Original Article

Machine learning model for circulating tumor DNA detection in chronic obstructive pulmonary disease patients with lung cancer

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Background: Patients with chronic obstructive pulmonary disease (COPD) have a high risk of developing lung cancer. Due to the high rates of complications from invasive diagnostic procedures in this population, detecting circulating tumor DNA (ctDNA) as a non-invasive method might be useful. However, clinical characteristics that are predictive of ctDNA mutation detection remain incompletely understood. This study aimed to investigate factors associated with ctDNA detection in COPD patients with lung cancer.

Methods: Herein, 177 patients with COPD and lung cancer were prospectively recruited. Plasma ctDNA was genotyped using targeted deep sequencing. Comprehensive clinical variables were collected, including the emphysema index (EI), using chest computed tomography. Machine learning models were constructed to predict ctDNA detection.

Results: At least one ctDNA mutation was detected in 54 (30.5%) patients. After adjustment for potential confounders, tumor stage, C-reactive protein (CRP) level, and milder emphysema were independently associated with ctDNA detection. An increase of 1% in the EI was associated with a 7% decrease in the odds of ctDNA detection (adjusted odds ratio =0.933; 95% confidence interval: 0.857–0.999; P=0.047). Machine learning models composed of multiple clinical factors predicted individuals with ctDNA mutations at high performance (AUC =0.774).

Conclusions: ctDNA mutations were likely to be observed in COPD patients with lung cancer who had an advanced clinical stage, high CRP level, or milder emphysema. This was validated in machine learning models with high accuracy. Further prospective studies are required to validate the clinical utility of our findings.

Keywords: Chronic obstructive pulmonary disease (COPD); circulating tumor DNA (ctDNA); emphysema; lung cancer; machine learning

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Introduction

Lung cancer often develops in patients with an underlying pulmonary disease. Of them, chronic obstructive pulmonary disease (COPD) is the most common disease, along with pulmonary fibrosis (1,2). Studies have documented that COPD is an established risk factor for lung cancer development, even in never-smokers or when smoking exposure is controlled (3-9). When lung cancer is radiologically suspected, patients with COPD have a higher likelihood of being diagnosed with lung cancer and have higher rates of complications from invasive procedures than those without COPD (10). In this context, some COPD patients with lung cancer do not receive histologic diagnosis even when the tumor stage is I or II (11), necessitating a non-invasive biomarker that could aid lung cancer diagnosis in this high-risk population.

Cell-free DNA (cfDNA) is a non-encapsulating DNA in the peripheral blood, which was first discovered in 1948 (12). Many types of tumors release small DNA fragments through a combination of apoptosis, necrosis, and secretion (13). Although circulating tumor DNA (ctDNA) comprises only a small fraction of the total blood cfDNA (0.01% to 10%), the genomic alterations of ctDNA are highly specific to those of the original tumor and have been widely studied along with the development of next-generation sequencing (NGS). Analyses of ctDNA in plasma are intensively studied in patients with advanced-stage lung cancer to guide and monitor genotype-directed therapies (14,15). However, attempts to integrate ctDNA analysis into the diagnosis of lung cancer have been faced with challenges (16,17). One of the major limitations is that not all tumors shed sufficient amounts of ctDNA into the peripheral circulation in earlier stages (18). Given the relatively low sensitivity and high cost of ctDNA analysis, it is important to identify patients who are more likely to benefit from it. Several predictors of ctDNA shedding have been established, including tumor size, stage, number and sites of metastasis, non-adenocarcinoma histology, and tumor necrosis (17-20). However, previous studies included lung cancer patients regardless of underlying COPD, and little is known about the clinical factors associated with ctDNA detection in COPD patients with lung cancer.

Thus, we analyzed ctDNA mutations in spirometry-confirmed COPD patients with newly diagnosed lung cancer and sought to investigate which COPD-related clinical and imaging variables are associated with ctDNA detection. In addition, we developed prediction models to identify patients who are most likely to benefit from ctDNA analysis using multivariable machine learning (ML) methods. We present this article in accordance with the STARD reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-633/rc).

Highlight box

Key findings

- In chronic obstructive pulmonary disease patients with lung cancer, circulating tumor DNA (ctDNA) mutations were likely to be observed in those with advanced clinical stage, high C-reactive protein level, and milder emphysema. This finding was validated in machine learning models with high accuracy.

What is known and what is new?

- It is known that factors including tumor size, stage, metastasis, histology, and tumor necrosis are predictive of ctDNA shedding.
- An inverse relationship between emphysema and ctDNA detection is novel finding of this study. An increase of 1% in the emphysema index was associated with a 7% decrease in the odds of ctDNA detection.

What is the implication, and what should change now?

- Although patients with severe emphysema are in great need for non-invasive diagnosis of lung cancer, ctDNA detection might have limited clinical utility in this population.

Methods

A detailed description of the methods can be found in Appendix 1.

Study population

From October 2017 to September 2020, 461 patients with

spirometry-defined COPD [post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7] aged ≥40 years were prospectively enrolled from a single referral hospital. All of them did not have a significant pulmonary fibrosis. After excluding patients whose blood samples did not pass quality control or had technical issues in sample processing or library preparation (N=43), who withdrew consent or did not collect blood samples (N=11), and who had a history of malignancy other than lung cancer (N=3), 404 patients were included in the study population. For the present study, we further excluded patients without lung cancer (N=209), those with missing variables (N=10), and never smokers (N=8). Finally, 177 COPD patients with newly diagnosed lung cancer were included in the analysis. This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center (IRB file No. SMC 2017-08-128). In addition, surgically resected lung cancer tissues from three patients were banked and provided by the Samsung Medical Center Biobank (IRB file No. SMC 2020-12-016). Informed consent was obtained from all the patients and the study was carried out in accordance with the Declaration of Helsinki (as revised in 2013).

Sequencing data processing and somatic mutation calling

Details of the sample preparation, DNA extraction and library preparation were described in the Appendix 1. For the library construction of plasma cfDNA, hybrid selection was performed using three customized baits (LungCancer v1, LiquidSCAN v2-PanCancer, or IVD v1.0, GENINUS, Seoul, Korea; Table S1). Each capture bait targeted 36, 38, and 46 cancer-related genes and covered 340, 117, and 174 kb genomic regions across the human genome.

All liquid biopsy sequencing data were aligned to the hg19 reference using BWA-mem (v0.7.5). GATK v4.0.0 (21) and SAMTOOLS v1.6 (22) were used for base quality recalibration and cross-validation of the unique molecular identifier (UMI) family, and for sorting sequence alignment map (SAM) and binary alignment map (BAM) files, respectively. After sequencing alignment, discordant paired and off-target sequencing reads were removed. Picard (v2.9.4) was used to group reads into the same UMI families and in-house python (v2.7.10) scripts were used for error suppression. The error suppression method was based on previous studies (20) with minor modifications. First, all bases were subjected to Phred quality filtering using a threshold Q of 30 and only positions where total depths were above 500× were considered for variant identification. To exclude germline mutations in the analysis, non-reference alleles present at a frequency greater than 1% in the matched white blood cell gDNA were removed. The error suppression method using UMIs was used to distinguish true somatic mutations from polymerase chain reaction (PCR) and sequencing errors. After applying the error suppression method to the sequencing data, the following selection steps were used to eliminate the remaining sequencing errors: (I) variants not significantly greater than the error found in the matched germline DNA (binomial Bonferroni-adjusted P<0.01) were filtered out; (II) variant candidates with a high strand bias (90% if supporting reads ≥20; Fisher's exact test, P<0.1 if supporting reads <20) were removed; (III) if the z-statistic of the variants was not significantly higher than the background error obtained from gDNA (Bonferroni-adjusted P<0.05), they were excluded from the analysis.

