate among clinical features, thus providing an excellent tool for patient stratification in clinical setting (4,5).

Research has also shown that CTC levels can be used to identify cancers with an accuracy exceeding ordinary histopathology diagnosis (6,7); however, the use of such methods has rarely been reported in lung cancer. Recently, a bioinformatics analysis revealed that CTCs could reveal non-small cell lung cancer (NSCLC) genomic characteristics and could potentially be used in pathological classification (8). However, the associations between CTCs and patients' clinical features were not described due to a lack of data on clinical information. The type of lung cancer is a significant factor affecting CTC levels; however, it is still unknown whether different subtypes of lung cancer, such as small cell lung cancer (SCLC) and metastatic lung cancer, have similar CTC levels and independent regulatory factors.

Further, given the multiple methods used to detect CTCs, and the mixed reference range due to the small sample sizes of previous research studies, no standard study (with matched surgical specimen confirmation) has explored in this area. To address these issues, we performed a large retrospective study to examine the baseline CTC levels of different lung cancer subtypes, including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), large cell carcinoma, SCLC, and cancers not otherwise specified. In addition, we investigated the factors affecting CTCs, including the clinical factors, imaging aspects, and genomic molecules. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-955/rc).

Highlight box

Key findings

- Circulating tumor cells (CTCs) are associated with different clinicopathologic and radiological features in different lung cancer subtypes.

What is known, and what is new?

- CTC levels are different in different cancers, and have been confirmed to be a useful diagnostic indicator.
- Our study revealed the specific clinicopathologic and radiological features affecting CTC levels in different lung cancer subtypes.

What is the implication, and what should change now?

- This study helps to elucidate the formation mechanism and relevant major regulation molecules of CTCs. We suggest to incorporate CTC assessments into routine clinical check-ups before surgery.

Methods

Patient cohort

This retrospective, single-center, cross-sectional study was performed in Shanghai, China. Between January 2020 and June 2023, 8,000 whole blood samples were collected from 8,000 patients who were hospitalized in Shanghai Pulmonary Hospital. All the patients were diagnosed with lung cancer (i.e., LUAD, LUSC, SCLC, large cell carcinoma, or other), and the diagnoses were confirmed by post-operative specimens obtained from general anesthesia surgery (wedge resection, segmentectomy, lobectomy, or other). Blood was collected from the patients after admission and before surgery. This study was independently approved by the Institutional Review Board of Shanghai Pulmonary Hospital, Shanghai, China (No. K24-539Y). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), International Conference on Harmonization Guidelines for Good Clinical Practice and local regulations. The patients provided informed consent to participate in the study. The patients were not compensated for participation in the study.

Blood sample and CTCs

Approximately 10 ml of whole blood was drawn from the patients before surgery and initiation of any systemic

treatment and stored in an additional vial of 10 mL CellSave tube (K2E EDTA, BD-Plymouth, PL6 7BP, Plymouth, UK). The blood was temporarily placed in a refrigerator and stored at 4 ℃ awaiting plasma isolation, which was performed within 2 hours of collection, CTC assessments were performed on the CellSave tube blood, and plasma centrifugation was performed at 1,000 g for 15 minutes. After centrifugation, the residual supernatant was removed. The CTC data were analyzed using the CellSearch Circulating Tumor Cell Kit (GENO, 20163400061, Nantong, China). If the sample was not immediately used, it was divided into small parts and stored at −80 ℃ to avoid repeated freezing. We tried not to use hemolytic or hyperlipidemic blood as much as possible.

Chest computed tomography (CT) scan and imaging features

All patients enrolled in the study underwent chest CT scans within 1 month before surgery at the Radiology Department using a Siemens Somatom Definition AS scanner (Siemens Medical Systems, Erlangen, Germany) or a Philips Brilliance 40 scanner (Philips Healthcare, Cleveland, USA). After CT, thin-slice chest high-resolution CT reconstructions were conducted at contiguous 1-mm slices. Pulmonary nodules with abnormally high density in the lungs with or without suspected morphologic abnormalities were detected. Next, lesions were identified using the CT multiplanar reconstruction. The radiographic parameters, including the nodule size, location, density, lobation (with or without), spiculated margin, vacuole sign, pleural indentation, and air bronchogram, were extracted by two experienced thoracic surgeons. All the CT features were generated in both the lung window (width 1,500 HU, level 600 HU) and mediastinal window (350 HU, 50 HU).

Tumor tissue molecular testing

The resected specimens were sent to the Pathology Department to identify the tumor pathological types and molecular markers. More specifically, paraffin-embedded tissues were prepared with a thickness of approximately 3 μm. The EnVision two-step method was used for immunohistochemistry detection, and the instructions of the staining kit were strictly followed. The primary antibodies were mouse anti-rat monoclonal antibody, including TTF-1, calretinin, CD34, CDX2, CK, CK7, CK20, desmin, E-cadherin, estrogen receptor (ER), napsin A, P53, progesterone receptor (PR), Ki-67, Villin, and WT-1. Details of the antibodies are provided in Table S1. To determine the lung cancer pathological type, two independent pathologists evaluated the sample slices, and if a disagreement arose, a consensus was reached as to the tissue diagnosis.

Measurement of gene mutation

The RainDrop Plus dPCR System (California, USA) was used to detect common gene mutations, including the epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), and v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutations. First, according to the DNA sequence of the deletion region, a pair of suitable primers were synthesized on both sides of the deletion fragment for the polymerase chain reaction (PCR). Next, agarose gel electrophoresis and ethidium bromide staining were used to observe whether there were specific amplification fragments under short-wave ultraviolet light.

Statistical analysis

The patients' clinical features, CT imaging values, and molecular data were presented in the form of the median, frequency, and percentage. For the continuous variables, the Mann-Whitney *U* test was used to compare the differences between the two groups. For the categorical variables, the Pearson's Chi-squared (χ^2 test) or Fisher's test was used to compare the differences between the two groups. A Pearson correlation analysis was used to explore the relevant factors when the data were quantitative and normally distributed, and a Spearman correlation analysis was used when the data were non-normally distributed. A univariate logistic regression analysis was performed to investigate the significant factors associated with CTC, and all the significant factors in the univariate logistic regression were included in the multivariate logistic regression to identify the independent factors. The statistical analyses were performed with R software (version 4.0.3). The P values were two-sided, and a P value <0.05 was considered statistically significant.

