Clinical utility of combined ctDNA and CTC assays in the diagnosis of primary lung cancer

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Background & Aim

Background

- **Liquid biopsy** : a promising non-invasive test for the detection of malignancy.
- **Advantages:**
  - ➢ Less burdensome than a tissue biopsy
  - ➢ A more comprehensive cross-section of heterogeneous diseases
  - ➢ Insights into the molecular drivers of different primary tumors or metastasis
- **Previous studies using liquid biopsy:**
  - ➢ circulating tumor cells **(CTCs):** sensitivity 84-100%, alteration detected (EGFR, ALK and ROS1)
  - ➢ circulating tumor DNA **(ctDNA):** sensitivity 68-95%, alteration detected (almost EGFR)

Neumann MHD *et al. Comput Struct Biotechnol J* 2018;1(16):190–195

Durendez-Saez E. *et al. J Thorac Dis* 2017;9(Suppl 13):S1332

Aim

- To evaluate diagnostic performance of each and combined assays of ctDNA and CTC compared with traditional tumor markers including CEA, cyfra 21-1 and NSE.

Methods

Study population

- From March 15, 2018 through July 9, 2018
- Sing center, Prospective study

Inclusion criteria

- Patients with histology-proven lung cancer or a clinical suspicion of lung cancer
- Age >=18, < 80 years
- No previous history of cancer treatment within 5 years
- Patients who agree to participate

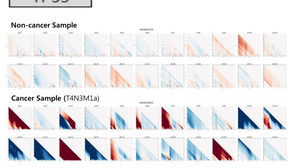
Exclusion criteria

- Patients who have been diagnosed with malignancy within 5 years
- Patients with previous history of lung cancer
- Patients who have uncontrolled coagulopathy

ctDNA

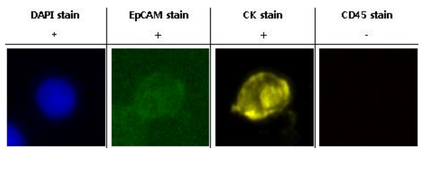
- Plasma ctDNA and PBMC DNA analysis
- Single nucleotide variants and Copy number alterations
- **Panel (EDScan-S)**

AKT1	ALK	AR	BRAF	CTNNB1	DDR2	EGFR	ERBB2	ESR1	FGFR1
FOXL2	GNAS	HRAS	IDH1	IDH2	KIT	KRAS	MAP2K1	MAP2K2	MET
MTOR	NRAS	NTRK1	NTRK3	PDGFRA	PIK3CA	RET	ROS1	SMAD4	TERT
TP53									



CTC

- Immunofluorescence staining : identify **CK or EpCAM-positive** and **CD45-negative** CTCs
- Cut-off: **2 cells or more (positive)**, 0 or 1 cell (negative)
- Smart Biopsy™ Cell Image Analyzer (Cat# CIA030, Cytogen, Inc., Seoul, Korea)



Statistical analysis

TEST \ DISEASE	Positive (+)	Negative (-)
Positive (+)	**A** True positive	**B** False positive
Negative (-)	**C** False negative	**D** True negative

- Sensitivity: **A/A+C**
- Specificity: **D/B+D**
- Positive predictive value (PPV) : **A/A+B**
- Negative predictive value (NPV) : **D/C+D**
- Accuracy: **A+D/ALL**

Results

Figure 1. Study population.

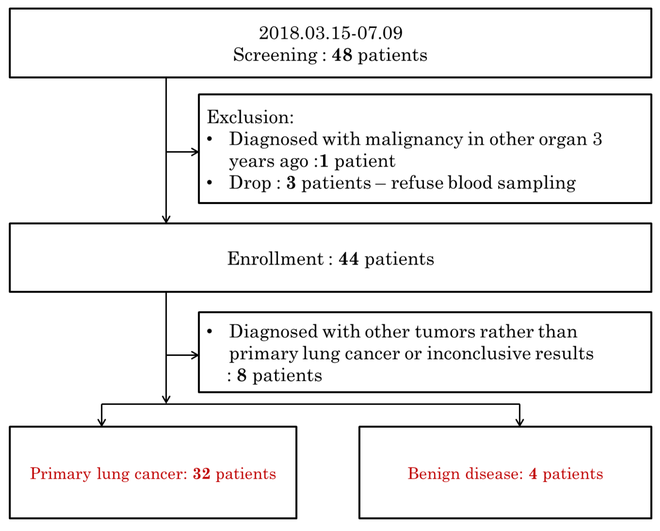


Table 1. Characteristics of the study population.

