Lung Cancer 134 (2019) 158–166

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Clinical implications of circulating cell-free DNA quantification and metabolic tumor burden in advanced non-small cell lung cancer

Myung Han Hyun[a,b,1], Eun Seong Lee[c,d,1], Jae Seon Eo[c,1], Sungeun Kim[c], Eun Joo Kang[a], Jae Sook Sung[b], Yoon Ji Choi[a], Kyong Hwa Park[a], Sang Won Shin[a], Sung Yong Lee[e], Yeul Hong Kim[a,b,*]

[a] Division of Medical Oncology, Department of Internal Medicine, Korea University Medical Center, Korea University College of Medicine, Seoul, Republic of Korea
[b] Cancer Research Institute, Korea University, Seoul, Republic of Korea
[c] Department of Nuclear Medicine, Korea University Medical Center, Korea University College of Medicine, Seoul, Republic of Korea
[d] Department of Molecular Medicine and Biopharmaceutical Science, WCU Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea
[e] Division of Pulmonary, Allergy and Critical Care Medicine, Department of Internal Medicine, Korea University Medical Center, Korea University College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
Non-small-cell lung carcinoma
Cell-free nucleic acids quantification
Metabolic tumor volume
Total lesion glycolysis
Prognosis

ABSTRACT

Objectives: This study unravels the significance of cell-free DNA (cfDNA) quantification as a promising measure of the biological behavior/aggressiveness of tumors. Metabolic tumor volume (MTV) and total lesion glycolysis (TLG) measured by positron emission tomography/computed tomography scan enable a precise assessment of metabolic tumor burden. However, their clinical implications in identifying patients who need more aggressive treatment in advanced non-small cell lung cancer (NSCLC) are not fully understood.

Materials and methods: In the current prospective trial, we analyzed 101 newly diagnosed advanced NSCLC (stage III-IV) patients with measurable baseline MTV, TLG, and cfDNA quantification. The best cut-offs for cfDNA levels, MTV, and TLG to predict progression-free survival and overall survival were determined using X-tile analysis.

Results: There were significant positive correlations between cfDNA and MTV ($r = 0.488$, $p < 0.001$) and between cfDNA and TLG ($r = 0.554$, $p < 0.001$). High-cfDNA levels and high-MTV/TLG negatively correlated with overall survival (OS) (all $p < 0.001$). Patients with high-MTV showed similar median OS irrespective of their cfDNA levels (low-cfDNA vs. high-cfDNA = 9.2 vs 6.6 months; $p > 0.05$). However, patients with low-MTV and low-cfDNA levels showed longer OS than those with low-MTV and high-cfDNA levels (low-cfDNA vs. high-cfDNA = 49.3 vs 11.5 months; $p < 0.001$). The patient group with low-TLG also showed similar trends. The cfDNA level was an independent prognostic factor for OS by Cox-proportional hazard analysis.

Conclusion: Although the patients with high metabolic tumor burden had a poor prognosis, regardless of the biological behavior/aggressiveness of the tumor, patients with low metabolic tumor burden and high cfDNA levels showed a poor prognosis. Taken together, this study indicates a stronger prognostic value of baseline cfDNA levels in identifying patients with advanced NSCLC and personalizing their treatment strategies for better survival.

1. Introduction

Lung cancer is one of the most common malignancies, with 1.3 million cancer-related deaths annually, and a five-year survival rate less than 10% [1]. Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancers and hence requires critical attention to improve clinical outcomes by biomarker identification and developing targeted therapies. Due to insufficient tumor samples, inherent clinical risks to the patients, and restrictions due to invasive biopsy, assessment of cell-free DNA (cfDNA) in plasma or serum called liquid biopsy has emerged as a

* Corresponding author at: Division of Medical Oncology, Department of Internal Medicine, Korea University College of Medicine, 73, Inchon-ro, Seongbuk-gu, Seoul, 02841, Republic of Korea.
E-mail address: yhk0215@korea.ac.kr (Y.H. Kim).
[1] These authors contributed equally to this article.

https://doi.org/10.1016/j.lungcan.2019.06.014
Received 4 March 2019; Received in revised form 10 May 2019; Accepted 12 June 2019

promising non-invasive prognostic marker in cancer [2,3].

Cancer cells shed their circulating cfDNA into the bloodstream through both passive (apoptosis, necrosis, and cell lysis) and active (spontaneous release of DNA fragments) processes [4,5]. In a tumor microenvironment, the level of cfDNA secreted from the tumor and the surrounding tissues corresponds to the degree of biological behavior/aggressiveness of the tumor [6,7]. In line with recent developments in detecting tumor-derived cfDNA, the quantification of cfDNA in blood is plausible as a simple and reliable biomarker with potential diagnostic applications [8,9]. Previous clinical investigations indicated the diagnostic and prognostic value of cfDNA levels in NSCLC patients [10–12]. So far, high cfDNA levels have been associated with increased biological behavior/aggressiveness of the tumor and poor OS [12,13].

Although there is no gold standard for assessing metabolic tumor burden, functional imaging by ^{18}F-fluorodeoxyglucose (FDG) PET/CT is used as a reliable technique to date [14,15]. The PET/CT data were calculated by semi-automated software using pre-existing cut-off values; then, nuclear medicine physicists manually generated a 3D volume of interest (VOI) of each primary site lesion and metastasis. Subsequently, the volume inside the boundary was defined as metabolic tumor volume (MTV). The total lesion glycolysis (TLG) was calculated by multiplying MTV by mean standardized uptake value (SUV) and reflected both tumor volume and metabolic activity. Hence, by measuring MTV and TLG, the metabolic tumor burden was theoretically calculated with accuracy [14,15].

To date, the biological behavior/aggressiveness of tumor as measured by cfDNA levels and metabolic tumor burden measured by PET/CT scan correlate with survival in advanced NSCLC patients [16,17]. However, the significance of the levels of cfDNA and the metabolic tumor burden in identifying patients who need more aggressive treatment is still poorly understood. The objective of this prospective trial was to evaluate these clinical implications to assess prognosis in advanced NSCLC patients.

2. Materials and methods

2.1. Patients selection and study design

This study was performed at the Korea University Anam Hospital and Guro Hospital from June 2010 to June 2016. The inclusion criteria for patients were the pathological diagnosis of NSCLC and computable MTV or TLG levels from the baseline PET/CT scan. Patients who underwent chemotherapy or had a history of any other malignancy were excluded. Of the initial 142 study subjects, forty-one patients were excluded due to non-computable MTV or TLG levels or insufficient cfDNA data. The remaining 101 patients were enrolled. Among all groups present, 81 patients with stage IV adenocarcinoma (ADC) were also analyzed to reduce heterogeneity and possible selection bias. The median interval of measurement between baseline cfDNA quantification and PET/CT scan was 7 days (interquartile [IQR] = 4–14 days). The median follow-up duration was 19.4 months (range, 13.8–30.0).

The study was performed in compliance with the Declaration of Helsinki, following approval from the Institutional Review Board of Korea University Medical Center (ED14110). Informed consent was obtained from all patients, stored in the hospital database, and used for research at each hospital.