Finally, the mutation candidates were selected according to the following conditions: Allele frequencies ≥0.15% and alternative allele counts ≥5 were selected. For tissue specimens, somatic variants were identified using different criteria: total depth ≥100× and allele frequency ≥2%. In the case of insertions or deletions, variants with an allele frequency ≥5% were selected. Variants were annotated using variant effect predictor (VEP) (v102) (23) and nonsynonymous variants were used in this analysis.

Clinical variables

Demographic and clinical information were obtained from electronic medical records, including age, sex, body mass index (BMI), smoking status, tumor stage (24) and centrality (25). Regarding COPD, modified Medical Research Council (mMRC) grade, COPD assessment test (CAT), pulmonary function tests (26,27), and chest CT parameters were collected. Using automatic segmentation software (Aview, Coreline Soft, Seoul, Korea) (28,29), we measured the emphysema index (EI), defined as the percentage of lung area with CT attenuation values <–950 HU in the whole lung at inspiration. WBC count and high-sensitivity C-reactive protein (hsCRP) levels were also measured in blood samples.

Statistical analysis

Logistic regression (LR) analyses were performed to analyze the clinical factors associated with the detection of ctDNA. In multivariable LR models (Models 1–5), we used a panel as an adjusted variable. To estimate the prediction score of

ctDNA detection in COPD patients, we used the sum of beta coefficients of significant variables from Model 5. To predict ctDNA detection using the variables, we considered four binary classifying machine learning (ML) models [logistic regression (LR), elastic net logistic regression (EN), random forest (RF), and support vector machine (SV)]. After splitting the dataset into training and test sets within the frame of leave-one-out cross-validation, we selected variables as features for ML models that showed significant association (P<0.1) with the presence of ctDNA mutation in a univariable LR model within each training set. The hyperparameters for EN, RF, and SV models were optimized by using grid search 5 cross-validation for accuracy in each training set. EN model was tuned by alpha from 0.0001 to 100, and L1 ratios between 0.0 and 1. RF model was allowed to have 10 to 1,000 estimators, maximum depth between 6 and 12, minimum samples per leaf between 8 and 18, and minimum samples per split between 8 and 20. SV model was allowed to use either radial or linear kernels, with gamma and C parameters between 0.001 to 100. To evaluate each model, we estimated the area under the receiver operating characteristics (ROC) curve (AUC), accuracy, sensitivity, specificity, and positive predictive value in the test set, and represented the performance of each model using an ROC curve plot. The model with the highest AUC was selected as the best prediction model for the ctDNA detection.

Results

Clinical characteristics of COPD patients with lung cancer

The clinical variables of patients with COPD and treatment-naïve lung cancer (N=177) are summarized in *Table 1*. Overall, the mean (standard deviation, SD) age was 69.8 (6.7) years, and most patients were male (94.4%) with former (67.8%) or current (32.2%) smoking exposure. Symptomatic burden measured using the mMRC dyspnea scale and CAT was relatively mild. Pulmonary function tests showed that the mean FEV_1 was 70.3% pred, and the majority of patients (90.4%) had FEV_1 ≥50% pred. The mean (SD) EI was 4.43% (6.8%) and 24 (13.6%) patients had an EI ≥10%. The clinical stages of lung cancer were classified as stage I (41.8%), II (17.5%), III (29.9%), and IV (10.7%), respectively. There were eight patients whose lung cancers were not histologically confirmed, mainly due to poor lung function, although the clinical diagnosis of lung cancer was unequivocal. Most patients (92.9%) had non-small cell lung cancer (NSCLC) and adenocarcinoma accounted for 40% of NSCLC cases.

Detection of ctDNA mutations in overall study population

Among the 177 patients with COPD and treatment-naïve lung cancer, at least one ctDNA mutation was detected in 54 patients (30.5%). Detection rate was 8.1%, 25.8%, 52.8%, and 63.2% in stage I, II, III, and IV, respectively. The median number of detected mutations per patient was 2 (range, 1–8) and the median VAF of the mutations was 6.0% (range, 0.7–85.3%). The most frequently mutated genes were *TP53* (70%), *RB1* (19%), *CSMD3* (15%), *KEAP1* (9%), and *LRP1B* (9%) (*Figure 1A*). *TP53* was the most frequently mutated gene in both adenocarcinoma (52.9%) and squamous cell carcinoma (69.6%). Among the 54 patients, 19 underwent surgical resection without neoadjuvant treatment. Tumor tissues and adjacent normal lung tissues were banked in three patients. To confirm that ctDNA mutations identified by our pipeline were derived from tumor tissues, we compared ctDNA mutations and mutations identified in tumor tissues of the same patient (*Figure 1B*). All ctDNA mutations (16 mutations within 6 genes) were also detected in tumor tissues across the three patients, while 62.5% (10/16) of tumor tissue mutations were detected in ctDNA, suggesting that ctDNA mutations are derived from the tumor tissues and can be used in the subsequent analyses as tumor mutations in COPD patients with lung cancer.

Clinical factors associated with ctDNA detection

To identify ctDNA detection-associated factors, we first compared the variables of patients with ctDNA detection (N=54) and those without ctDNA detection (N=123) using univariable models (*Table 1*). As a result, patients with ctDNA mutations (N=54) had higher mMRC grade, lower EI, higher CRP, larger tumor size, more advanced clinical stages, more centrally located tumors, and a higher prevalence of small cell lung cancer (SCLC) than patients without ctDNA detection (*Table 1*). As different sequencing panels were used in our mutation data, we further conducted multivariable LR analyses with the same variables adjusted for sequencing panel type (*Table 2*), considering different types of EI (continuous variable in Model 2, binary variable using cut-off of 10% in Model 3, or continuous variable of tumor located lobes in Model 4). Tumor stages were most strongly associated with ctDNA detection in all the models {adjusted odds ratio (OR) comparing stage II, III, and IV to stage I: 3.82 [95% confidence interval (CI): 1.14–13.58], 9.01 (95% CI: 3.23–28.61), and 15.52 (95% CI: 4.15–66.14), respectively, in Model 2}. Lower EI values of the total lung

Table 1 Characteristics of COPD patients with lung cancer according to ctDNA detection