Results

Participants' characteristics

This study cohort comprised 8,000 patients with lung cancer confirmed by surgical pathology, and 8,000 corresponding blood samples were collected. The patients had a median age [interquartile range] of 65 [33–78] years, and 3,681 (46.01%) were male. A majority of the patients had LUAD (n=5,128, 64.10%), followed by LUSC (n=2,226, 27.83%), SCLC (n=248, 3.10%), large cell carcinoma (n=99, 1.24%), metastatic tumor (n=70, 0.88%), and a cancer not otherwise specified (n=229, 2.86%). Of the patients, 2,142 (26.78%) were active smokers, and 18.71% (n=1,497) had hypertension, 5.04% (n=403) had coronary heart disease, and 16.40% had diabetes (n=1,312).

Characteristics of CTCs in LUAD

The full clinical, radiological, and molecular characteristics of the 5,128 patients with early-stage LUAD are presented in *Table 1*. The average CTC level of these patients was 10.04±5.42 (range, 2.76–34.91) pg/mL, and the median was 8.85 pg/mL. Based on the Pearson correlation analysis, no significant associations were found between age, gender, underlying diseases (hypertension, coronary heart disease, and diabetes), and CTC level (P>0.053) (*Table 1*).

To assess the potential effects of the radiological features on CTCs, we compared the tumor density, size, distance from pleura, lobation, spiculation, vascular convergence, pleural indentation, and air bronchogram, etc. The analysis of these imaging characteristics revealed that the presence of tumor lobation (r=0.680, P=0.04) and air bronchogram (r=0.712, P=0.03) were positively associated with CTCs.

To further explore the factors affecting CTCs, we investigated the clinicopathologic and genomic characteristics using the pathological specimens resected from surgery. The results showed that the expression of CK (r=0.701, P=0.03), CD68 (r=0.749, P=0.03), and P40 (r=0.821, P=0.02) were significantly associated with CTCs. A previous study reported that the CTC levels were affected by EGFR mutation status in NSCLC (9). Therefore, we also calculated the coefficients between the CTCs and EGFR, ALK, ROS1, KRAS, and BRAF mutations. However, the mutation data results were not statistically significant, except in relation to the EGFR mutation (r=0.634, P=0.047) (*Table 2*). The patients were divided into the CTC-high (CTC-H) and CTC-low (CTC-L) groups based on the median. We conducted a logistic regression to explore the clinical, pathologic, and genomic characteristics associated with CTCs. The multivariable logistic regression analysis indicated that CD68 [odds ratio (OR) =1.56, 95% confidence interval (CI): 0.78–3.77, P=0.045] and P40 (OR =3.87, 95% CI: 1.53–4.68, P=0.009) expression were independent factors affecting CTCs (*Table 3*).

Characteristics of CTCs in LUSC

The full clinical, radiological, and molecular characteristics of the 2,226 patients with LUSC are presented in *Table 3*. The average CTC level of these patients was 13.12±6.20 (5.90–32.81) pg/mL, and the median was 11.59 pg/mL. No significant associations were found between age, gender, underlying diseases (hypertension, coronary heart disease, and diabetes), and CTC level.

In relation to the radiological features, tumor size (r=0.849, P=0.02), tumor spiculation (r=0.670, P=0.03), pleural indentation (r=0.562, P=0.046), and air bronchogram (r=0.713, P=0.02) were found to be significantly associated with CTC levels (*Table 4*).

In relation to the genomic characteristics, CK8/18 (r=−0.691, P=0.03), GATA6 (r=0.655, P=0.03), and leucocyte common antigen (LCA) (r=−0.046, P=0.81) were found to be significantly associated with CTC levels. No significant associations were found between the EGFR, ALK, ROS1, KRAS, and BRAF mutation and CTCs (*Table 5*).

Similarly, the patients were also divided into CTC-H and CTC-L groups based on the median. The multivariable logistic regression analysis revealed that tumor size (OR =2.29, 95% CI: 0.13–5.46, P=0.01), pleural indentation (OR =1.66, 95% CI: 0.39–4.33, P=0.02), and air bronchogram (OR =1.37, 95% CI: 0.79–2.87, P=0.04) were independent factors affecting CTCs in LUSC (*Table 3*).

Characteristics of CTCs in SCLC

In terms of its radiological features, compared to NSCLC, SCLC has few distinguishing differences, especially in patients without any clinical symptoms. All the SCLC patients presented with a single nodule, ranging from 9.20–56 millimeters, with no distant metastasis. The full clinical, radiological, and molecular characteristics of the 248 patients with SCLC are presented in *Table 6*. The average CTC level of these patients was 10.31±2.02 (range, 3.26–22.26) pg/mL, and the median was 10.67 pg/mL. The expression levels of TTF1 (r=−0.289, P=0.64), CgA (r=−0.350, P=0.58), CK7 (r=−0.707, P=0.18), CD56

Table 1 Baseline clinical characteristics and correlation analysis of CTCs in patients with LUAD (n=5,128)

Characteristic	Value	Correlation coefficient	P
Gender		0.101	0.21
Male	1,902 (37.09)		
Female	3,226 (62.91)		
Age (years)	59.96±9.88	–0.001	>0.99
Underlying diseases			
Hypertension	891 (17.38)	0.125	0.12
Diabetes	928 (18.10)	0.034	0.67
Coronary heart disease	32 (6.24)	0.074	0.36
Active smoker	1,581 (30.83)	–0.057	0.48
Radiological features			
Tumor size (mm)	23.35±12.35	0.151	0.058
Tumor lobation	2,774 (54.10)	0.680	0.043*
Air bronchogram	291 (5.67)	0.712	0.03*
Location			
RUL	1,965 (38.32)	0.064	0.43
RML	417 (8.13)	0.085	0.29
RLL	736 (14.35)	0.095	0.24
LUL	1252 (24.41)	–0.052	0.52
LLL	758 (14.78)	0.018	0.83
Density (HU)	–186.95±258.89	0.099	0.21
Spiculated margin	3,677 (71.70)	0.000	>0.99
Vacuole sign	435 (8.49)	–0.013	0.87
Pleural indentation	1,613 (31.45)	–0.039	0.63
Necrosis	359 (7.00)	0.032	0.70
Vascular wall invasion	548 (10.69)	–0.092	0.25
Pleural infiltration	1,032 (20.12)	–0.011	0.89
Perineural invasion	129 (2.52)	–0.002	0.98
Intravascular tumor thrombus	1,354 (26.40)	–0.041	0.61
Positive incision margin	8 (0.16)	–0.021	0.83
Positive lymph node	1,161 (22.64)	–0.090	0.26
Genetic mutation			
EGFR-mutated	2,588 (50.47)	0.634	0.047*
ALK-mutated	297 (5.79)	0.150	0.06
ROS1-mutated	748 (14.59)	0.155	0.052
KRAS-mutated	672 (13.10)	0.148	0.06
BRAF-mutated	309 (6.03)	0.132	0.10