2.2. Analysis of cfDNA quantification

The serum samples were harvested in 10-mL serum separator tubes. Following centrifugation for 10 min at 2000 × *g* at 4 °C, the supernatants were transferred and again centrifuged for 5 min at 16,000 × *g* and 4 °C. The resulting serum was frozen at −80 °C until use. The cfDNA was extracted from serum aliquots (500 μL) using the QIAamp circulating nucleic acid kit (Qiagen, Hilden, Germany). The extraction efficiency of cfDNA was measured using an Agilent High Sensitivity DNA kit and the Bioanalyzer 2100 instrument (Agilent Technologies, Santa Clara, CA). Additional purification was performed using Agencourt AMPure XP (BeckMan Coulter, Brea, CA). The isolated DNA was quantified with the Agilent High Sensitivity DNA kit and measured in a Qubit 2.0 Fluorometer.

2.3. Analysis of PET/CT images

All patients underwent combined PET/CT scanning under a routine clinical protocol with Gemini TF/16 channel PET/CT scanners (Philips Medical Systems, Cleveland, OH). After fasting for at least 6 h, the radiotracer (5–6 MBq/kg) FDG was administrated intravenously and scanning was performed after 60 min. Unenhanced CT scans were acquired first for attenuation correction, and then PET scans (1 min per bed) were acquired.

The SUV, MTV, and TLG were extracted using MIRADA XD3 software (MIRADA Medical, Oxford, UK) by drawing a VOI on the PET image [18]. A fixed SUV cut-off value of 2.5 was used for the semi-automated contouring system to exclude physiologic FDG uptake of the non-tumor tissue. The manual correction of the boundaries of the automated VOI was performed by nuclear medicine physicists who were blinded to the cfDNA levels of the subjects. The MTV was defined as the total tumor volume inside the boundaries of the manually corrected VOI. TLG was calculated as the product of MTV and the SUV mean.

2.4. Statistics

The cfDNA quantification data in all the NSCLC and stage IV ADC patients were expressed as median and IQR ranges. The correlation between the cfDNA levels and MTV/TLG was assessed by Spearman's rank correlation test. The clinical outcomes were assessed by progression free survival (PFS) and overall survival (OS). The Kaplan-Meier survival curve for PFS and OS was constructed, and the statistical differences were measured by the log-rank test. The optimal cut-off values for cfDNA quantification, MTV, and TLG were determined using X-tile software. X-tile software is specialized for the analysis of survival data, which is divided into matched training and validation sets (1:1 ratio). Then, X-tile plots show the $\chi 2$ log-rank values with a Kaplan-Meier curve and identify a minimal p-value with histogram. Since the small number of events favored the use of the OS, which is closer to the PFS, the OS cut-off was determined as the optimal cut-off [19]. Multivariate Cox proportional hazard model was used to investigate the hazard ratios (HR) for the impact of cfDNA levels on survival outcomes by adjusted covariates including age, sex, performance status, Charlson comorbidity index, clinical stage, and computable MTV/TLG levels. The statistical analyses were performed using X-tile software v 3.6.1 (Yale University, New Haven, CT), SPSS v 24.0 (SPSS Inc., Chicago, IL), and R software v 3.2.2 (R Core Team, Vienna, Austria).

3. Results

3.1. Baseline characteristics according to patients' cfDNA quantification

This study was conducted according to the guidelines of the REporting recommendations for tumor MARKer prognostic studies (REMARK) (Supplementary Table S1) [20]. The detailed baseline clinicopathological characteristics and PET/CT parameters according to the cfDNA concentration are listed in Table 1. The median cfDNA levels of the entire cohort, stage III, stage IV, and stage IV ADC only patients were 70 ng/mL (IQR = 40–161 ng/mL), 64 ng/mL (IQR = 28–87 ng/mL), 72 ng/mL (IQR = 41–165 ng/mL), and 65 ng/mL (IQR = 38–158 ng/mL), respectively. There was no significant difference in cfDNA level between stage III and IV patients ($p = 0.273$) (data not shown). Overall, the baseline characteristics between patients with low- and high-cfDNA levels were similar (Table 1). However, patients with high-cfDNA levels showed significantly higher MTV and TLG levels

Table 1
Baseline clinicopathologic characteristics and PET/CT parameters in NSCLC patients.

Variables	All NSCLC patients (n = 101)				Stage IV ADC only (n = 81)			
	Total (n = 101)	Low cfDNA quantification† (n = 51)	High cfDNA quantification† (n = 50)	P value	Total (n = 81)	Low cfDNA quantification† (n = 44)	High cfDNA quantification† (n = 37)	P value
Age, years*	66 (58–73)	65 (57–73)	66 (60–72)	0.921	65 (57–74)	65 (56–74)	65 (57–72)	0.868
Male sex, n (%)	64 (63)	33 (65)	31 (62)	0.778	50 (62)	28 (64)	22 (60)	0.700
ECOG status ≤ 1, n (%)	78 (77)	40 (78)	38 (76)		60 (74)	33 (75)	27 (73)	
ECOG status > 1, n (%)	23 (23)	11 (22)	12 (24)	0.771	21 (26)	11 (25)	10 (27)	0.836
CCI ≤ 7, n (%)	18 (18)	8 (16)	10 (20)		9 (11)	3 (7)	6 (16)	
CCI > 7, n (%)	83 (82)	43 (84)	40 (80)	0.571	72 (89)	41 (93)	31 (84)	0.180
Histology type ADC, n (%)	88 (87)	48 (94)	40 (80)		81 (100)	44 (100)	37 (100)	
Histology type Sqcc, n (%)	10 (10)	3 (6)	7 (14)		0 (0)	0 (0)	0 (0)	
Histology type others, n (%)	3 (3)	0 (0)	3 (6)	0.070	0 (0)	0 (0)	0 (0)	N/A
EGFR mutation positive, n (%)	54 (60)	30 (65)	24 (55)		49 (65)	28 (67)	21 (62)	
EGFR mutation negative, n (%)	36 (40)	16 (35)	20 (45)	0.302	27 (35)	14 (33)	13 (38)	0.657
Clinical stage III, n (%)	11 (11)	7 (14)	4 (8)		0 (0)	0 (0)	0 (0)	
Clinical stage IV, n (%)	90 (89)	44 (86)	46 (92)	0.356	81 (100)	44 (100)	37 (100)	N/A
Bone metastasis, n (%)	50 (50)	21 (41)	29 (58)	0.091	46 (57)	21 (48)	25 (67)	0.073
Other metastasis except bone, n (%)	40 (44)	23 (52)	17 (37)	0.144	35 (43)	23 (52)	12 (32)	0.073
PET/CT parameters								
SUVmax, g/mL*	8 (7–11)	8 (6–10)	9 (7–12)	0.169	8 (7–11)	8 (6–11)	8 (7–10)	0.985
MTV, mL*	73 (24–252)	49 (8–123)	105 (50–290)	0.002	73 (21–247)	51 (10–169)	100 (45–273)	0.037
MTV > 100 mL, n (%)	42 (42)	15 (30)	27 (54)	0.012	32 (40)	14 (32)	18 (49)	0.123
TLG, g*	294 (81 – 1071)	169 (35–498)	415 (244–1330)	0.003	268 (77–959)	170 (37–836)	337 (194–1149)	0.064
TLG > 500 g, n (%)	35 (35)	12 (24)	23 (46)	0.018	27 (33)	12 (27)	15 (41)	0.207

PET/CT, positron emission tomography/computed tomography; NSCLC, non-small cell lung cancer; ADC, adenocarcinoma; cfDNA, cell free DNA; ECOG, eastern cooperative oncology group; CCI, Charlson's comorbidity index; Sqcc, squamous cell carcinoma; EGFR, epidermal growth factor receptor; SUV, standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; N/A, not available.