Clinical variables	Overall (N=177)	ctDNA not detected (N=123)	ctDNA detected (N=54)	Univariable OR (95% CI)	P
Age (years)	69.8 (6.7)	70.3 (6.6)	68.5 (6.8)	0.96 (0.91–1.01)	0.082
Sex, male	167 (94.4)	115 (93.5)	52 (96.3)	1.81 (0.43–12.26)	0.440
Smoking					
Former	120 (67.8)	88 (71.5)	32 (59.3)	Reference	
Current	57 (32.2)	35 (28.5)	22 (40.7)	1.73 (0.88–3.38)	0.111
BMI (kg/m^2)	23.2 (2.8)	23.3 (2.7)	23.1 (3.0)	0.98 (0.87–1.10)	0.731
mMRC ≥2	48 (27.1)	26 (21.1)	22 (40.7)	2.56 (1.28–5.16)	0.008
CAT total ≥10	99 (55.9)	65 (52.8)	34 (63.0)	1.52 (0.79–2.96)	0.21
Pulmonary function					
FVC, % pred	88.9 (13.7)	89.0 (14.6)	88.7 (11.6)	1.00 (0.98–1.02)	0.907
FEV_1, % pred	70.3 (15.4)	69.7 (15.7)	71.5 (14.7)	1.01 (0.99–1.03)	0.489
FEV_1/FVC, %	55.6 (10.1)	54.8 (10.3)	57.5 (9.3)	1.03 (1.00–1.07)	0.089
FEV_1 <50% pred, n	17 (9.6)	13 (10.6)	4 (7.4)	0.68 (0.18–2.02)	0.502
EI					
% total lung	4.43 (6.8)	5.07 (7.2)	2.98 (5.6)	0.94 (0.88–1.00)	0.041
≥10%, n	24 (13.6)	21 (17.1)	3 (5.6)	0.29 (0.07–0.88)	0.027
% tumor-located lobe (N=175)	4.24 (8.2)	5.11 (9.5)	2.22 (3.4)	0.92 (0.83–0.98)	0.010
CRP (mg/dL)	1.06 (1.9)	0.66 (1.4)	2.00 (2.6)	1.41 (1.18–1.72)	<0.001
Tumor size (mm)	37.2 (19.5)	32.1 (15.1)	48.9 (23.1)	1.05 (1.03–1.07)	<0.001
Clinical stage of lung cancer					
I	74 (41.8)	68 (55.3)	6 (11.1)	Reference	
II	31 (17.5)	23 (18.7)	8 (14.8)	3.94 (1.24–13.15)	0.020
III	53 (29.9)	25 (20.3)	28 (51.9)	12.69 (4.98–37.32)	<0.001
IV	19 (10.7)	7 (5.7)	12 (22.2)	19.43 (5.85–73.40)	<0.001
Centrally located tumor	71 (40.1)	39 (31.7)	32 (59.3)	3.13 (1.63–6.14)	<0.001
Histology (N=169)					
NSCLC	157 (92.9)	112 (97.4)	45 (83.3)	Reference	
SCLC	12 (7.1)	3 (2.6)	9 (16.7)	7.47 (2.12–34.82)	<0.001
Histology of NSCLC (N=157)					
Non-adenocarcinoma	94 (59.9)	66 (58.9)	28 (62.2)	Reference	
Adenocarcinoma	63 (40.1)	46 (41.1)	17 (37.8)	0.87 (0.42–1.76)	0.703
Sequencing panel					
IVDv1	82 (46.3)	58 (47.2)	24 (44.4)	Reference	
LCv1	41 (23.2)	27 (22.0)	14 (25.9)	1.25 (0.55–2.78)	0.583
PCv2	54 (30.5)	38 (30.9)	16 (29.6)	1.02 (0.47–2.15)	0.964

Values indicate the number of number (%) or mean (standard deviation) for categorical and continuous variables, respectively. COPD, chronic obstructive pulmonary disease; ctDNA, circulating tumor DNA; OR, odds ratio; CI, confidence interval; BMI, body mass index; mMRC, modified medical research council; CAT, COPD assessment test; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; EI, emphysema index; CRP, c-reactive protein; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

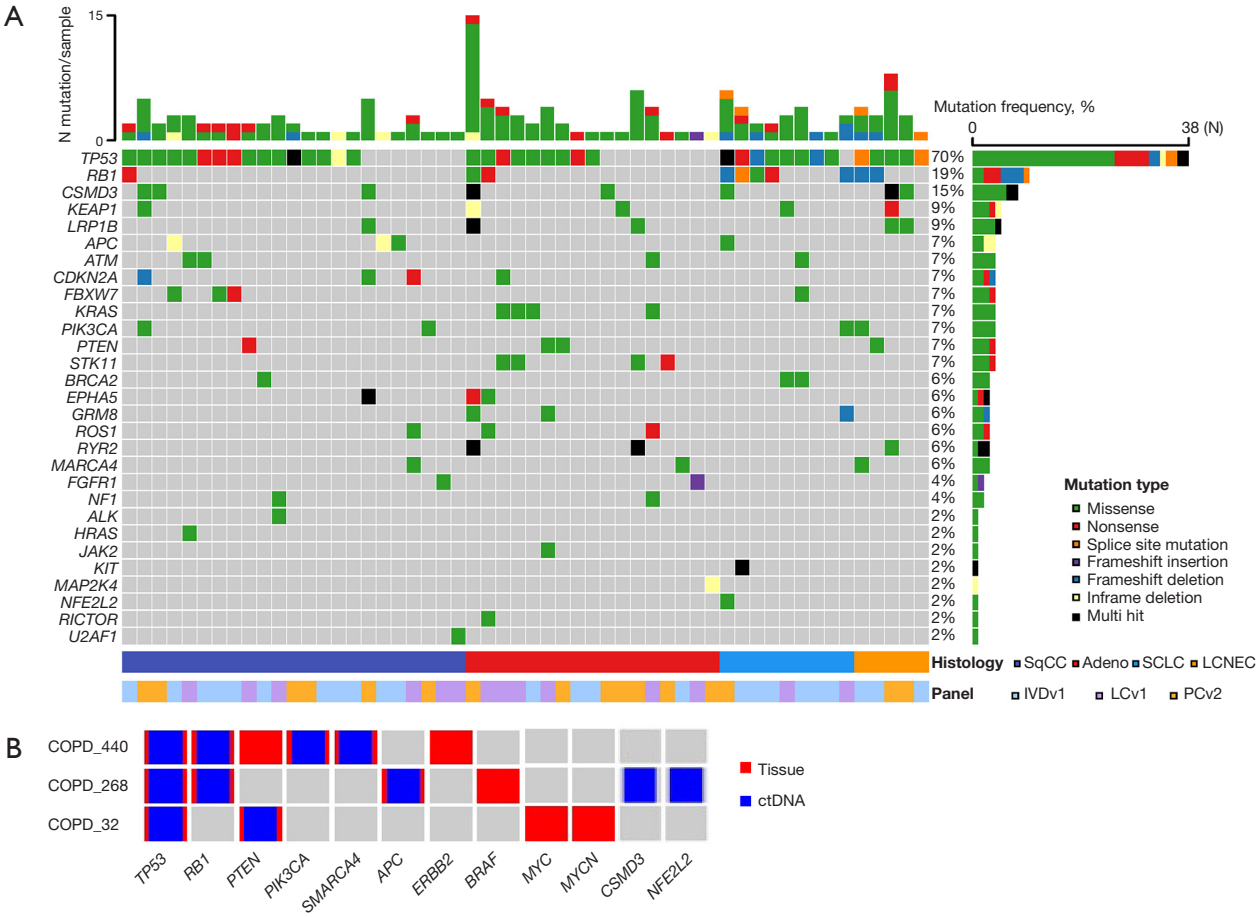


Figure 1 Mutations identified in ctDNA of 54 patients with COPD and lung cancer. (A) Overview of the mutated genes in patients with ctDNA detected. (B) Comparison of genetic alteration between ctDNA and surgically resected tumor tissues in three patients. For one patient (COPD_268) who had a missense mutation in *NFE2L2* and *CSMD3* in ctDNA, these two mutations could not be compared with those in the tissue because *NFE2L2* and *CSMD3* were not included in the panel used for tissue sequencing. SqCC, squamous cell carcinoma; Adeno, adenocarcinoma; SCLC, small cell lung cancer; LCNEC, large cell neuroendocrine carcinoma; COPD, chronic obstructive pulmonary disease; ctDNA, circulating tumor DNA.

and higher CRP levels were also significantly associated with ctDNA detection. In Model 2, a 1% increase in EI of the total lung was associated with a 7% decrease in the odds of ctDNA detection (adjusted OR: 0.933, 95% CI: 0.857–0.999, P=0.047).