Data are presented as n (%), or mean ± standard deviation. *, P<0.05. CTC, circulating tumor cell; LUAD, lung adenocarcinoma; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; HU, Hounsfield unit; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

Table 2 Correlations between CTC and molecular expression in LUAD

Molecular expression	Negative, N (%)	Positive, N (%)	Unknown N (%)	Correlation coefficient	P
TTF-1 (10%)	513 (10.00)	4,615 (90.00)	0.000	–0.050	0.54
Calretinin (5%)	123 (2.40)	70 (1.37)	4,935 (96.24)	–0.042	0.60
CD34 (20%)	33 (0.64)	140 (2.73)	4,955 (96.63)	–0.062	0.44
CD56 (5%)	322 (6.28)	642 (12.52)	4,164 (81.20)	0.083	0.30
CD68 (30%)	95 (1.85)	129 (2.52)	4,904 (95.63)	0.749	0.03*
CDX2 (50%)	152 (2.96)	99 (1.93)	4,877 (95.11)	–0.044	0.58
C-MET (25%)	312 (6.08)	1,677 (32.70)	3,139 (61.21)	–0.092	0.25
CgA (10%)	31 (0.60)	98 (1.91)	4,999 (97.48)	0.421	0.19
CK (10%)	269 (5.25)	1,764 (34.40)	3,095 (60.35)	0.701	0.03*
CK20 (10%)	34 (0.66)	260 (5.07)	4,837 (94.33)	0.044	0.59
CK7 (10%)	32 (0.62)	291 (5.67)	4,805 (93.70)	0.101	0.21
CK8/18 (10%)	112 (2.18)	4,004 (78.08)	1,012 (19.73)	0.013	0.87
Desmin (1%)	189 (3.69)	116 (2.26)	4,823 (94.05)	0.102	0.20
E-cadherin (25%)	418 (8.15)	2,769 (54.00)	1,941 (37.85)	0.014	0.86
ER (25%)	366 (71.37)	111 (2.16)	4,651 (90.70)	0.454	0.11
GATA3 (50%)	225 (4.39)	33 (6.44)	4,870 (94.97)	0.119	0.14
GATA6 (10%)	37 (0.72)	1,931 (37.66)	3,160 (61.62)	0.012	0.88
GPA33 (1%)	332 (6.47)	97 (1.89)	4,699 (91.63)	–0.031	0.70
HER2 (50%)	910 (17.75)	2,161 (42.14)	2,057 (40.11)	0.013	0.87
HNF4α (1%)	349 (6.81)	484 (9.44)	4,295 (83.76)	–0.008	0.92
Ki-67 (25%)	42 (0.82)	839 (16.36)	4,247 (82.82)	0.106	0.19
LCA (10%)	181 (3.53)	52 (1.01)	4,895 (95.46)	–0.081	0.31
MTAP (50%)	37 (0.72)	78 (1.52)	5,013 (97.76)	–0.087	0.28
MUC4 (5%)	506 (9.87)	10 (0.20)	4,612 (89.94)	–0.110	0.17
Napsin A (25%)	1,129 (22.02)	3,967 (77.36)	32 (0.62)	0.032	0.69
P40 (10%)	2,104 (41.03)	276 (5.38)	2,748 (53.59)	0.821	0.02*
P53 (50%)	843 (16.44)	2,243 (43.74)	2,042 (39.82)	–0.091	0.25
Pou2F3 (1%)	289 (5.64)	194 (3.78)	4,645 (90.58)	–0.005	0.95
PD-L1 (1%)	801 (15.62)	4,020 (78.39)	307 (5.99)	–0.037	0.65
PR (5%)	980 (19.11)	76 (1.48)	4,072 (79.41)	0.080	0.32
SALL4 (25%)	632 (12.32)	3,456 (67.39)	1,040 (20.28)	–0.149	0.06
SMARCA4 (25%)	2,159 (42.10)	2,705 (52.75)	264 (5.15)	0.055	0.49
Villin (10%)	1,937 (37.77)	2,923 (57.00)	268 (5.23)	–0.028	0.73
WT-1 (1%)	204 (3.98)	9 (0.18)	4,915 (95.85)	–0.076	0.35
ZEB1 (10%)	1,512 (29.49)	2,205 (43.00)	1,411 (27.52)	0.103	0.20
β-catenin (50%)	1,881 (36.68)	1,589 (29.80)	1,658 (32.33)	0.485	0.67

For molecular expression, the percentage in parentheses represents the threshold for positive and negative expressions. *, P<0.05. CTC, circulating tumor cell; LUAD, lung adenocarcinoma; TTF-1, thyroid transcription factor-1; CK, cytokeratin; C-MET, cellular-mesenchymal to epithelial transition factor; LCA, leucocyte common antigen; MTAP, methylthioadenosine phosphorylase; ER, estrogen receptor; PD-L1, programmed cell death ligand 1; PR, progesterone receptor; SALL4, spalt-like transcription factor 4; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; WT-1, Wilms tumor 1; ZEB1, zinc finger E-box binding homeobox 1.

Table 3 The multivariable logistic analysis of factors associated with CTCs in LUAD and LUSC

Variables	CTCs		
	OR	95% CI	P value
LUAD			
CD68	1.56	0.78–3.77	0.045
P40	3.87	1.53–4.68	0.009
LUSC			
Tumor size	2.29	0.13–5.46	0.01
Pleural indentation	1.66	0.39–4.33	0.02
Air bronchogram	1.37	0.79–2.87	0.04

CTC, circulating tumor cell; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OR, odds ratio; CI, confidence interval.

(r=–0.354, P=0.56), CDX2 (r=–0.272, P=0.50), GATA3 (r=–0.667, P=0.22), and Pou2F3 (r=–0.297, P=0.43), and the presence of spiculation (r=–0.439, P=0.18) were negatively correlated with CTCs, but none reached statistical significance. The expression levels of SMARCA4 (r=–0.166, P=0.36), Ki-67 (r=–0.095, P=0.98), and GATA6 (r=–0.171, P=0.44), and tumor size (r=–0.331 P=0.08) were positively correlated with CTCs, but none reached statistical significance (*Table 7*).