† Optimal cut-off value for cfDNA level (70 ng/mL) was identified using X-tile software analysis (Supplementary Figs. S2) [19].

* Data are expressed as the median, followed by the interquartile range in parentheses.

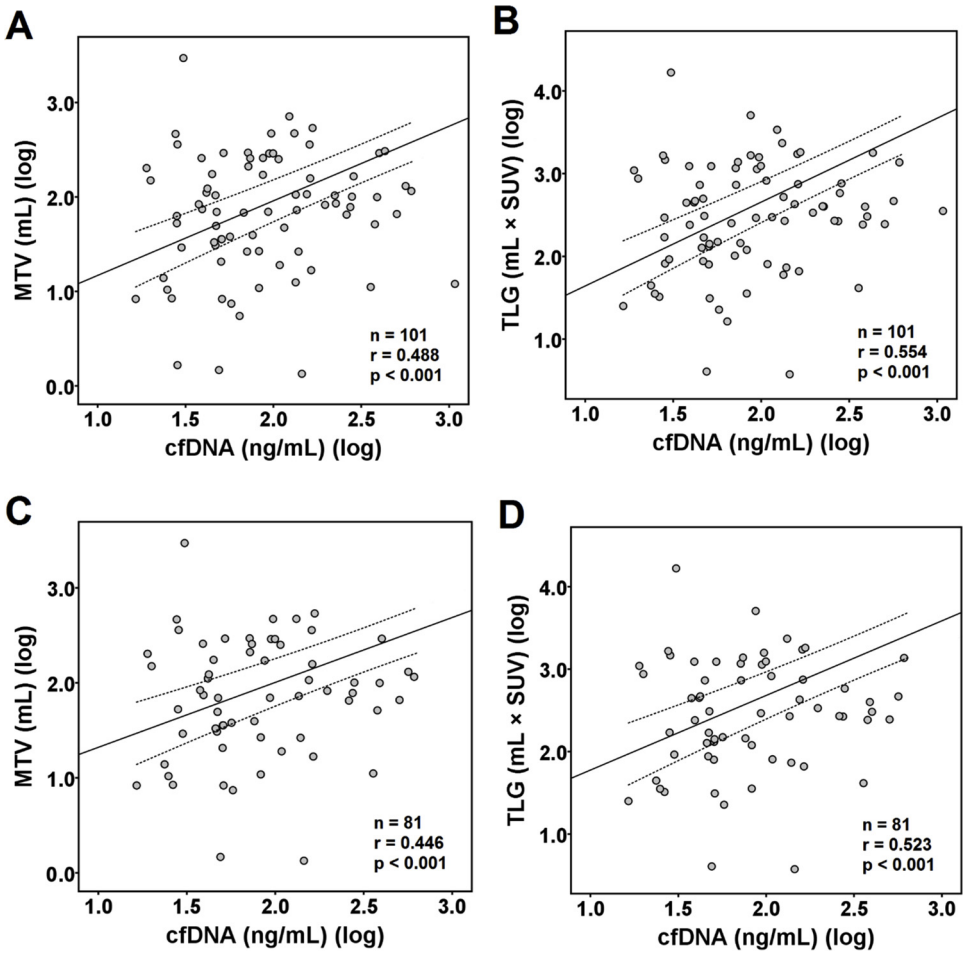

Fig. 1. Scatter plots to demonstrate the relationship between the level of cfDNA and PET parameters like MTV and TLG in NSCLC patients.

Relationship between MTV, TLG, and cfDNA levels. The correlation between cfDNA levels and the MTV and TLG ($r = 0.488$, $p < 0.001$; $r = 0.554$, $p < 0.001$; respectively) in NSCLC patients ([A] and [B] respectively). The correlation between the cfDNA levels and the MTV and TLG ($r = 0.446$, $p < 0.001$; $r = 0.523$, $p < 0.001$; respectively) in stage IV adenocarcinoma patients ([C] and [D] respectively). Solid line indicates the linear regression line and dashed lines represent the 95% confidential intervals.

cfDNA, cell free DNA; PET, positron emission tomography; MTV, metabolic tumor volume; TLG, total lesion glycolysis; NSCLC, non-small cell lung cancer; SUV, standardized uptake value.

compared to those with low-cfDNA levels. The stage IV ADC group exhibited no significant differences in the baseline characteristics or PET/CT parameters, irrespective of their cfDNA levels (Table 1).

3.2. Correlation between cfDNA levels and the metabolic tumor burden

There were significant positive correlations between cfDNA and MTV ($r = 0.488$, $p < 0.001$) and between cfDNA and TLG ($r = 0.554$, $p < 0.001$) (Fig. 1A and B). In the stage IV ADC group, the cfDNA levels showed a significant positive correlation with baseline MTV ($r = 0.446$, $p < 0.001$) and baseline TLG ($r = 0.523$, $p < 0.001$) (Fig. 1C and D). Additionally, MTV and TLG were consistent with radiology ($r = 0.959$, $p < 0.001$) (data not shown)

3.3. Survival analyses of cfDNA quantification and metabolic tumor burden

The median PFS was 7.4 months, and the median OS was 19.9 months, in all NSCLC patients. Stage IV ADC patients showed a median PFS of 8.2 months and a median OS of 19.9 months. The optimal cut-off values for cfDNA concentration, MTV, and TLG levels according to X-tile software analysis were 70 ng/mL, 100 ml, and 500 g, respectively (Supplementary Figs. S1–S3). The median PFS (high-cfDNA vs low-cfDNA; 4.5 vs 14.9 months; $p < 0.001$) and median OS (high-cfDNA vs low-cfDNA; 8.2 vs 49.3 months; $p < 0.001$) of patients with high-cfDNA levels were significantly shorter than those of patients with low-cfDNA levels (Fig. 2A and B). The patients with high-MTV had a shorter median PFS (high-MTV vs low-MTV; 3.8 vs 15.0 months; $p < 0.001$) and median OS (high-MTV vs low-MTV; 7.4 vs 49.3 months; $p < 0.001$) than those with low-MTV (Fig. 2C and D). Likewise, high-TLG correlated with shorter median PFS (high-TLG vs low-TLG; 3.6 vs 14.9 months; $p < 0.001$) and median OS (high-TLG vs low-TLG; 7.4 vs 49.3 months; $p = 0.001$) than low-TLG (Fig. 2E and F). In the subgroup analysis, stage IV ADC patients with high-cfDNA levels also exhibited poor PFS and OS compared to those with low-cfDNA levels (Fig. S4A and B). Additionally, a similar survival pattern was observed in stage IV ADC patients according to MTV and TLG levels (Supplementary Fig. S4C–F).