Prediction of ctDNA detection using machine learning models

To predict ctDNA mutation detection using multiple variables, we selected variables with P<0.1 from the univariable LR models as the features of four ML prediction models (LR, EN, RV, SV) (*Table 3* and Table S2). As shown in *Figure 2*, the LR model showed the highest AUC (0.774) with an accuracy of 71.8%, sensitivity of 42.6%, and specificity of 84.6% for predicting the presence of ctDNA mutations. We further estimated the prediction score per sample to show the effect of the significant variables on the risk of ctDNA detection in COPD patients with lung cancer using the beta coefficients of the multivariable LR model (Model 5, composed of variables with P<0.05 of the Model 2 adjusted by panel). After classifying samples based on the risk scores, we found that 82.4% of the patients in the highest (10th) decile group had ctDNA mutations while all patients in the lowest (1st) decile group had no ctDNA mutations (*Figure 3* and Table S3).

Table 2 Multivariable models for clinical factors associated with ctDNA detection in COPD patients with lung cancer

Clinical variables	Model 1*		Model 2†		Model 3†		Model 4†	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	0.96 (0.91–1.01)	0.081						
Sex	1.73 (0.41–11.8)	0.482						
Smoking								
Former	Reference							
Current	1.71 (0.86–3.38)	0.126						
BMI (kg/m^2)	0.98 (0.87–1.10)	0.710						
mMRC ≥2	2.57 (1.27–5.23)	0.009	2.05 (0.88–4.79)	0.095	1.97 (0.86–4.52)	0.108	2.09 (0.89–4.93)	0.091
CAT total ≥10	1.50 (0.78–2.94)	0.221						
FEV_1 <50% pred	0.67 (0.18–2.00)	0.484						
EI								
% total lung	0.94 (0.88–1.00)	0.043	0.93 (0.86–0.999)	0.047				
≥10%, n	0.29 (0.07–0.89)	0.029			0.29 (0.06–1.07)	0.064		
% tumor located lobe	0.92 (0.83–0.98)	0.009					0.93 (0.83–1.00)	0.061
CRP (mg/dL)	1.41 (1.18–1.74)	<0.001	1.39 (1.12–1.78)	0.002	1.42 (1.14–1.83)	0.001	1.39 (1.11–1.79)	0.003
Clinical stage of lung cancer								
I	Reference		Reference		Reference		Reference	
II	3.96 (1.25–13.23)	0.020	3.82 (1.14–13.58)	0.029	4.10 (1.24–14.41)	0.021	3.55 (1.06–12.59)	0.040
III	12.87 (4.99–38.30)	<0.001	9.01 (3.23–28.61)	<0.001	10.17 (3.75–31.67)	<0.001	8.10 (2.90–25.71)	<0.001
IV	19.16 (5.75–72.59)	<0.001	15.52 (4.15–66.14)	<0.001	17.43 (4.73–73.20)	<0.001	14.23 (3.63–63.06)	<0.001
Centrally located tumor	3.14 (1.63–6.17)	0.001	1.75 (0.79–3.86)	0.164			1.77 (0.80–3.93)	0.160
Sequencing panel								
IVDv1.0	–		Reference		Reference		Reference	
LCv1			0.78 (0.28–2.09)	0.620	0.77 (0.28–2.06)	0.602	0.83 (0.30–2.25)	0.719
PCv2			0.88 (0.34–2.25)	0.790	0.92 (0.36–2.32)	0.864	0.87 (0.33–2.22)	0.767

*, adjusted only for sequencing panels. †, variables with P<0.05 from Model 1 were used in forward selection. The selected variables were then used in the construction of Models 2, 3, and 4. For the EI, continuous and binary values of EI of the total lung areas were used in Models 2 and 3, respectively. Continuous values of EI of the tumor located in lobes were used in Model 4. In Model 4, 175 samples were used as described in *Table 1*. ctDNA, circulating tumor DNA; COPD, chronic obstructive pulmonary disease; OR, odds ratio; CI, confidence interval; BMI, body mass index; mMRC, modified medical research council; CAT, COPD assessment test; FEV_1, forced expiratory volume in 1 second; EI, emphysema index; CRP, C-reactive protein.

Prognostic values of ctDNA detection

During the median follow-up of 20.7 (interquartile range, 10.9–31.8) months, 51 (28.8%) patients with lung cancer died. The proportion of patients who died was significantly higher in those with ctDNA detection than in those without ctDNA detection (51.9% *vs.* 18.7%, P<0.001). In an unadjusted Cox regression model, ctDNA detection was associated with an increased risk of death [unadjusted hazard ratio (HR): 3.27, 95% CI: 1.87–5.72; Figure S1). However, after adjustment for major confounders, including tumor stage and histology, this association was not statistically significant. In a subgroup of patients with stage I and II lung cancer (N=105), ctDNA detection was independently associated with increased mortality (fully adjusted HR:

Table 3 Performance of prediction models for ctDNA detection using machine learning

Performance	LR	EN	SV	RF
Accuracy (%)	71.8	65.5	71.8	70.1
Specificity (%)	84.6	72.4	94.3	92.7
Sensitivity (%)	42.6	50.0	20.4	18.5
PPV (%)	54.8	44.3	61.1	52.6
AUC	0.774	0.678	0.663	0.711

AUC, area under the receiver-operating-characteristics curve; ctDNA, circulating tumor DNA; EN, elastic net regression; LR, logistic regression; PPV, positive predictive value; RF, random forest; SV, support vector machine.

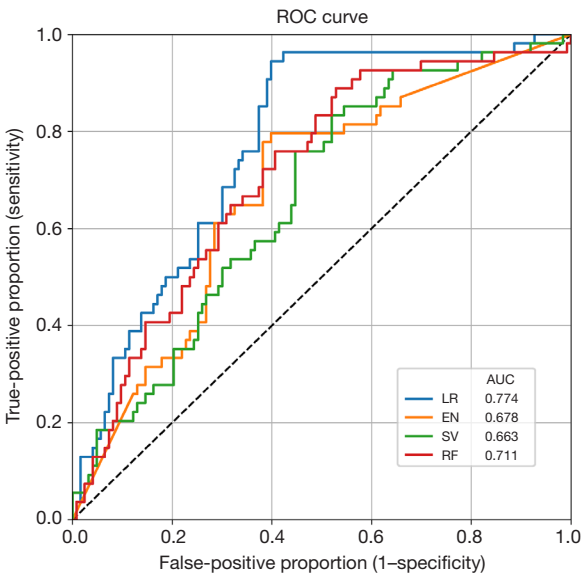


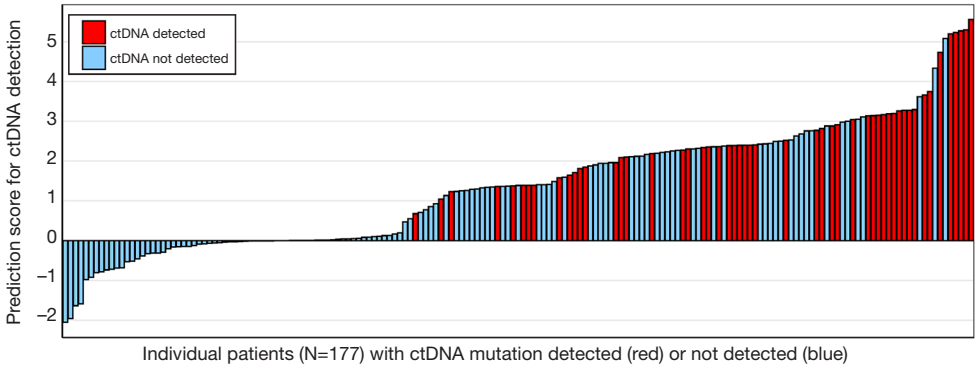
Figure 2 ROC curves of four machine learning prediction models for ctDNA detection in COPD patients with lung cancer. ROC, receiver operating characteristic; AUC, area under the ROC curve; LR, logistic regression; EN, elastic net regression; SV, support vector machine; RF, random forest; ctDNA, circulating tumor DNA; COPD, chronic obstructive pulmonary disease.