Characteristics of CTCs in large cell carcinoma

The full clinical, radiological, and molecular characteristics of the 99 patients with large cell carcinoma are presented in *Table 8*. The average CTC level of these patients was 11.55±2.43 (range, 3.19–21.98) pg/mL, and the median was 11.76 pg/mL. As *Table 9* shows, the expression levels of SMARCA4 (r=0.811, P=0.01), CgA (r=0.719, P=0.04), CD56 (r=0.730, P=0.03), and GATA6 (r=0.623, P=0.046) were significantly associated with CTCs. The results of the radiological features analysis showed that nodule lobation (r=0.641, P=0.04) and spiculation (r=0.736, P=0.03) were significantly associated with CTC levels. The univariate and multivariable logistic regression analyses found that no factors were significantly associated with CTCs (*Table 9*).

Characteristics of CTCs in metastatic lung cancer

Given that metastatic lung cancers present molecular and cellular features distinct from different original malignancies, we systematically explored the clinical and radiological features, but not the molecular characteristics. Most lung metastases (n=48, 68.57%) manifested as multiple nodules on CT imaging, and the most common primary cancer were gastrointestinal cancer (n=29, 41.43%), lung cancer (n=17, 24.29%), breast cancer (n=5, 7.14%), nasopharyngeal carcinoma (n=4, 5.71%), and other (n=15, 21.43%). The average CTC level of these patients was 12.35±3.45 (range, 4.53–25.16) pg/mL, and the median was 10.40 pg/mL (*Table 10*). The correlation analysis showed that positive lymphoid node status was the only factor significantly associated with CTC level (r=0.829, P=0.02) (*Table 11*).

Discussion

Despite tertiary medical institutions paying increased attention to CTCs as a valuable auxiliary method to detect lung cancer and predict patient prognosis, robust data on the prevalence of and factors affecting CTCs in distinct pathological subtypes of lung cancer are limited. We conducted a large retrospective cohort of lung cancer patients who received surgery with horizontal multi-clinical records. We found that CTC presents in different subsets of lung cancer, and may be regulated by distinct clinical and genomic features. Our findings support previous findings that CTC is significantly associated with imaging characteristics and covers a comprehensive clinical-genomic landscape (10,11). These results suggest that it may be beneficial to add plasma CTC testing to the standard diagnostic check-up for lung cancer or to confirm the subtype before surgery in clinical settings.

Previous studies preliminarily investigated CTCs, clinical variables, and radiomics features prior to surgery, and found that the use of CTCs could increase the proportion of patients being accurately diagnosed (12,13). Such results could also increase the accuracy of a precise diagnosis and enable targeted therapy. To further enhance understandings and improve the timely prediction, CTC testing should be performed earlier as a check-up for suspected lung cancer as it was in our study.

We also identified additional clinical and imaging factors related to CTCs that have pronounced diagnostic and prognostic effects. The presence of these factors, including the tumor size, spiculation, pleural indentation, and air bronchogram, suggest that further CTC-driven protocols using blood in the clinic could be used for patient stratification and prediction.

Our study demonstrated that CTCs in different lung

Table 4 Baseline clinical characteristics and correlation analysis of CTCs in patients with LUSC (n=2,226)

Characteristic	Value	Correlation coefficient	P
Gender		0.033	0.86
Male	1,791 (80.46)		
Female	435 (19.54)		
Age (years)	64.26±6.01	0.228	0.22
Underlying diseases			
Hypertension	352 (15.81)	–0.265	0.15
Diabetes	360 (16.17)	–0.122	0.52
Coronary heart disease	105 (4.72)	0.601	0.54
Active smoker	581 (26.10)	–0.230	0.21
Radiological features			
Tumor size (mm)	30.01±19.11	0.849	0.02*
Tumor lobation	1,461 (65.63)	0.329	0.07
Air bronchogram	487 (21.88)	0.713	0.02*
Location			
RUL	903 (40.57)	0.129	0.49
RML	156 (7.01)	0.142	0.45
RLL	647 (29.07)	0.061	0.75
LUL	239 (10.74)	0.181	0.33
LLL	281 (12.62)	0.053	0.78
Density (HU)	49.35±16.42	0.200	0.70
Spiculated margin	1,183 (53.14)	0.670	0.03*
Vacuole sign	61 (2.74)	0.196	0.29
Pleural indentation	417 (18.73)	0.562	0.046*
Necrosis	833 (37.42)	0.024	0.90
Vascular wall invasion	69 (3.10)	–0.115	0.54
Pleural infiltration	44 (1.98)	0.080	0.67
Perineural invasion	28 (1.26)	0.092	0.62
Intravascular tumor thrombus	37 (1.66)	0.148	0.39
Positive incision margin	4 (0.18)	–0.096	0.61
Positive lymph node	766 (34.41)	0.106	0.57
Genetic mutation			
EGFR-mutated	342 (15.36)	0.298	0.10
ALK-mutated	93 (4.18)	0.105	0.57
ROS1-mutated	35 (1.57)	0.196	0.48
KRAS-mutated	192 (8.63)	0.069	0.71
BRAF-mutated	73 (3.28)	0.120	0.29

Data are presented as n (%), or mean ± standard deviation. *, P<0.05. CTC, circulating tumor cell; LUAD, lung adenocarcinoma; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; HU, Hounsfield unit; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