3.4. Significance of MTV or TLG on prognostic value for cfDNA quantification

Patients with high-MTV showed a similar median PFS (low-cfDNA and high-MTV vs high-cfDNA and high-MTV; 4.0 vs 3.6 months; $p > 0.05$) and median OS (low-cfDNA and high-MTV vs high-cfDNA and high-MTV; 9.2 vs 6.6 months; $p > 0.05$), irrespective of their cfDNA levels. However, patients with low-MTV and low-cfDNA levels showed longer median PFS (low-cfDNA and low-MTV vs high-cfDNA and low-MTV; 16.8 vs 7.1 months; $p < 0.001$) and median OS (low-cfDNA and low-MTV vs high-cfDNA and low-MTV; 49.3 vs 11.5 months; $p < 0.001$) than those with low-MTV and high-cfDNA levels (Fig. 3A and B).

Likewise, NSCLC patients with high-TLG showed a similar median PFS between low and high-cfDNA levels (low-cfDNA and high-TLG vs high-cfDNA and high-TLG; 4.0 vs 3.2 months; $p > 0.05$). However, the median PFS (low-cfDNA and low-TLG vs high-cfDNA and low-TLG; 16.8 vs 5.3 months; $p < 0.001$) and median OS (low-cfDNA and low-TLG vs high-cfDNA and low-TLG; 49.3 vs 10.6 months; $p < 0.001$) of the patients with low-TLG and low-cfDNA levels were longer than those with low-TLG and high-cfDNA levels. The median OS of patients with high-TLG and low-cfDNA levels was slightly longer than those with

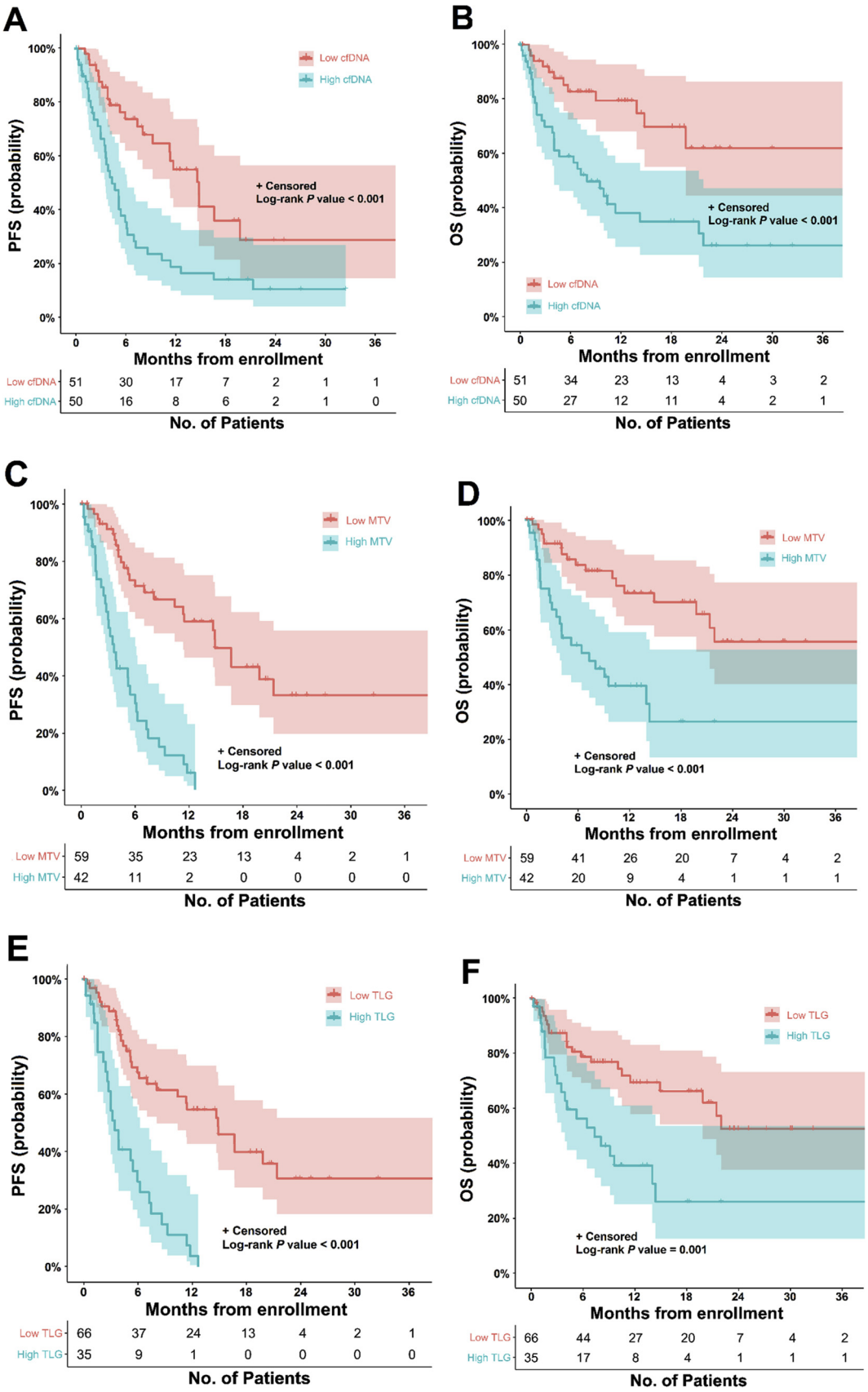

Fig. 2. Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) according to cfDNA quantification, MTV, and TLG levels in NSCLC patients.

PFS and OS according to the baseline cfDNA quantification (≤70 ng/mL vs. > 70 ng/mL) in NSCLC patients ((a) and (b); respectively), baseline MTV level (≤100 cm^3 vs. > 100 cm^3) ((c) PFS, (d) OS, respectively) and TLG level (> 500 g vs. ≤500 g) ((e) PFS, (f) OS, respectively) in NSCLC patients. Optimal cut-off values for cfDNA level (70 ng/mL), MTV level (100 cm3/mL) and TLG level (500 g) were identified using X-tile software analysis (Supplementary Figs. S2–S4) [19].

cfDNA, cell free DNA; MTV, metabolic tumor volume; TLG, total lesion glycolysis; NSCLC, non-small cell lung cancer; PFS, progression free survival; OS, overall