7.91, 95% CI: 1.55–40.36). VAF (per 1% increase) was also significantly associated with an increased risk of death in patients with stage I and II lung cancer (fully adjusted HR: 1.25, 95% CI: 1.01–1.56) (Table S4).

Discussion

Despite having a high risk of developing lung cancer, patients with COPD experience higher rates of complications from invasive diagnostic procedures compared to those without COPD. To focus on these high-risk populations, this study exclusively included patients with COPD with newly diagnosed lung cancer and analyzed ctDNA mutations using targeted deep sequencing. At least one ctDNA mutation was detected in 30.5% of patients (8.1% in stage I to 63.2% in stage IV). Of the comprehensively collected clinical and imaging variables, advanced clinical stage, lesser degree of emphysema, and increased CRP levels were associated with ctDNA detection among COPD patients with lung cancer. While this finding must be further validated, ML models with cross-validation demonstrated a satisfactory performance in identifying patients with ctDNA mutations, suggesting a potential clinical utility of ctDNA analysis assisted by a prediction model. We also confirmed that ctDNA detection and VAF levels were prognostic factors for poor overall survival (OS), particularly in early stage lung cancer patients.

We found an inverse relationship between emphysema and ctDNA detection, which is a novel finding. *Figure 4* shows representative cases of two patients with similar smoking exposure and lung function and the same stage IIB squamous cell carcinomas, which were located centrally. However, one patient with an EI of 1% had ctDNA mutations detected (*RB1* and *TP53*) whereas the other patient, with an EI of 10%, was negative for ctDNA mutation. Given that the major process of ctDNA shedding is tumor cell apoptosis and release into the bloodstream, it might be attributable to impaired pulmonary vasculature in the emphysematous lung. Earlier histological studies reported vascular alterations in emphysema (30). Another study showed that endothelial dysfunction (decreased expression of *VEGF*) is associated with the extent of emphysema (31). Indeed, the cross-sectional area of small pulmonary vessels is inversely correlated with the extent of emphysema (32). Thus, the lower rate of ctDNA shedding



Decile	Risk score range	ctDNA detected (%)
1	(–2.05) – (–0.31)	0
2	(–0.31) – (–0.01)	5.6
3	(–0.01) – (0.04)	5.6
4	(0.04)–0.93	11.1
5	0.93–1.39	27.8
6	1.39–2.09	44.4
7	2.09–2.36	38.9
8	2.36–2.69	47.1
9	2.69–3.19	52.9
10	3.19–5.56	82.4

Figure 3 Distribution of prediction scores for ctDNA detection in all individual COPD patients with lung cancer and proportion of patients with ctDNA mutations detected per decile group according to risk scores. The prediction score for ctDNA detection is a score for an individual patient estimated by sum of beta coefficients of variables from Model 5, which is composed of variables with P<0.05 of the multivariable Model 2 (please refer Table S3). ctDNA, circulating tumor DNA; COPD, chronic obstructive pulmonary disease.

in patients with severe emphysema in this study might be due to loss of vessels. This inverse correlation between emphysema and ctDNA detection in COPD patients with lung cancer suggests that ctDNA might have limited clinical utility in patients with severe emphysema who are in greater need for non-invasive diagnosis of lung cancer. Negative ctDNA mutation in this population cannot exclude lung cancer diagnosis. Therefore, it should be used as complementary to other modalities, such as chest CT scans.

Despite the limitations regarding insufficient shedding of ctDNA, several factors related to ctDNA detection have been reported. This study showed that advanced clinical stage is strongly associated with ctDNA detection in COPD patients with lung cancer, which was consistent with the correlation between tumor stage and ctDNA detection in many previous studies, as well as the number of metastatic sites (19). Based on several studies using NGS of multiple recurrent genetic alterations in lung cancer for the purpose of non-invasive diagnosis or residual disease detection (16,20,33,34), the sensitivity for stages II and III was up to 100% but the sensitivity was 50% or less for stage I NSCLC (16,34). Tumor size has been consistently associated with ctDNA shedding and a minimum tumor volume of 10 cm^3, which corresponds to a nodule diameter of 2.6 cm (T1c stage), is required to quantify VAF of 0.1% (20). In addition, ^{18}F-FDG avidity or metabolic tumor volume on positron emission tomography-CT scans were positively associated with ctDNA detection and VAF levels (17,20,35). Other radiologic parameters associated with the ctDNA detection rate include necrosis and nodule density (17). Among histological parameters, non-adenocarcinoma histology, SCLC, Ki67 proliferation index, necrosis, and lymphovascular invasion are known to predict ctDNA detection (20,36,37). This study did not include histological parameters in the multivariable models because we aimed to determine clinical factors predicting ctDNA detection before or even without histological confirmation as patients with COPD often have a high complication risk of invasive procedures.

Regarding the prognostic value of ctDNA detection, this study confirmed the findings of previous studies by showing that ctDNA detection and VAF levels are associated with shorter OS, particularly in early stages (17,38-40). Poor survival with positive ctDNA in early-stage lung cancer might stem from the higher recurrence rate after the surgery. Numerous studies have shown that preoperative and postoperative ctDNA detection was associated with shorter recurrence-free survival and OS after curative surgery (41-43). Accordingly, previous studies suggested the presence of ctDNA mutations from liquid biopsy into cancer staging as TNM "B" tumor staging, as ctDNA detection may reflect the presence of micrometastasis or minimal residual disease beyond a mere reproduction of information from tissues (44). Moreover, recent studies have shown that specific mutational profile or tumor mutational burden in ctDNA can also predict poor clinical outcome with polyclonal metastasis pattern and treatment response to immune checkpoint inhibitors (45,46).