Table 5 Correlations between CTC and molecular expression in LUSC

Molecular expression	Negative, N (%)	Positive, N (%)	Unknown, N (%)	Clinical information	P
TTF-1 (10%)	1,127 (50.63)	646 (29.02)	453 (20.35)	–0.200	0.28
Calretinin (5%)	83 (3.73)	36 (1.62)	2,107 (94.65)	–0.004	0.98
CD34 (20%)	91 (4.09)	39 (1.75)	2,096 (94.16)	–0.115	0.55
CD56 (5%)	98 (4.40)	43 (1.93)	2,085 (93.67)	0.145	0.44
CD68 (30%)	137 (6.15)	209 (9.39)	1,880 (84.46)	–0.156	0.40
CDX2 (50%)	61 (2.74)	26 (1.17)	2,139 (96.09)	–0.189	0.31
c-MET (25%)	37 (1.66)	45 (2.02)	2,144 (96.32)	0.179	0.34
CgA (10%)	53 (2.38)	40 (1.80)	2,133 (95.82)	–0.042	0.83
CK (10%)	767 (34.46)	1,044 (46.90)	415 (18.64)	0.148	0.43
CK20 (10%)	198 (8.89)	210 (9.43)	1,818 (81.67)	0.039	0.84
CK7 (10%)	29 (1.30)	288 (12.94)	1,909 (85.76)	0.233	0.21
CK8/18 (10%)	10 (0.45)	213 (9.57)	2,003 (89.98)	–0.691	0.03
Desmin (1%)	176 (7.91)	17 (0.76)	2,033 (91.33)	–0.060	0.75
E-cadherin (25%)	117 (5.26)	63 (2.83)	2,046 (91.91)	0.099	0.60
ER (25%)	112 (5.03)	48 (2.16)	2,066 (92.81)	0.304	0.10
GATA3 (50%)	71 (3.19)	834 (37.47)	1,321 (59.34)	0.026	0.89
GATA6 (10%)	344 (15.45)	402 (18.06)	1,480 (66.49)	0.655	0.03*
GPA33 (1%)	79 (3.55)	92 (4.13)	2,056 (92.36)	0.170	0.36
HER2 (50%)	101 (4.54)	279 (12.53)	1,846 (82.93)	–0.006	0.98
HNF4α (1%)	49 (2.20)	178 (8.00)	1,999 (89.80)	–0.002	0.99
Ki-67 (25%)	146 (6.56)	514 (23.09)	1,566 (70.35)	0.214	0.25
LCA (10%)	57 (2.56)	74 (3.32)	2,095 (94.12)	–0.046	0.81
MTAP (50%)	38 (1.71)	20 (0.90)	2,168 (97.39)	0.559	0.047*
MUC4 (5%)	18 (0.81)	55 (2.47)	2,153 (96.72)	0.169	0.37
Napsin A (25%)	376 (16.89)	967 (43.44)	883 (39.67)	0.154	0.24
P40 (10%)	140 (6.29)	1,984 (89.13)	102 (4.58)	0.066	0.73
P53 (50%)	702 (31.54)	1,330 (59.75)	194 (8.72)	0.270	0.14
Pou2F3 (1%)	154 (6.92)	53 (2.38)	2,019 (90.70)	0.055	0.77
PD-L1 (1%)	543 (24.39)	1,263 (56.74)	420 (18.87)	–0.012	0.95
PR (5%)	21 (0.94)	47 (2.11)	2,158 (96.95)	–0.101	0.59
SALL4 (25%)	663 (29.78)	1,116 (50.13)	447 (20.08)	0.177	0.34
SMARCA4 (25%)	418 (18.78)	1,456 (65.41)	352 (15.81)	0.178	0.35
Villin (10%)	164 (7.37)	23 (1.03)	2,039 (91.60)	–0.089	0.63
WT-1 (1%)	70 (3.14)	89 (4.00)	2,067 (92.86)	0.080	0.67
ZEB1 (10%)	528 (23.72)	451 (20.26)	1,247 (56.02)	–0.093	0.62
β-catenin (50%)	111 (4.99)	742 (33.33)	1,373 (61.68)	0.215	0.25

For molecular expression, the percentage in parentheses represents the threshold for positive and negative expressions. *, P<0.05. CTC, circulating tumor cell; LUSC, lung squamous cell carcinoma; TTF-1, thyroid transcription factor-1; CK, cytokeratin; C-MET, cellular-mesenchymal to epithelial transition factor; LCA, leucocyte common antigen; MTAP, methylthioadenosine phosphorylase; ER, estrogen receptor; PD-L1, programmed cell death ligand 1; PR, progesterone receptor; SALL4, spalt-like transcription factor 4; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; WT-1, Wilms tumor 1; ZEB1, zinc finger E-box binding homeobox 1.

Table 6 Baseline clinical characteristics and correlation analysis of CTCs in patients with small cell carcinoma (n=248)

Characteristic	Value	Correlation coefficient	P
Gender		−0.047	0.80
Male	176 (70.97)		
Female	72 (29.03)		
Age (years)	58.20±7.79	0.336	0.58
Underlying diseases			
Hypertension	36 (14.52)	−0.068	0.71
Diabetes	29 (11.69)	0.143	0.44
Coronary heart disease	9 (3.63)	0.074	0.69
Active smoker	45 (18.15)	0.527	0.23
Radiological features			
Tumor size (mm)	28.20±7.79	−0.331	0.08
Tumor lobation	185 (74.60)	0.058	0.75
Air bronchogram	23 (9.27)	−0.232	0.20
Location			
RUL	38 (15.32)	0.338	0.58
RML	20 (8.06)	−0.022	0.91
RLL	24 (9.68)	0.271	0.16
LUL	76 (30.65)	0.183	0.40
LLL	90 (36.29)	−0.057	0.77
Density (HU)	33.50±24.45	0.095	0.61
Spiculated margin	32 (12.90)	−0.439	0.18
Vacuole sign	16 (6.45)	0.062	0.75
Pleural indentation	62 (25.00)	0.018	0.76
Necrosis	8 (3.23)	0.074	0.69
Vascular wall invasion	70 (28.23)	−0.169	0.36
Pleural infiltration	61 (24.60)	−0.111	0.55
Perineural invasion	63 (25.40)	−0.222	0.22
Intravascular tumor thrombus	96 (38.71)	0.116	0.53
Positive incision margin	0.000	0.303	0.09
Positive lymph node	167 (67.34)	−0.118	0.52
Genetic mutation		0.258	0.58
EGFR-mutated	23 (9.27)	0.216	0.26
ALK-mutated	2 (0.81)	0.322	0.07
ROS1-mutated	3 (1.21)	−0.155	0.40
KRAS-mutated	7 (2.82)	0.014	0.94
BRAF-mutated	0.000	0.275	0.13

Data are presented as n (%), or mean ± standard deviation. CTC, circulating tumor cell; LUAD, lung adenocarcinoma; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; HU, Hounsfield unit; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

Table 7 Correlations between CTC and molecular expression in lung small cell carcinoma