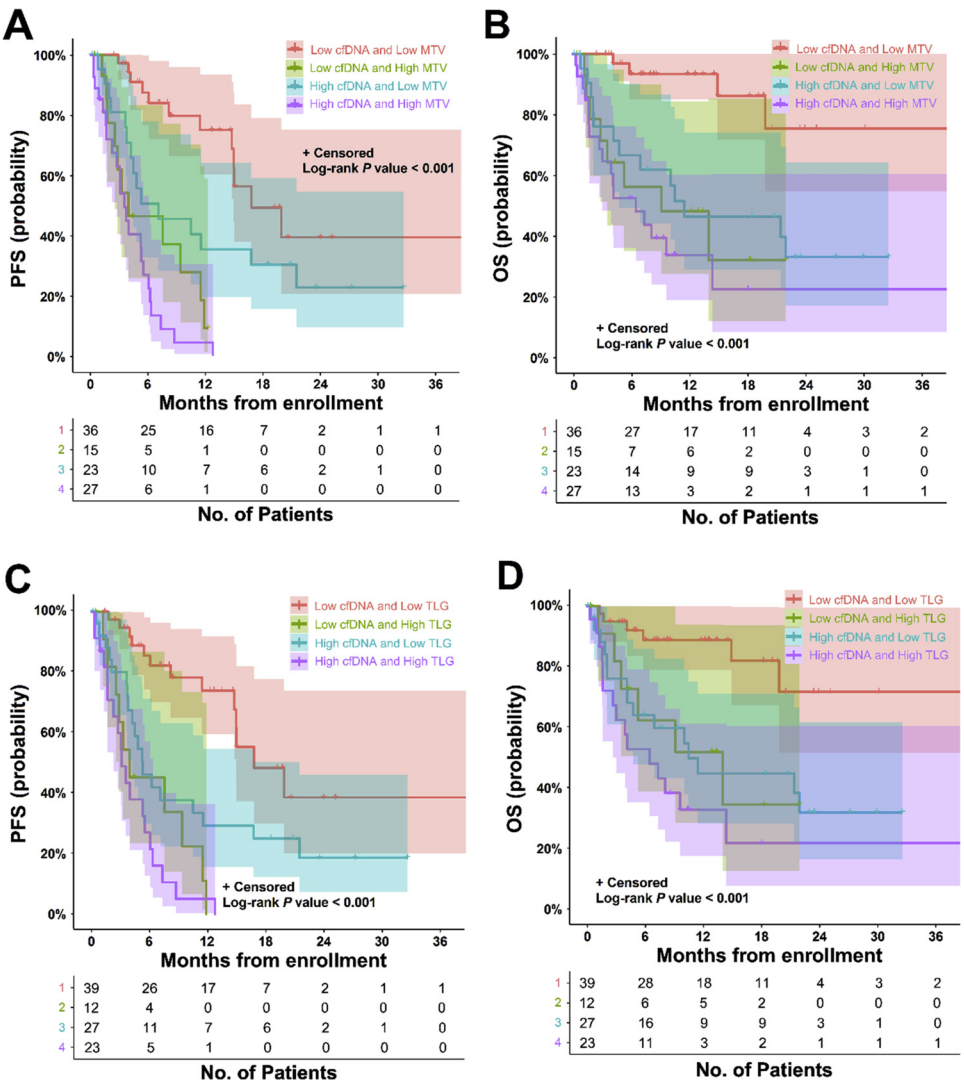

Fig. 3. Kaplan-Meier estimates of PFS and OS according to the cfDNA quantification classified to MTV and TLG levels in NSCLC patients. Kaplan-Meier survival curve according to the baseline cfDNA quantification (≤70 ng/mL vs. > 70 ng/mL) and MTV (≤100 cm^3 vs. > 100 cm^3) ((a) PFS, (b) OS, respectively) and TLG (> 500 g vs. ≤500 g) ((c) PFS, (d) OS, respectively) in NSCLC patients. Optimal cut-off values for cfDNA level (70 ng/mL), MTV level (100 cm3/mL) and TLG level (500 g) were identified using X-tile software analysis (Supplementary Figs. S2–S4) [19].
PFS, progression free survival; OS, overall survival; cfDNA, cell free DNA; MTV, metabolic tumor volume; TLG, total lesion glycolysis; NSCLC, non-small cell lung cancer.

high-TLG and high-cfDNA levels (low-cfDNA and low-TLG vs high-cfDNA and low-TLG; 14.1 vs 6.6 months; p < 0.05) (Fig. 3C and D). Stage IV ADC patients had identical survival patterns (p < 0.001) (Supplementary Fig. S5).

3.5. Univariate and multivariate analyses

A Cox regression analysis of the prognostic factors of OS in NSCLC patients was performed (Table 2). In univariate analysis, the cfDNA levels were significantly associated with poor OS. (All NSCLC, HR = 3.00, p = 0.001; Stage IV ADC only, HR = 2.98, p = 0.003) In both cases, the multivariate Cox regression analysis showed that the HR for OS in the high-cfDNA group increased significantly compared to low-cfDNA (All NSCLC, HR = 3.22, p = 0.001; Stage IV ADC only, HR = 3.61, p = 0.001). After adjusting for MTV and TLG in the multivariate analysis, the HRs for high-cfDNA were similar in both NSCLC and stage IV ADC patients (p < 0.01) (Table 2).

4. Discussion

Since circulating cfDNA is postulated to be released from tumor lysis and necrosis in the tumor microenvironment, measurement of cfDNA levels is a promising method for assessing the biological behavior/aggressiveness of the tumor [21,22]. To date, high cfDNA levels have been associated with poor prognosis in NSCLC patients [8,23,24]. Numerous studies, including the recent meta-analysis (17 studies with 1723 patients), have shown poor survival outcome in patients with high-cfDNA compared to those with low-cfDNA levels [13]. The utilization of MTV and TLG levels from FDG PET/CT scanning offers a precise estimation of the metabolic tumor burden, theoretically [25,26]. Previously, an MTV risk stratification system was reported to have prognostic value independent of the clinical stage and other prognostic variables in NSCLC [27]. However, the clinical importance of the biological behavior/aggressiveness of the tumor as measured by quantifying cfDNA and metabolic tumor burden has not been well established.

In the present study, we observed that the cfDNA levels were correlated with metabolic tumor burden as assessed by MTV and TLG in NSCLC patients. In other words, patients with a higher metabolic tumor burden were likely to have higher cfDNA concentration. To date, only two studies have evaluated the relationship between cfDNA levels and metabolic tumor burden in NSCLC. Nygaard et al. demonstrated the relationship between cfDNA levels and MTV or TLG in 53 stage III-IV NSCLC patients [16]. They reported that the level of cfDNA did not correlate with MTV and TLG levels. Morbelli et al. also reported similar results in 37 stage III-IV NSCLC patients [17]. Quantitative analysis was used in a recent study to show that increased cfDNA correlated with the number of metastatic sites and lesions, and the sum of measurable lesion diameters in 64 NSCLC patients with EGFR mutations [28]. Although both MTV and TLG are concordant with cfDNA levels, their clinical implications on NSCLC prognosis are quite different.

Table 2
Univariate and multivariate analysis of hazard ratios for OS in NSCLC patients.