In addition, the most frequently mutated genes in our data were also significantly mutated in lung adenocarcinoma (*TP53*, *RB1*, and *KEAP1*) and lung squamous cell carcinoma

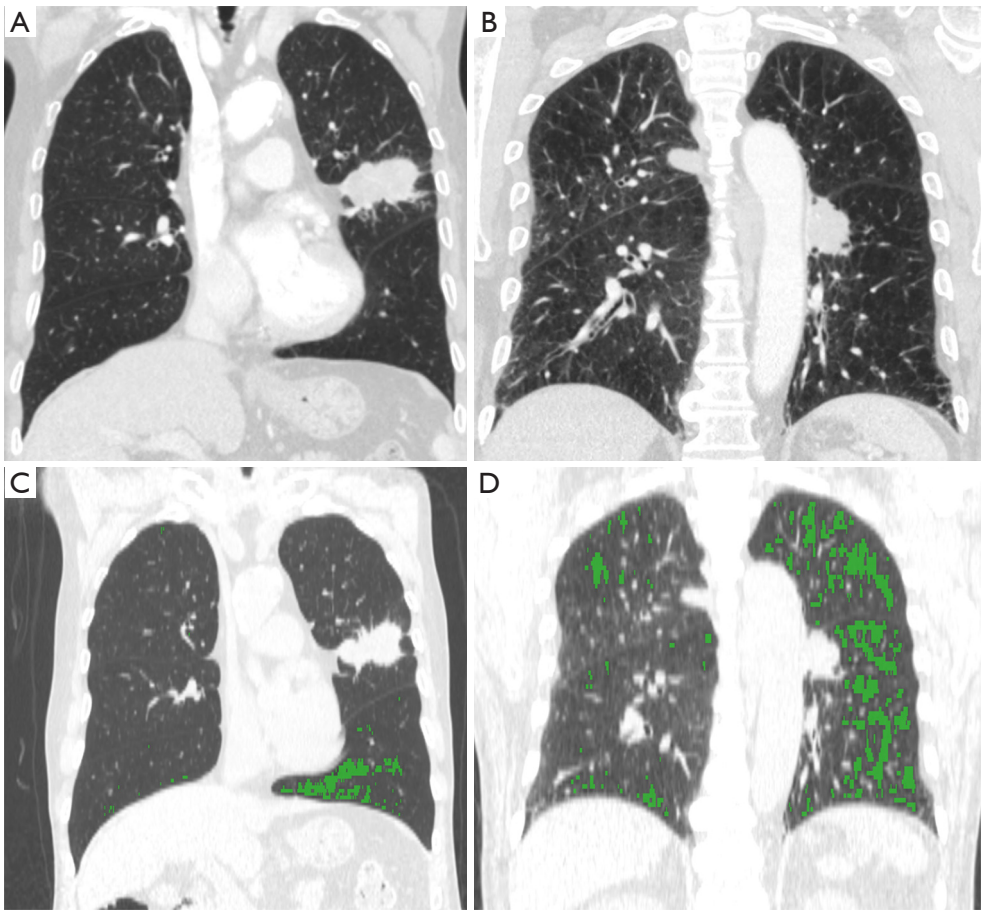


Figure 4 Representative cases of two COPD patients with lung cancer, with or without ctDNA mutation detection. (A) This 80-year-old male patient had 36 pack-year of smoking history and spirometry-confirmed COPD (post bronchodilator FEV_1/FVC =0.57, FEV_1 89% pred). Emphysema index in chest CT was 1.14%. He was diagnosed with squamous cell carcinoma (clinical stage T2bN1M0) and ctDNA mutations were detected for *RB1* and *TP53*. (B) This 70-year-old male patient had 42 pack-year of smoking history and spirometry-confirmed COPD (post bronchodilator FEV_1/FVC =0.42, FEV_1 67% pred). Emphysema index in chest CT was 10.39%. He was diagnosed with squamous cell carcinoma (clinical stage T2aN1M0) and ctDNA mutations were not detected. (C,D) Corresponding color map of emphysema index for two patients. COPD, chronic obstructive pulmonary disease; ctDNA, circulating tumor DNA; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; CT, computed tomography.

(*TP53* and *RB1*) in a previous large-scale study using whole exome sequencing (47). Similarly, *TP53* was the most frequently mutated gene in both lung adenocarcinoma [52.9% *vs.* 54.1%; our study *vs.* Campbell *et al.* (47)] and lung squamous cell carcinoma (69.6% *vs.* 86.4%).

The strength of this study is the use of data from targeted deep sequencing and the adoption of ML models to predict ctDNA detection in an individual patient with COPD and lung cancer. The model can assist in non-invasive lung cancer diagnosis by estimating a probability of ctDNA detection. For example, based on the prediction scores, more than 82% of patients with the top 10% score had ctDNA mutations, suggesting that the diagnosis of lung cancer can be established using ctDNA in these patients. On the other hand, for some patients with low prediction scores (low probability of ctDNA detection), clinicians should also utilize other diagnostic tests rather than solely rely on the ctDNA analysis. In addition, this study only included patients with spirometry-confirmed COPD, who are at a higher risk of developing lung cancer compared to matched smokers, and collected comprehensive information regarding COPD, such as COPD symptoms, lung function, and quantitatively measured emphysema on CT.

This study also has several limitations. First, it was

conducted in a single referral center and the study results were not externally validated. To address this limitation, we adopted machine learning models with cross-validation. In addition, as this study focused on COPD patients recruited from pulmonology clinics, our cohort predominantly consisted of men and smokers (>90%), which is consistent with the multicenter studies from Korea that are based on pulmonology clinics (48,49). This may limit the generalizability of our findings to other populations. As all patients were current or former smokers, the relatively lower prevalence of *EGFR* mutations in our cohort might be attributable to smoking and COPD (36,50). The lack of never-smokers made it impossible to explore the association between smoking exposure and ctDNA detection. Similarly, due to the unavailability of occupational information, the association between occupational exposure and ctDNA detection was not investigated. Second, genotyping in this study was limited to the pre-determined genes that were included in the panel, which are relatively fewer in number compared to previous studies (17,33). Thus, ctDNA detection might be underestimated compared to targeted sequencing with more genes, whole exome, or whole genome sequencing. Moreover, due to the difference in the genes between the panels used, the data regarding individual mutational features were not included in the current analysis. Nevertheless, we used panels as an adjusted covariate in multivariable models to minimize the effect of different panels on the outcomes. Finally, the mutations between ctDNA and tumor tissues were compared in only three patients. However, considering that all mutations from ctDNA were detected in the tumor tissues, which is consistent with previous reports (17,51,52), it was appropriate to use our ctDNA mutation data as a surrogate for tumor mutation data from the other patients in our analyses.

Conclusions

Using NGS of targeted genes, this study showed that approximately one-third of COPD patients shed ctDNA at the time of lung cancer diagnosis. In addition to the well-known correlation with the tumor stages, we found that patients with severe emphysema were less likely to have ctDNA detected, despite the presence of lung cancer. We also constructed ML models to predict ctDNA detection with high accuracy. Further studies incorporating individual mutational features and detailed radiologic parameters are needed to improve the prediction model for ctDNA detection and to develop prediction models for lung cancer diagnosis in COPD patients.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-633/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-633/dss

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-633/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-633/coif). M.J.A. served as an unpaid editorial board member of *Translational Lung Cancer Research* from October 2021 to September 2023. Seung-Ho Shin and D.P. were employees, and W.Y.P. is the CEO and stakeholder of Geninus Inc. D.P. is also an employee of Planit Healthcare Inc. The other 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 approved by the IRB of Samsung Medical Center (IRB file no. SMC 2017-08-128). In addition, surgically resected lung cancer tissues from three patients were banked and provided by the Samsung Medical Center Biobank under approval of IRB of Samsung Medical Center (IRB file no. SMC 2020-12-016). Informed consent was obtained from all the patients including the consent for publication and the study was carried out in accordance with the Declaration of Helsinki (as revised in 2013). The representative case images were considered anonymized as identifying

information was not included.