Molecular expression	Negative, N (%)	Positive, N (%)	Unknown, N (%)	Correlation coefficient	P
TTF-1 (10%)	21 (8.47)	153 (61.69)	74 (29.84)	–0.289	0.64
Calretinin (5%)	10 (4.03)	0.000	238 (95.97)	0.116	0.59
CD34 (20%)	71 (28.63)	30 (12.10)	147 (59.27)	0.289	0.11
CD56 (5%)	33 (13.31)	202 (81.45)	13 (5.24)	–0.354	0.56
CD68 (30%)	31 (12.50)	4 (1.61)	213 (85.89)	0.022	0.89
CDX2 (50%)	5 (2.02)	0.000	243 (97.98)	–0.272	0.50
c-MET (25%)	37 (14.92)	6 (2.42)	205 (82.66)	–0.031	0.87
CgA (10%)	47 (18.95)	146 (58.87)	55 (22.18)	–0.350	0.58
CK (10%)	66 (26.61)	93 (37.50)	89 (35.89)	–0.164	0.34
CK20 (10%)	43 (17.34)	11 (4.44)	194 (28.23)	–0.120	0.52
CK7 (10%)	49 (19.76)	142 (57.26)	57 (22.98)	–0.707	0.18
CK8/18 (10%)	26 (10.48)	0.000	222 (89.52)	0.230	0.71
Desmin (1%)	7 (2.82)	0.000	241 (97.18)	–0.108	0.56
E-cadherin (25%)	104 (41.94)	27 (10.89)	117 (47.185)	0.464	0.50
ER (25%)	14 (5.65)	0.000	234 (94.35)	–0.102	0.58
GATA3 (50%)	8 (3.23)	0.000	240 (96.77)	–0.667	0.22
GATA6 (10%)	174 (70.16)	50 (20.16)	24 (9.68)	–0.171	0.44
GPA33 (1%)	22 (8.87)	0.000	226 (91.13)	–0.036	0.85
HER2 (50%)	35 (14.11)	15 (6.05)	198 (79.84)	0.242	0.19
HNF4α (1%)	12 (4.84)	0.000	236 (95.16)	–0.073	0.70
Ki-67 (25%)	28 (11.29)	170 (68.55)	50 (20.16)	–0.095	0.98
LCA (10%)	23 (9.27)	0.000	225 (90.73)	0.080	0.67
MTAP (50%)	18 (7.26)	0.000	230 (92.74)	–0.373	0.54
MUC4 (5%)	16 (6.45)	0.000	232 (93.55)	0.419	0.48
Napsin A (25%)	194 (78.23)	3 (1.21)	51 (20.56)	–0.152	0.91
P40 (10%)	39 (15.73)	10 (4.03)	199 (80.24)	0.026	0.81
P53 (50%)	56 (22.58)	159 (64.11)	33 (13.31)	–0.447	0.23
Pou2F3 (1%)	30 (12.90)	53 (21.37)	165 (66.53)	–0.297	0.43
PD-L1 (1%)	136 (54.84)	58 (23.39)	54 (21.77)	–0.010	0.96
PR (5%)	17 (6.85)	2 (0.81)	229 (92.34)	0.011	0.96
SALL4 (25%)	133 (53.63)	25 (10.08)	90 (36.29)	0.049	0.79
SMARCA4 (25%)	82 (33.06)	34 (13.71)	132 (53.23)	–0.166	0.36
Villin (10%)	32 (12.90)	0.000	216 (87.10)	0.081	0.66
WT-1 (1%)	41 (16.53)	0.000	207 (83.47)	–0.079	0.71
ZEB1 (10%)	8 (3.22)	0.000	240 (96.77)	–0.167	0.43
β-catenin (50%)	48 (19.35)	42 (16.94)	158 (63.71)	–0.046	0.80

For molecular expression, the percentage in parentheses represents the threshold for positive and negative expressions. CTC, circulating tumor cell; TTF-1, thyroid transcription factor-1; CK, cytokeratin; C-MET, cellular-mesenchymal to epithelial transition factor; LCA, leucocyte common antigen; MTAP, methylthioadenosine phosphorylase; ER, estrogen receptor; PD-L1, programmed cell death ligand 1; PR, progesterone receptor; SALL4, spalt-like transcription factor 4; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; WT-1, Wilms tumor 1; ZEB1, zinc finger E-box binding homeobox 1.

Table 8 Baseline clinical characteristics and correlation analysis of CTCs in patients with large cell carcinoma (n=99)

Characteristic	Value	Correlation coefficient	P
Gender		–0.235	0.70
Male	56 (56.57)		
Female	43 (43.43)		
Age (years)	65.67±9.01	0.368	0.54
Underlying diseases			
Hypertension	13 (13.13)	0.183	0.77
Diabetes	14 (14.14)	–0.135	0.83
Coronary heart disease	3 (3.03)	0.611	0.27
Active smoker	19 (19.19)	0.374	0.54
Radiological features			
Tumor size (mm)	18.63±10.44	–0.066	0.82
Tumor lobation	61 (61.62)	0.641	0.04*
Air bronchogram	20 (20.20)	–0.239	0.39
Location			
RUL	32 (32.32)	–0.297	0.30
RML	8 (8.08)	0.293	0.27
RLL	23 (23.23)	0.048	0.86
LUL	19 (19.19)	0.378	0.15
LLL	17 (17.17)	–0.228	0.40
Density (HU)	–23.25±150.17	0.476	0.83
Spiculated margin	54 (54.55)	0.736	0.03*
Vacuole sign	6 (6.06)	–0.271	0.34
Pleural indentation	62 (62.63)	–0.500	0.67
Necrosis	19 (19.19)	–0.130	0.62
Vascular wall invasion	4 (4.04)	0.211	0.42
Pleural infiltration	21 (21.21)	–0.292	0.26
Perineural invasion	5 (5.05)	0.120	0.64
Intravascular tumor thrombus	32 (32.32)	–0.467	0.059
Positive incision margin	1 (1.01)	0.694	0.80
Positive lymph node	28 (28.28)	0.138	0.67
Genetic mutation			
EGFR-mutated	5 (5.05)	–0.083	0.79
ALK-mutated	5 (5.05)	0.365	0.22
ROS1-mutated	0.000	–0.123	0.69
KRAS-mutated	40 (40.40)	0.076	0.61
BRAF-mutated	0.000	0.234	0.20

Data are presented as n (%), or mean ± standard deviation. *, P<0.05. CTC, circulating tumor cell; LUAD, lung adenocarcinoma; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; HU, Hounsfield unit; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

Table 9 Correlations between CTC and molecular expression in lung large cell carcinoma