	Univariate analysis		Multivariate analysis without MTV or TLG		Multivariate analysis including MTV		Multivariate analysis including TLG	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
All NSCLC patients (n = 101)								
cfDNA quantification (> 70 ng/mL)	3.03 (1.58–5.79)	0.001*	3.22 (1.60–6.48)	0.001*	2.61 (1.26–5.39)	0.010*	2.84 (1.37–5.89)	0.005*
Age (> median)	1.52 (0.83–2.78)	0.173	1.08 (0.57–2.04)	0.821	1.03 (0.54–1.95)	0.937	1.09 (0.58–2.07)	0.793
Sex (Male)	1.14 (0.61–2.15)	0.679	1.05 (0.55–1.98)	0.887	0.95 (0.50–1.82)	0.883	1.01 (0.53–1.91)	0.984
ECOG (> 1)	2.33 (1.20–4.52)	0.012*	2.75 (1.33–5.69)	0.006*	2.34 (1.12–4.89)	0.023*	2.40 (1.12–5.14)	0.025*
CCI (> 7)	1.33 (0.59–3.00)	0.495	1.60 (0.57–4.52)	0.377	1.54 (0.55–4.30)	0.653	1.57 (0.56–4.42)	0.390
Histology (ADC)	0.38 (0.17–0.85)	0.018*	0.47 (0.21–1.08)	0.076	0.59 (0.25–1.39)	0.223	0.53 (0.22–1.25)	0.146
Clinical stage (IV)	1.52 (0.54–4.28)	0.426	1.12 (0.30–4.21)	0.871	1.36 (0.36–5.18)	0.512	1.22 (0.32–4.64)	0.769
MTV (> 100 mL)	3.16 (1.71–5.83)	< 0.001*	N/A	N/A	1.95 (0.94–4.06)	0.074	N/A	N/A
TLG (> 500 g)	2.60 (1.43–4.74)	0.002*	N/A	N/A	N/A	N/A	1.47 (0.72–3.00)	0.288
Stage IV ADC only (n = 81)								
cfDNA quantification (> 70 ng/mL)	2.98 (1.45–6.12)	0.003*	3.61 (1.70–7.66)	0.001*	2.96 (1.36–6.41)	0.006*	3.21 (1.47–7.00)	0.003*
Age (> median)	1.29 (0.65–2.53)	0.468	0.88 (0.43–1.83)	0.738	0.86 (0.41–1.79)	0.689	0.90 (0.43–1.87)	0.772
Sex (Male)	1.30 (0.63–2.68)	0.484	1.17 (0.55–2.52)	0.685	1.10 (0.51–2.36)	0.809	1.14 (0.53–2.44)	0.740
ECOG (> 1)	2.19 (1.05–4.56)	0.037*	2.71 (1.22–6.02)	0.015*	2.26 (1.00–5.10)	0.050*	2.35 (1.01–4.46)	0.047*
CCI (> 7)	1.16 (0.40–3.32)	0.788	1.65 (0.54–5.00)	0.379	1.58 (0.53–4.76)	0.412	1.61 (0.54–4.86)	0.395
MTV (> 100 mL)	2.72 (1.36–5.41)	0.004*	N/A	N/A	1.87 (0.88–3.96)	0.101	N/A	N/A
TLG (> 500 g)	2.23 (1.13–4.42)	0.021*	N/A	N/A	N/A	N/A	1.45 (0.68–3.08)	0.334

OS, overall survival; NSCLC, non-small cell lung cancer; MTV, metabolic tumor volume; TLG, total lesion glycolysis; HR, hazard ratio; CI, confidential interval; cfDNA, cell free DNA; ECOG, eastern cooperative oncology group; CCI, Charlson's comorbidity index; ADC, adenocarcinoma; N/A, not available.

Our study indicated poor OS in high-MTV or -TLG patients. Consistently, previous studies have reported that high baseline metabolic tumor parameters were independently associated with survival in NSCLC [29–31]. Chen et al. (105 stage I-IV NSCLC patients) and Zaizen et al. (68 stage III/IV NSCLC patients) reported that high TLG was associated with poor OS [29,30]. Liao et al. also showed that the highest tertile-MTV and -TLG patients had poorer OS than those of the lowest tertile in 169 stage I-IV NSCLC patients [31].

Furthermore, we tried to analyze the prognostic value of cfDNA levels combined with MTV and TLG for NSCLC. As anticipated, we observed poor prognosis for patients with high metabolic tumor burden irrespective of their cfDNA levels. Interestingly, our studies indicated poorer prognosis in patients with high-cfDNA than low-cfDNA in low metabolic tumor burden patients. Hence, monitoring baseline cfDNA levels to predict survival might be crucial for patients with low metabolic tumor burden. Lastly, high-cfDNA levels were independently associated with poor OS in a multivariate cox regression test after adjusting for MTV and TLG. Therefore, baseline metabolic tumor burden is not a significant confounder on the prognostic value of cfDNA levels.

In summary, the results of this study provide initial support for the clinical implications of tumor biological behavior/aggressiveness, measured by cfDNA quantification and metabolic tumor burden according to MTV/TLG using FDG-PET/CT scans, for the survival of patients with advanced NSCLC. We recommend that metabolic tumor burden should be initially considered for the therapeutic strategy for advanced NSCLC patients. High-MTV or high-TLG were associated with poor prognosis, and these patients would need more aggressive treatments, regardless of the biological behavior/aggressiveness of the tumor. The next consideration is tumor biologic aggressiveness/behavior. We identified that low-cfDNA and low-MTV or low-TLG showed a better prognosis than low-cfDNA and high-MTV or high-TLG. (Fig. 4) Therefore, our results suggest that monitoring cfDNA quantification as an early, accessible biomarker to identify patients with low metabolic tumor burden and who need more aggressive treatment strategies may be effective in informing treatment regimens.

This study has several limitations. First, FDG uptake in a tumor lesion by PET/CT can be biased since small lesions with low activity might be excluded. The inflammatory lesions or non-neoplastic conditions could also be included by chance, which might lead to overestimation of the tumor volume. Therefore, we carefully defined cut-off values for neoplastic conditions during MTV/TLG calculation by using patients' radiologic data (other than PET/CT) and patients' clinicopathologic information. In addition, we quantified the MTV and TLG by visually estimating the hypermetabolic foci and confirmed the results with two independent specialists who were blinded to the cfDNA data.

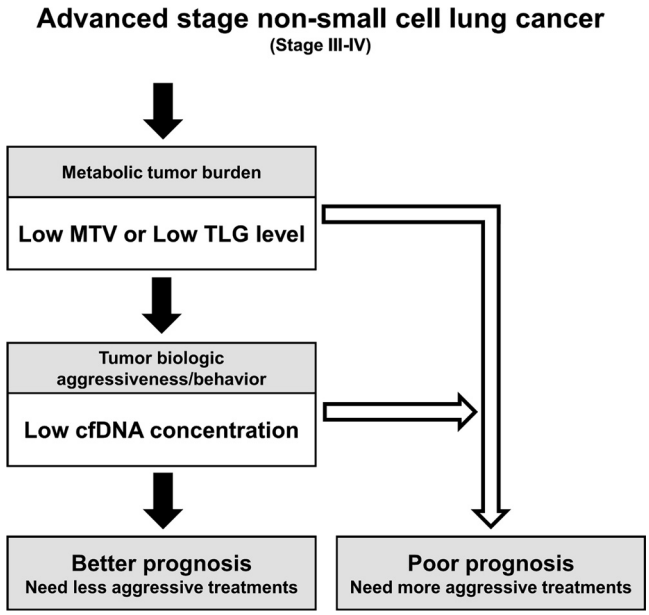

Fig. 4. Therapeutic strategy for advanced stage non-small cell lung cancer according to metabolic tumor burden and tumor biologic aggressiveness/behavior.
Black arrows designate positive results, whereas white arrows designate negative results.
MTV, metabolic tumor volume; TLG, total lesion glycolysis; cfDNA, cell free DNA.