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Appendix 1 Supplementary methods

Sample preparation and DNA extraction

Whole blood samples were collected using a BCT (Streck Inc., Omaha, NE, USA). Plasma was prepared using three centrifugation steps with increasing centrifugal force. After centrifugation, plasma and plasma-depleted whole blood was stored at –80 ℃ until cfDNA extraction. cfDNA was extracted from plasma using a QIAamp Circulating Nucleic Acid Kit (Qiagen, Santa Clarita, CA, USA). Genomic DNA (gDNA) was isolated from blood samples using a QIAamp DNA Mini Kit (Qiagen, Santa Clarita, CA, USA). DNA concentration and purity were quantified using an Infinite M200 Pro NanoQuant (Tecan, Switzerland) and a Picogreen fluorescence assay on a Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Fragment size distribution was measured using a 4200 TapeStation instrument (Agilent Technologies, Santa Clara, CA, USA). An AllPrep DNA/RNA Mini Kit (Qiagen, Santa Clarita, CA, USA) was used to purify gDNA from frozen tissues. After extraction, DNA was quantified and fragmented in the same manner as gDNA from plasma-depleted whole blood, and ≤100 ng of sheared DNA was used for library preparation.

Library preparation

Purified gDNA was sonicated (7 min, 0.5% duty, intensity of 0.1, and 50 cycles/burst) into 150–200 bp fragments using a Covaris S2 (Covaris Inc. Woburn, MA, USA). gDNA and plasma DNA libraries were created using a KAPA Hyper Prep Kit (Kapa Biosystems, Woburn, MA, USA). Briefly, after completing end repair and A-tailing according to the manufacturer's protocol, we performed adaptor ligation at 4 ℃, overnight, using a customized adapter (Integrated Device Technology, San Jose, CA, USA). For the library construction of plasma cfDNA, hybrid selection was performed using three customized baits (LungCancer v1, LiquidSCAN v2-PanCancer, or IVD v1.0, GENINUS, Seoul, Korea, Table S1). Each capture bait targeted 36, 38, and 46 cancer-related genes and covered 340, 117, and 174 kb genomic regions across the human genome.

Detection of somatic mutations

First, all bases were subjected to Phred quality filtering using a threshold Q of 30 and only positions where total depths were above 500× were considered for variant identification. To exclude germline mutations in the analysis, non-reference alleles present at a frequency greater than 1% in the matched white blood cell gDNA were removed. The error suppression method using UMIs was used to distinguish true somatic mutations from PCR and sequencing errors. After applying the error suppression method to the sequencing data, the following selection steps were used to eliminate the remaining sequencing errors: (I) variants not significantly greater than the error found in the matched germline DNA (binomial Bonferroni-adjusted $P<0.01$) were filtered out; (II) variant candidates with a high strand bias (90% if supporting reads 20; Fisher's exact test, $P<0.1$ if supporting reads <20) were removed; (III) if the z-statistic of the variants was not significantly higher than the background error obtained from gDNA (Bonferroni-adjusted $P<0.05$), they were excluded from the analysis.

Finally, the mutation candidates were selected according to the following conditions: Allele frequencies ≥0.15% and alternative allele counts ≥5 were selected. For tissue specimens, somatic variants were identified using different criteria: total depth ≥100× and allele frequency ≥2%. In the case of insertions or deletions, variants with an allele frequency ≥5% were selected. Variants were annotated using VEP (v102) (23) and nonsynonymous variants were used in this analysis.

Clinical variables

Demographic and clinical information were obtained from electronic medical records, including age, sex, body mass index (BMI), and smoking status. Tumors were staged using the eighth edition of the American Joint Committee on Cancer (24) and central location was defined as 'within the inner one-third of the hemithorax by concentric lines arising from the midline' (25).

Regarding COPD, dyspnea was measured using the modified Medical Research Council (mMRC) grade, symptom burden measured using the COPD assessment test (CAT), pulmonary function tests (26,27), and chest CT parameters were collected.

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All spirometry tests were performed in a pulmonary function lab, using a Vmax 22 system (SensorMedics, Yorba Linda, CA, USA) according to the American Thoracic Society/European Respiratory Society criteria (26). Absolute values were obtained, and the percentages of predicted values were calculated using a reference equation obtained from a representative South Korean sample (27). All chest CT scans were analyzed using automatic segmentation software (Aview, Coreline Soft, Seoul, Korea) (28,29). We measured whole lung volume at inspiration and the emphysema index (EI), defined as the percentage of lung area with CT attenuation values <–950 HU in the whole lung at inspiration. We also measured the EI of the tumor-located lobe. At the time of blood sampling for cfDNA analysis, white blood cell count and high-sensitivity C-reactive protein (hsCRP) were measured together.

Statistical analysis

To analyze the clinical factors associated with the detection of ctDNA in the study participants, we performed logistic regression analyses for continuous variables (age, BMI, EI, and CRP) and categorical variables (sex, mMRC ≥2, CAT ≥10, FEV1 <50% pred, EI 10%, central location, sequencing panels, and tumor stages). Odds ratios (ORs), 95% confidence intervals (CIs), and p-values were obtained from each analysis. In multivariable logistic regression models (Models 1–5), we used a panel type as an adjusted variable because three different panels were used to generate the mutation data. Variables with P<0.05, in Model 1, were included in the multivariable models (Models 2–4) after forward variable selection. Model 5 was constructed by including variables with P<0.05 in Model 2 adjusted by panel. To estimate the prediction score of ctDNA detection in COPD patients, we used the sum of beta coefficients of significant variables from Model 5 (P<0.05; EI (%), CRP, and tumor stage).

To predict ctDNA detection using the variables, we considered four binary classifying ML models [logistic regression (LR), elastic net logistic regression (EN), random forest (RF), and support vector machine (SV)]. After splitting the dataset into training and test sets within the frame of leave-one-out cross-validation, we selected variables as features for ML models that showed significant association (P<0.1) with the presence of ctDNA mutation in a univariable logistic regression model within each training set. The hyperparameters for EN, RF, and SV models were optimized by using grid search 5 cross-validation for accuracy in each training set. EN model was tuned by alpha from 0.0001 to 100, and L1 ratios between 0.0 and 1. RF model was allowed to have 10 to 1,000 estimators, maximum depth between 6 and 12, minimum samples per leaf between 8 and 18, and minimum samples per split between 8 and 20. SV model was allowed to use either radial or linear kernels, with gamma and C parameters between 0.001 to 100. To evaluate each model, we estimated the area under the receiver operating characteristics (ROC) curve (AUC), accuracy, sensitivity, specificity, and positive predictive value in the test set, and represented the performance of each model using an ROC curve plot. The model with the highest AUC was selected as the best prediction model for the shedder.

The Kaplan–Meier method was used to estimate the overall survival (OS). Data of patients who were alive or those who could not be traced during follow-up were censored for OS at the time they were last known to be alive. Hazard ratios (HRs) and 95% CIs were calculated using the Cox proportional hazards model. All analyses were performed using R 3.6.0, Stata 14.0, and Python 3.8.8.