Molecular expression	Negative, N (%)	Positive, N (%)	Unknown, N (%)	Correlation coefficient	P
TTF-1 (10%)	33 (33.33)	42 (42.42)	24 (24.24)	–0.357	0.16
Calretinin (5%)	5 (5.05)	2 (2.02)	92 (92.93)	0.553	0.70
CD34 (20%)	10 (10.10)	3 (3.03)	86 (86.87)	0.250	0.12
CD56 (5%)	34 (34.34)	44 (44.44)	21 (21.21)	0.730	0.03*
CD68 (30%)	15 (15.15)	9 (9.09)	75 (75.76)	–0.061	0.82
CDX2 (50%)	7 (7.07)	2 (2.02)	90 (90.91)	0.169	0.52
C-MET (25%)	16 (16.16)	6 (6.06)	77 (77.78)	0.034	0.56
CgA (10%)	20 (20.20)	41 (41.41)	38 (38.38)	0.719	0.04*
CK (10%)	6 (6.06)	47 (47.47)	46 (46.46)	–0.140	0.59
CK20 (10%)	11 (11.11)	3 (3.03)	85 (85.86)	–0.408	0.10
CK7 (10%)	26 (26.26)	4 (4.04)	69 (69.70)	–0.059	0.82
CK8/18 (10%)	27 (27.27)	50 (50.51)	22 (22.22)	0.078	0.77
Desmin (1%)	13 (13.13)	7 (7.07)	79 (79.80)	0.388	0.52
E-cadherin (25%)	3 (3.03)	1 (1.01)	95 (95.96)	0.162	0.23
ER (25%)	12 (12.12)	0.000	87 (87.88)	0.316	0.20
GATA3 (50%)	14 (14.14)	0.000	85 (85.86)	–0.343	0.18
GATA6 (10%)	51 (55.56)	23 (23.23)	25 (25.25)	0.623	0.046*
GPA33 (1%)	16 (16.16)	0.000	83 (83.84)	0.236	0.51
HER2 (50%)	12 (12.12)	6 (6.06)	80 (80.81)	0.178	0.86
HNF4α (1%)	8 (8.08)	0.000	91 (91.92)	0.557	0.22
Ki-67 (25%)	15 (15.15)	84 (84.85)	0.000	0.119	0.67
LCA (10%)	6 (6.06)	0.000	93 (93.94)	–0.086	0.74
MTAP (50%)	4 (4.04)	0.000	95 (95.96)	0.164	0.33
MUC4 (5%)	2 (2.02)	0.000	97 (97.98)	0.569	0.78
Napsin A (25%)	47 (47.47)	52 (52.53)	0.000	0.307	0.11
P40 (10%)	18 (18.18)	0.000	81 (81.82)	0.704	0.07
P53 (50%)	10 (10.10)	66 (66.67)	23 (23.23)	0.278	0.59
Pou2F3 (1%)	5 (5.05)	0.000	94 (94.95)	–0.030	0.91
PD-L1 (1%)	28 (28.28)	37 (37.37)	34 (34.34)	–0.064	0.81
PR (5%)	11 (11.11)	1 (1.01)	87 (87.88)	0.318	0.23
SALL4 (25%)	51 (51.52)	13 (13.13)	35 (35.35)	0.259	0.37
SMARCA4 (25%)	45 (45.45)	54 (54.55)	0.000	0.811	0.01*
Villin (10%)	9 (9.09)	0.000	90 (90.91)	0.082	0.16
WT-1 (1%)	17 (17.17)	0.000	82 (82.83)	–0.130	0.61
ZEB1 (10%)	5 (5.05)	0.000	94 (94.95)	0.454	0.059
β-catenin (50%)	36 (36.36)	0.000	63 (63.64)	0.426	0.09

For molecular expression, the percentage in parentheses represents the threshold for positive and negative expressions. *, P<0.05. CTC, circulating tumor cell; TTF-1, thyroid transcription factor-1; CK, cytokeratin; C-MET, cellular-mesenchymal to epithelial transition factor; LCA, leucocyte common antigen; MTAP, methylthioadenosine phosphorylase; ER, estrogen receptor; PD-L1, programmed cell death ligand 1; PR, progesterone receptor; SALL4, spalt-like transcription factor 4; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; WT-1, Wilms tumor 1; ZEB1, zinc finger E-box binding homeobox 1.

Table 10 Baseline clinical characteristics and correlation analysis of CTCs in patients with metastatic cancer (n=70)

Characteristic	Value	Correlation coefficient	P
Gender		0.782	0.07
Male	21 (30.00)		
Female	49 (70.00)		
Age (year)	48.83±12.37	0.606	0.20
Underlying diseases			
Hypertension	7 (10.00)	–0.027	0.78
Diabetes	12 (17.14)	–0.031	0.76
Coronary heart disease	4 (5.71)	0.136	0.16
Active smoker	22 (31.43)	–0.019	0.85
Radiological features			
Tumor size (mm)	26.87±17.55	0.187	0.06
Tumor lobation	2 (2.86)	0.024	0.81
Air bronchogram	3 (4.29)	0.126	0.12
Location			
RUL	28 (40.00)	0.106	0.28
RML	4 (5.71)	0.145	0.65
RLL	13 (18.57)	0.014	0.89
LUL	10 (14.29)	0.142	0.15
LLL	15 (21.43)	0.124	0.20
Density (HU)	–234.25±350.29	–0.056	0.57
Spiculated margin	23 (32.86)	–0.076	0.43
Vacuole sign	6 (8.57)	–0.088	0.37
Pleural indentation	12 (17.14)	0.041	0.68
Necrosis	8 (11.43)	–0.001	>0.99
Vascular wall invasion	5 (7.14)	–0.134	0.17
Pleural infiltration	4 (5.71)	–0.121	0.22
Perineural invasion	3 (4.29)	–0.117	0.23
Intravascular tumor thrombus	1 (1.43)	–0.018	0.96
Positive incision margin	0.000	–	–
Positive lymph node	9 (12.86)	0.829	0.02
Genetic mutation			
EGFR-mutated	8 (11.43)	–0.018	0.87
ALK-mutated	5 (7.14)	–0.092	0.35
ROS1-mutated	0.000	–0.032	0.74
KRAS-mutated	3 (4.29)	–0.068	0.49
BRAF-mutated	6 (8.57)	–0.094	0.33

Data are presented as n (%), or mean ± standard deviation. CTC, circulating tumor cell; LUAD, lung adenocarcinoma; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; HU, Hounsfield unit; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

Table 11 Correlations between CTC and molecular expression in lung metastatic cancer