Secondly, serum was used to isolate cfDNA rather than plasma. Compared to plasma, cfDNA isolated from serum tends to be at a higher

concentration because of the extra release of DNA during the coagulation process [32]. cfDNA from serum has superior correlation and concordance rates according to various quantification methods [33]. In addition, we analyzed total cfDNA concentration rather than investigating allelic frequencies of NSCLC oncogenic drivers. However, to reduce the bias introduced by interfering factors, we used stringent purification methods to minimize the contamination from serum samples and to obtain high quality cfDNA. Thirdly, only Korean patients were analyzed with the retrospective study design, and hence, ethnic differences might alter the results. Larger prospective trials including various races are needed for further validation.

In conclusion, cfDNA levels might serve as a prognostic factor that reflects the biological behavior/aggressiveness of the tumor in advanced NSCLC patients. Since patients with low metabolic tumor burden had a different prognosis according to cfDNA levels, monitoring their baseline cfDNA levels and metabolic tumor burden might help to identify patients who need aggressive treatment strategies.

Author contributions

Conception/design: Myung Han Hyun, Eun Seong Lee, Jae Seon Eo, Yeul Hong Kim.

Provision of study materials or patients: Myung Han Hyun, Eun Seong Lee, Jae Seon Eo, Yeul Hong Kim.

Providing study materials or patients: Eun Joo Kang, Yoon Ji Choi, Kyong Hwa Park, Sang Won Shin, Sung Yong Lee, Yeul Hong Kim.

Collection and/or assembly of data: Jae Seon Eo, Sungeun Kim, Eun Joo Kang, Jae Sook Sung, Yoon Ji Choi, Kyong Hwa Park, Sang Won Shin, Sung Yong Lee.

Data analysis and interpretation: Myung Han Hyun, Eun Seong Lee, Jae Seon Eo, Yeul Hong Kim.

Manuscript writing: Myung Han Hyun, Eun Seong Lee, Jae Seon Eo, Eun Joo Kang, Yoon Ji Choi, Kyong Hwa Park, Sang Won Shin, Seong Yong Lee, Yeul Hong Kim.

Final approval of manuscript: Myung Han Hyun, Eun Seong Lee, Jae Seon Eo, Sungeun Kim, Eun Joo Kang, Jae Sook Sung, Yoon Ji Choi, Kyong Hwa Park, Sang Won Shin, Sung Yong Lee, Yeul Hong Kim.

Source of funding and support

This research was supported in part by the Korea Health Technology R&D Project of the Korea Health Industry Development Institute, Republic of Korea (HI14C0066; to Y. H. Kim); the Bio & Medical Technology Development Program of the National Research Foundation, Republic of Korea (NRF-2015M3A9D7031070; to Y. H. Kim); and Korea University Grant (K1813121; to J. S. Eo).

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.06.014.