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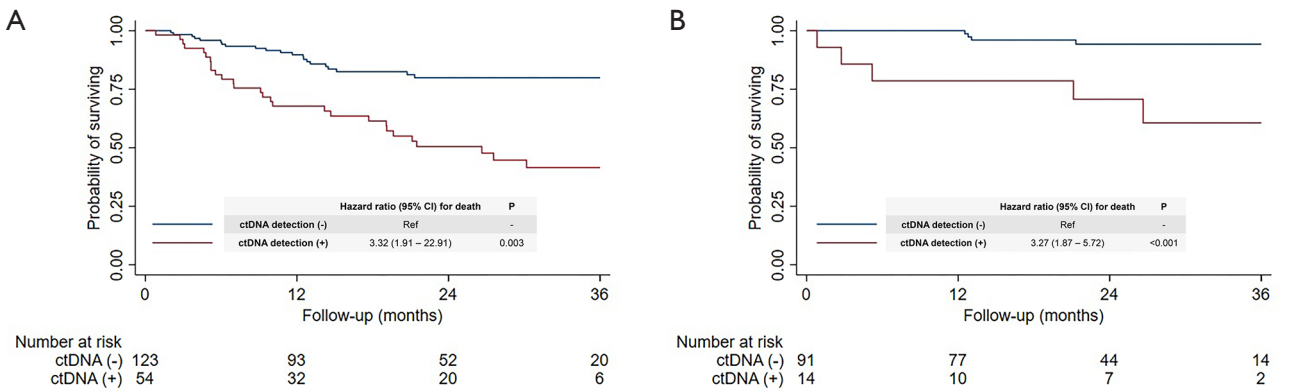

Figure S1 Overall survival according to ctDNA detection in COPD patients with lung cancer of (A) all stages (N=177) and of early stage (N=105).

Table S1 List of cancer-related genes included in targeted deep sequencing panels

Panels	List of genes							
Lung cancer v1	*AKT1*	*ALK*	*ARAF*	*ATM*	*BRAF*	*BRCA1*	*BRCA2*	*CDKN2A*
	EGFR	*ERBB2*	*FGFR1*	*FGFR2*	*FGFR3*	*HRAS*	*IDH1*	*IDH2*
	JAK2	*KEAP1*	*KIT*	*KRAS*	*MAP3K1*	*MDM2*	*MET*	*MYC*
	MYCL	*MYCN*	*NF1*	*NFE2L2*	*NRAS*	*NTRK1*	*NTRK2*	*NTRK3*
	PDGFRA	*PIK3CA*	*PTEN*	*RAF1*	*RB1*	*RET*	*RICTOR*	*ROS1*
	SMARCA4	*STK11*	*TP53*	*TSC1*	*U2AF1*			
LiquidSCAN v2—pan cancer	*AKT1*	*APC*	*BRAF*	*CBFB*	*CDH1*	*CDKN1B*	*CDKN2A*	*CSMD3*
	CTNNB1	*EGFR*	*EPHA5*	*ERBB2*	*ESR1*	*FBXW7*	*FGFR2*	*GATA3*
	GRM8	*HIST1H3B*	*KEAP1*	*KRAS*	*LRP1B*	*MAP2K4*	*MAP3K1*	*MYC*
	NFE2L2	*NRAS*	*NTRK3*	*PIK3CA*	*PIK3R1*	*PPP2R1A*	*PTEN*	*RB1*
	RUNX1	*RYR2*	*SMAD4*	*STK11*	*TBX3*	*TP53*		
IVD v1.0	*AKT1*	*ALK*	*APC*	*AR*	*ATM*	*BRAF*	*BRCA1*	*BRCA2*
	CDH1	*CDKN2A*	*CTNNB1*	*EGFR*	*ERBB2*	*ESR1*	*FBXW7*	*FGFR3*
	GNAS	*HRAS*	*HSPH1*	*KIT*	*KRAS*	*MET*	*MTOR*	*MYC*
	NF1	*NOTCH1*	*NRAS*	*PDGFRA*	*PIK3CA*	*POLE*	*PTEN*	*RB1*
	RET (fusion)	*ROS1(fusion)*	*SMAD4*	*SMARCA4*	*STK11*	*TP53*		

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Table S2 Performance of prediction models for ctDNA detection using machine learning according to different variables for the emphysema index

Performance	LR			EN			SV			RF		
	Model a*	Model b	Model c	Model a	Model b	Model c	Model a	Model b	Model c	Model a	Model b	Model c
Accuracy (%)	71.8	71.8	70.3	68.4	65.5	68.0	66.1	71.8	58.3	71.2	70.1	68.6
Specificity (%)	85.4	84.6	83.6	81.3	72.4	75.4	88.6	94.3	78.7	93.5	92.7	91.8
Sensitivity (%)	40.7	42.6	39.6	38.9	50.0	50.9	14.8	20.4	11.3	20.4	18.5	15.1
PPV (%)	55.0	54.8	51.2	47.7	44.3	47.4	36.4	61.1	18.8	57.9	52.6	44.4
AUC	0.767	0.774	0.754	0.650	0.678	0.642	0.557	0.663	0.539	0.719	0.711	0.692

*, for the EI, continuous and binary values were used in model a and model b, respectively, and continuous value of EI of the tumor located in lobes was used in model c. LR, logistic regression; EN, elastic net regression; SV, support vector machine; RF, random forest; PPV, positive predictive value; AUC, area under the receiver operating characteristic curve; EI, emphysema index.

Table S3 Prediction score of the 10th decile group of COPD patients with lung cancer according to Model 5

Sample	ctDNA mutation	EI (%) of total lung	CRP (mg/dL)	Tumor stage	Prediction score	Decile group
COPD_352	Detected	1.098	9.43	3	5.560	10th
COPD_444	Detected	2.556	8.94	3	5.303	10th
COPD_261	Detected	0.067	8.43	3	5.275	10th
COPD_17	Detected	8.031	7.75	4	5.232	10th
COPD_34	Detected	0.054	8.2	3	5.196	10th
COPD_407	Not detected	1.806	6.24	4	5.082	10th
COPD_31	Detected	0.204	4.96	4	4.734	10th
COPD_102	Not detected	7.173	6.95	3	4.335	10th
COPD_393	Detected	1.925	2.42	4	3.749	10th
COPD_227	Detected	0.778	3.9	3	3.661	10th
COPD_190	Not detected	7.626	4.97	3	3.621	10th
COPD_186	Detected	5.823	3.72	3	3.295	10th
COPD_117	Detected	0.030	2.67	3	3.279	10th
COPD_216	Detected	3.620	3.28	3	3.275	10th
COPD_450	Detected	0.708	0.8	4	3.260	10th
COPD_340	Detected	0.601	0.6	4	3.197	10th
COPD_32	Detected	0.283	2.46	3	3.191	10th

Prediction score = −0.060*EI (%) + 0.347*CRP +1.389*Tumor_stage2 + 2.354*Tumor_stage3 + 3.025*Tumor_stage4.

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Table S4 Risk of all-cause mortality in COPD patients with lung cancer according to ctDNA detection or VAF (%)

Stage	Unadjusted		Adjusted*	
	HR for death	P	HR for death	P
All stages (N=177)				
ctDNA detection	3.27 (1.87–5.72)	<0.001	1.39 (0.71–2.70)	0.337
VAF (%)	1.04 (1.02–1.05)	<0.001	1.00 (0.98–1.03)	0.687
Stage I, II (N=105)				
ctDNA detection	3.32 (1.91–22.91)	0.003	7.91 (1.55–40.36)	0.013
VAF (%)	1.19 (1.08–1.31)	<0.001	1.25 (1.01–1.56)	0.042
Stage III, IV (N =72)				
ctDNA detection	0.96 (0.51–1.79)	0.886	1.27 (0.63–2.57)	0.511
VAF (%)	1.02 (1.00–1.03)	0.108	1.02 (0.99–1.04)	0.185

*, adjusted for age, smoking (current vs. former), BMI, FEV_1 % pred, emphysema index of total lung (%), CRP, clinical stage of lung cancer, central location, and small cell histology. In a subgroup analysis by early and advanced stages, clinical stage was not adjusted. BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; ctDNA, circulating tumor DNA; EI, emphysema index; HR, hazard ratio; VAF, variant allele frequency.

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