Molecular expression	Negative, N (%)	Positive, N (%)	Unknown, N (%)	Correlation coefficient	P
TTF-1 (10%)	50 (71.43)	14 (20.00)	6 (8.57)	–0.447	0.37
Calretinin (5%)	4 (5.71)	0.000	66 (94.29)	0.049	0.62
CD34 (20%)	23 (32.86)	19 (27.14)	28 (40.00)	0.186	0.056
CD56 (5%)	8 (11.43)	0.000	62 (88.57)	0.083	0.40
CD68 (30%)	20 (28.57)	31 (44.29)	19 (27.14)	0.010	0.92
CDX2 (50%)	14 (20.00)	47 (67.14)	9 (12.86)	0.137	0.16
C-MET (25%)	16 (22.86)	6 (8.57)	48 (68.57)	–0.161	0.10
CgA (10%)	10 (14.29)	0.000	60 (85.71)	–0.060	0.54
CK (10%)	5 (7.14)	0.000	65 (92.86)	0.127	0.19
CK20 (10%)	11 (15.71)	1 (1.43)	58 (82.86)	0.088	0.37
CK7 (10%)	19 (27.14)	39 (55.71)	12 (17.14)	–0.015	0.88
CK8/18 (10%)	8 (11.43)	5 (7.14)	57 (81.43)	0.123	0.21
Desmin (1%)	15 (21.43)	0.000	55 (78.57)	–0.051	0.60
E-cadherin (25%)	35 (50.00)	18 (25.71)	17 (24.29)	–0.103	0.29
ER (25%)	2 (2.86)	5 (7.14)	63 (90.00)	0.078	0.43
GATA3 (50%)	21 (30.00)	12 (17.14)	37 (52.86)	0.039	0.69
GATA6 (10%)	36 (51.43)	7 (10.00)	27 (38.57)	–0.084	0.39
GPA33 (1%)	3 (4.29)	0.000	67 (95.71)	–0.022	0.82
HER2 (50%)	0.000	5 (7.14)	65 (92.86)	0.057	0.56
HNF4α (1%)	9 (12.86)	0.000	61 (87.14)	–0.018	0.85
Ki-67 (25%)	20 (28.57)	42 (60.00)	8 (11.43)	–0.062	0.53
LCA (10%)	5 (7.14)	1 (1.43)	64 (91.43)	0.017	0.86
MTAP (50%)	7 (10.00)	0.000	63 (90.00)	–0.134	0.17
MUC4 (5%)	20 (28.57)	13 (18.57)	37 (52.86)	0.118	0.23
Napsin A (25%)	32 (45.71)	6 (8.57)	32 (45.71)	0.238	0.16
P40 (10%)	12 (17.14)	0.000	58 (82.86)	–0.001	0.99
P53 (50%)	11 (15.71)	24 (34.29)	35 (50.00)	–0.024	0.81
Pou2F3 (1%)	7 (10.00)	1 (1.43)	62 (88.57)	–0.118	0.23
PD-L1 (1%)	2 (2.86)	0.000	68 (97.14)	0.013	0.89
PR (5%)	33 (47.14)	12 (17.14)	25 (35.71)	0.017	0.87
SALL4 (25%)	15 (21.43)	9 (12.86)	46 (65.71)	–0.048	0.62
SMARCA4 (25%)	24 (34.29)	17 (24.29)	29 (41.43)	–0.017	0.86
Villin (10%)	11 (15.71)	49 (70.00)	10 (14.29)	–0.016	0.87
WT-1 (1%)	10 (14.29)	3 (4.29)	57 (81.43)	–0.044	0.66
ZEB1 (10%)	12 (17.14)	0.000	58 (82.86)	0.053	0.59
β-catenin (50%)	20 (28.57)	15 (21.43)	35 (50.00)	0.145	0.14

For molecular expression, the percentage in parentheses represents the threshold for positive and negative expressions. CTC, circulating tumor cell; TTF-1, thyroid transcription factor-1; CK, cytokeratin; C-MET, cellular-mesenchymal to epithelial transition factor; LCA, leucocyte common antigen; MTAP, methylthioadenosine phosphorylase; ER, estrogen receptor; PD-L1, programmed cell death ligand 1; PR, progesterone receptor; SALL4, spalt-like transcription factor 4; SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin; WT-1, Wilms tumor 1; ZEB1, zinc finger E-box binding homeobox 1.

cancer subtypes share distinct radiological and clinical features. Cho *et al.* (14) reported that a larger tumor diameter, solid nodule density and nodal metastasis were associated with the incidence of NSCLC. Conversely, our results indicated that these factors were not associated with CTCs. Similar to the LUAD cohort, strong correlations were found between CTCs and air bronchogram in the LUSC cohort, which suggests that air bronchogram may be a predictive factor for CTC. It may be that LUSC and LUAD cancer cells usually spread along the alveolar wall and alveolar septa, and involve the alveolar septa. This process maintains the integrity and normal bronchial lumen of the lobules, resulting in no significant changes in gas content, and thus the formation of a more natural bronchial inflation sign. Taken together, our findings showed that radiological variables in LUAD/LUSC and CTCs may be modulated by the bronchial or alveolar lesions.

Using complete cytological morphology and rich multi-omics information, and advanced diagnostic technology, CTCs have been used to detect the EGFR mutation and guide optimal EGFR-TKI therapy for NSCLC (15,16). Similarly, we found that CTCs define a subset of lung cancers associated with the EGFR mutation. A large-scale systematic review confirmed their diagnostic value in detecting the EGFR mutation in NSCLC, and a recent study demonstrated that CTC testing can be used to eliminate wasteful therapies and side effects in lung cancer patients with longitudinal undetectable minimal residual disease (17,18). Remarkably, our study showed that CTC levels increased as the EGFR mutation increased in patients with LUAD, which indicates that high CTCs may indicate a good response to EGFR-TKIs. However, the specific mechanisms of the lung cancer bypassed or alternative pathway activation, and histological/phenotypic transformation, should be examined in further studies.

There are several limitations in this study. First, as a retrospective study, this study had a number of inherent drawbacks, such as selection bias. Second, the results are based on a Chinese population, and the applicability of the results to other populations is under investigation. Third, while the associations between CTCs and genomic expressions were explored, the specific mechanisms regulating CTCs remain uncertain. Finally, CTCs can be isolated and analyzed using a number of methods, including droplet digital PCR, beads, emulsion, amplification, and magnetics (BEAMing), and tagged-amplicon deep sequencing. Our methods were based on beads, and we did not examine the differences between our method and any other methods. Therefore, the scientific generalization requires further evaluation.

Conclusions

This study demonstrated that tumor properties, radiological features, and genomic characteristics are significantly associated with CTCs, and vary in different subtypes of lung cancer. Our findings may help to elucidate the generation mechanism and relevant major regulation molecules of CTCs. We suggest to incorporate CTC testing into routine clinical check-ups before surgery.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-955/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-955/coif). The authors have no conflicts of interest to declare.

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. This study was independently approved by the Institutional Review Board of Shanghai Pulmonary Hospital, Shanghai, China (No. K24-539Y). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), International Conference on Harmonization Guidelines for Good Clinical Practice and local regulations. The patients provided informed consent to participate in the study.

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