References

[1] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (1) (2013) 11–30.
[2] H. Akca, A. Demiray, A. Yaren, F. Bir, A. Koseler, R. Iwakawa, G. Bagci, J. Yokota, Utility of serum DNA and pyrosequencing for the detection of EGFR mutations in non-small cell lung cancer, Cancer Genet. 206 (3) (2013) 73–80.
[3] G. Sozzi, D. Conte, L. Mariani, S. Lo Vullo, L. Roz, C. Lombardo, M.A. Pierotti, L. Tavecchio, Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients, Cancer Res. 61 (12) (2001) 4675–4678.
[4] P.B. Gahan, R. Swaminathan, Circulating nucleic acids in plasma and serum. Recent developments, Ann. N. Y. Acad. Sci. 1137 (2008) 1–6.
[5] J.C. Rogers, D. Boldt, S. Kornfeld, A. Skinner, C.R. Valeri, Excretion of deoxyribonucleic acid by lymphocytes stimulated with phytohemagglutinin or antigen, Proc. Natl. Acad. Sci. U. S. A. 69 (7) (1972) 1685–1689.
[6] S. Jahr, H. Hentze, S. Englisch, D. Hardt, F.O. Fackelmayer, R.D. Hesch, R. Knippers, DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells, Cancer Res. 61 (4) (2001) 1659–1665.
[7] H. Schwarzenbach, C. Alix-Panabieres, I. Muller, N. Letang, J.P. Vendrell, X. Rebillard, K. Pantel, Cell-free tumor DNA in blood plasma as a marker for circulating tumor cells in prostate cancer, Clin. Cancer Res. 15 (3) (2009) 1032–1038.
[8] O. Gautschi, C. Bigosch, B. Huegli, M. Jermann, A. Marx, E. Chasse, D. Ratschiller, W. Weder, M. Joerger, D.C. Betticher, R.A. Stahel, A. Ziegler, Circulating deoxyribonucleic acid as prognostic marker in non-small-cell lung cancer patients undergoing chemotherapy, J. Clin. Oncol. 22 (20) (2004) 4157–4164.
[9] L. Sorber, K. Zwaenepoel, V. Deschoolmeester, P.E. Van Schil, J. Van Meerbeeck, F. Lardon, C. Rolfo, P. Pauwels, Circulating cell-free nucleic acids and platelets as a liquid biopsy in the provision of personalized therapy for lung cancer patients, Lung Cancer 107 (2017) 100–107.
[10] C. Tissot, A.C. Toffart, S. Villar, P.J. Souquet, P. Merle, D. Moro-Sibilot, M. Perol, J. Zavadil, C. Brambilla, M. Olivier, S. Couraud, Circulating free DNA concentration is an independent prognostic biomarker in lung cancer, Eur. Respir. J. 46 (6) (2015) 1773–1780.
[11] H. Kimura, K. Kasahara, M. Kawaishi, H. Kunitoh, T. Tamura, B. Holloway, K. Nishio, Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer, Clin. Cancer Res. 12 (13) (2006) 3915–3921.
[12] R. Sirera, R.M. Bremnes, A. Cabrera, E. Jantus-Lewintre, E. Sanmartin, A. Blasco, N. Del Pozo, R. Rosell, R. Guijarro, J. Galbis, J.J. Sanchez, C. Camps, Circulating DNA is a useful prognostic factor in patients with advanced non-small cell lung cancer, J. Thorac. Oncol. 6 (2) (2011) 286–290.
[13] S. Cargnin, P.L. Canonico, A.A. Genazzani, S. Terrazzino, Quantitative analysis of circulating cell-free DNA for correlation with lung cancer survival: a systematic review and meta-analysis, J. Thorac. Oncol. 12 (1) (2017) 43–53.
[14] J.G. Bazan, F. Duan, B.S. Snyder, D. Horng, E.E. Graves, B.A. Siegel, M. Machtay, B.W. Loo Jr., Metabolic tumor volume predicts overall survival and local control in patients with stage III non-small cell lung cancer treated in ACRIN 6668/RTOG 0235, Eur. J. Nucl. Med. Mol. Imaging 44 (1) (2017) 17–24.
[15] C.C. Vu, R. Matthews, B. Kim, D. Franceschi, T.V. Bilfinger, W.H. Moore, Prognostic value of metabolic tumor volume and total lesion glycolysis from (1)(8)F-FDG PET/CT in patients undergoing stereotactic body radiation therapy for stage I non-small-cell lung cancer, Nucl. Med. Commun. 34 (10) (2013) 959–963.
[16] A.D. Nygaard, P.C. Holdgaard, K.L. Spindler, N. Pallisgaard, A. Jakobsen, The correlation between cell-free DNA and tumour burden was estimated by PET/CT in patients with advanced NSCLC, Br. J. Cancer 110 (2) (2014) 363–368.
[17] S. Morbelli, A. Alama, G. Ferrarazzo, S. Coco, C. Genova, E. Rijavec, F. Bongioanni, F. Biello, M.G. Dal Bello, G. Barletta, M. Massollo, I. Vanni, R. Piva, A. Nieri, M. Bauckneht, G. Sambuceti, F. Grossi, Circulating tumor DNA reflects tumor metabolism rather than tumor burden in chemotherapy-naive patients with advanced non-small cell lung cancer (NSCLC): an 18F-FDG PET/CT study, J. Nucl. Med. 58 (November (11)) (2017) 1764–1769.
[18] J.H. Hong, H.H. Kim, E.J. Han, J.H. Byun, H.S. Jang, E.K. Choi, J.H. Kang, R. Yoo Ie, Total lesion glycolysis using (1)(8)F-FDG PET/CT as a prognostic factor for locally advanced esophageal cancer, J. Korean Med. Sci. 31 (1) (2016) 39–46.
[19] R.L. Camp, M. Dolled-Filhart, D.L. Rimm, X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization, Clin. Cancer Res. 10 (21) (2004) 7252–7259.
[20] L.M. McShane, D.G. Altman, W. Sauerbrei, S.E. Taube, M. Gion, G.M. Clark, N.C.I.E.W.G.o.C.D. Statistics Subcommittee of the, Reporting recommendations for tumor marker prognostic studies (REMARK), J. Natl. Cancer Inst. 97 (16) (2005) 1180–1184.
[21] H. Schwarzenbach, D.S. Hoon, K. Pantel, Cell-free nucleic acids as biomarkers in cancer patients, Nat. Rev. Cancer 11 (6) (2011) 426–437.
[22] M. van der Vaart, P.J. Pretorius, The origin of circulating free DNA, Clin. Chem. 53 (12) (2007) 2215.
[23] Y.J. Lee, K.A. Yoon, J.Y. Han, H.T. Kim, T. Yun, G.K. Lee, H.Y. Kim, J.S. Lee, Circulating cell-free DNA in plasma of never smokers with advanced lung adenocarcinoma receiving gefitinib or standard chemotherapy as first-line therapy, Clin. Cancer Res. 17 (15) (2011) 5179–5187.
[24] B.T. Li, A. Drilon, M.L. Johnson, M. Hsu, C.S. Sima, C. McGinn, H. Sugita, M.G. Kris, C.G. Azzoli, A prospective study of total plasma cell-free DNA as a predictive biomarker for response to systemic therapy in patients with advanced non-small-cell lung cancers, Ann. Oncol. 27 (1) (2016) 154–159.
[25] W. Huang, M. Fan, B. Liu, Z. Fu, T. Zhou, Z. Zhang, H. Gong, B. Li, Value of metabolic tumor volume on repeated 18F-FDG PET/CT for early prediction of survival in locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy, J. Nucl. Med. 55 (10) (2014) 1584–1590.
[26] E. Kurtipek, M. Cayci, N. Duzgun, H. Esme, Y. Terzi, S. Bakdik, M.S. Aygun, Y. Unlu, C. Burnik, T.T. Bekci, (18)F-FDG PET/CT mean SUV and metabolic tumor volume for mean survival time in non-small cell lung cancer, Clin. Nucl. Med. 40 (6) (2015) 459–463.
[27] Y. Pu, J.X. Zhang, H. Liu, D. Appelbaum, J. Meng, B.C. Penney, Developing and validating a novel metabolic tumor volume risk stratification system for supplementing non-small cell lung cancer staging, Eur. J. Nucl. Med. Mol. Imaging 45 (November (12)) (2018) 2079–2092.
[28] Y.J. Zhu, H.B. Zhang, Y.H. Liu, F.L. Zhang, Y.Z. Zhu, Y. Li, J.P. Bai, L.R. Liu, Y.C. Qu, X. Qu, X. Chen, Y. Li, G.J. Zheng, Quantitative cell-free circulating EGFR mutation

concentration is correlated with tumor burden in advanced NSCLC patients, Lung Cancer 109 (2017) 124–127.
[29] H.H. Chen, N.T. Chiu, W.C. Su, H.R. Guo, B.F. Lee, Prognostic value of whole-body total lesion glycolysis at pretreatment FDG PET/CT in non-small cell lung cancer, Radiology 264 (2) (2012) 559–566.
[30] Y. Zaizen, K. Azuma, S. Kurata, E. Sadashima, S. Hattori, T. Sasada, Y. Imamura, H. Kaida, A. Kawahara, T. Kinoshita, M. Ishibashi, T. Hoshino, Prognostic significance of total lesion glycolysis in patients with advanced non-small cell lung cancer receiving chemotherapy, Eur. J. Radiol. 81 (12) (2012) 4179–4184.
[31] S. Liao, B.C. Penney, K. Wroblewski, H. Zhang, C.A. Simon, R. Kampalath, M.C. Shih, N. Shimada, S. Chen, R. Salgia, D.E. Appelbaum, K. Suzuki, C.T. Chen, Y. Pu, Prognostic value of metabolic tumor burden on 18F-FDG PET in nonsurgical patients with non-small cell lung cancer, Eur. J. Nucl. Med. Mol. Imaging 39 (1) (2012) 27–38.
[32] S. Holdenrieder, P. Stieber, H. Bodenmuller, G. Fertig, H. Furst, N. Schmeller, M. Untch, D. Seidel, Nucleosomes in serum as a marker for cell death, Clin. Chem. Lab. Med. 39 (7) (2001) 596–605.
[33] S. Holdenrieder, P. Stieber, L.Y. Chan, S. Geiger, A. Kremer, D. Nagel, Y.M. Lo, Cell-free DNA in serum and plasma: comparison of ELISA and quantitative PCR, Clin. Chem 51 (8) (2005) 1544–1546.