	Total n=36
Age, years	65 (IQR, 57-70)
Male	23 (64%)
Lung cancer	**32 (89%)**
Adenocarcinoma	22 (69%)
Squamous cell carcinoma	7 (22%)
Large cell neuroendocrine carcinoma	1 (3%)
Other non-small cell lung cancer	1 (3%)
Small cell lung cancer, extensive disease	1 (3%)
Stage, NSCLC	**31**
I	11 (35%)
II	5 (16%)
III	9 (30%)
IV	6 (19%)

IQR: interquartile range

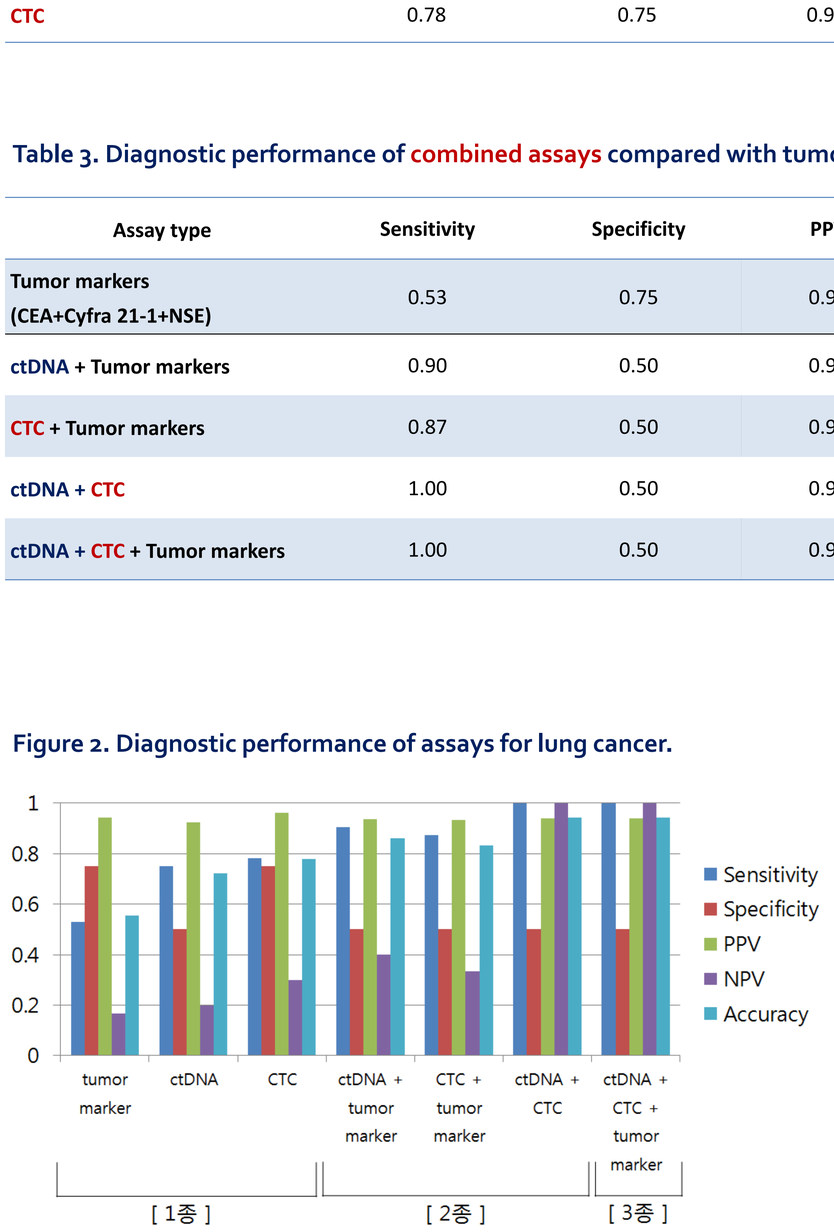
Table 2. Diagnostic performance of the assay compared with tumor markers for lung cancer

Assay type	Sensitivity	Specificity	PPV	NPV	Accuracy
CEA	0.25	1.00	1.00	0.14	0.33
Cyfra 21-1	0.40	1.00	1.00	0.17	0.47
NSE	0.25	0.75	0.88	0.11	0.30
Tumor markers (CEA+Cyfra 21-1+NSE)	0.53	0.75	0.94	0.16	0.55
ctDNA	0.75	0.50	0.92	0.20	0.72
CTC	0.78	0.75	0.96	0.30	0.77

Table 3. Diagnostic performance of combined assays compared with tumor markers for lung cancera

Assay type	Sensitivity	Specificity	PPV	NPV	Accuracy
Tumor markers (CEA+Cyfra 21-1+NSE)	0.53	0.75	0.94	0.16	0.55
ctDNA + Tumor markers	0.90	0.50	0.93	0.40	0.86
CTC + Tumor markers	0.87	0.50	0.93	0.33	0.83
ctDNA + CTC	1.00	0.50	0.94	1.00	0.94
ctDNA + CTC + Tumor markers	1.00	0.50	0.94	1.00	0.94

Figure 2. Diagnostic performance of assays for lung cancer.



Conclusions

- The **sensitivity** for lung cancer was **75%** (**ctDNA**) ,**78%** (**CTC**) and **100%** (**ctDNA + CTC**).
- The **diagnostic accuracy** was **72%** (**ctDNA**), **77%** (**CTC**) and **94%** (**ctDNA + CTC**).
- CTCs and ctDNA likely have **complementry roles** in the diagnosis of lung cancer.
- The combination of ctDNA and CTC assays can enhance the diagnostic sensitivity for the diagnosis of the primary lung cancer. (ClinicalTrials.gov number, NCT03479099.)