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Combination of Circulating Tumour DNA and ^{18}F-FDG PET/CT for Precision Monitoring of Therapy Response in Patients With Advanced Non-small Cell Lung Cancer: A Prospective Study

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Abstract. *Background/Aim: Circulating tumour DNA (ctDNA) represents an emerging biomarker in non-small cell lung cancer (NSCLC). We focused on the combination of ctDNA and positron emission tomography/computed tomography (PET/CT) in the follow-up monitoring of advanced-stage NSCLC patients treated with chemotherapy. Patients and Methods: Eighty-four patients were enrolled in this study. ^{18}F-fluorodeoxyglucose PET/CT and ctDNA assessments were performed at baseline and after two cycles of chemotherapy (follow-up). Results: There was a correlation of ctDNA with metabolic tumour volume (MTV), total lesion glycolysis (TLG), and iodine concentration (IC) at baseline (p=0.001, p=0.001, p=0.003) and at follow-up (p=0.006, p=0.002, p=0.001). The objective response was associated with follow-up ctDNA (p<0.001) and the change of all PET/CT parameters. ROC analyses showed that the combination of follow-up ctDNA with changes in SUVmax is very promising for the estimation of objective response and progression-free survival. Conclusion: The combination of ctDNA assessment with PET/CT is a promising approach for the follow-up monitoring of therapy response and prognosis estimation of advanced-stage NSCLC patients.*

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Key Words: Circulating tumour DNA, PET/CT, therapy response, non-small cell lung cancer, liquid biopsy.

Lung cancer is one of the leading cancer-related causes of morbidity and mortality worldwide (1, 2), with non-small cell lung cancer (NSCLC) being the most common histological type, representing more than 80% of all cases (3). The management of locally advanced or metastatic NSCLC has been markedly changing in recent years with the introduction of targeted therapies and immune checkpoint inhibitors leading to significant improvements in patient survival. The establishment of personalised medicine based on molecular biomarkers has also brought a significant progress. Despite great advances in the field of therapeutic strategies, it is apparent that further progress is needed in the development of novel diagnostic tools enabling precision monitoring or prediction of therapy response. Notably a precision assessment of early therapy response could enable modification of the treatment strategy to prevent patients from staying on an ineffective therapy.

Imaging methods play a key role in the clinical decision-making in patients with advanced stage NSCLC. The objective therapy response is routinely assessed by measurement of changes in tumour size using computed tomography (CT), which is the gold standard imaging method in the field. Response Evaluation Criteria in Solid Tumours (RECIST) based on CT assessment are well established due to their simplicity and high reproducibility (4). However, the measurement of the size of tumour lesions is itself considerably limited, particularly in the evaluation of early treatment response, and it seems that functional imaging methods revealing tumour vascularisation or metabolic activity can be more useful for this purpose. Positron emission tomography (PET) with 18-F fluorodeoxyglucose (^{18}F-FDG) coupled with CT (PET/CT) has emerged as a promising tool for assessing early treatment response as well as for its prediction in multiple cancers including NSCLC (5-7). Although the development of hybrid imaging techniques represented by PET/CT is making indisputable progress, several shortcomings remain due to the limited resolution and specificity of these examinations. Thus, additional non-invasive methods allowing for more accurate early treatment response assessments are needed. In parallel with imaging methods, molecular biology has also reached great progress in NSCLC, notably the concept of liquid biopsy. One major tool of liquid biopsy is the assessment of circulating tumour DNA (ctDNA), which is a small fraction of total circulating free DNA (cfDNA) that contains tumour-specific aberrations such as somatic DNA mutations, methylations, *etc*. (8). Recently, ctDNA monitoring has been applied as an effective non-invasive blood-based biomarker providing prognostic and predictive information in patients with advanced NSCLC treated with systemic therapy (9-11).

The aim of this prospective study was to evaluate the role of combination of ctDNA analysis with ^{18}F-FDG PET/CT in the monitoring of therapy response in patients with advanced NSCLC treated with first-line chemotherapy. At first, we focused on correlation between ctDNA levels and PET/CT parameters and their dynamics during the course of therapy, and second, we focused on their association with objective response and survival of patients.

Patients and Methods

Study design and treatment. This prospective single-centre observational study enrolled patients with newly diagnosed cytologically or histologically confirmed locally-advanced (III) or metastatic (IV) stage NSCLC treated with first-line chemotherapy between 2017 and 2021. A detailed entry clinical examination included ^{18}F-FDG PET/CT using single-source dual energy (DE) CT scan. Tumour tissue was assessed for the presence of tumour-specific somatic mutations using a preselected panel of the most commonly mutated genes in NSCLC (see below). All the patients were treated according to the current clinical guidelines using first-line chemotherapy regimens including: carboplatin plus paclitaxel plus bevacizumab, cisplatin or carboplatin plus pemetrexed, carboplatin plus docetaxel plus bevacizumab and cisplatin plus vinorelbine. Chemotherapy was administered intravenously at the standard approved doses. Clinical follow-up controls including physical examination, plain chest X-ray and routine laboratory tests were performed every 3 weeks. Early follow-up 18-FDG PET/CT was performed after 2 cycles of chemotherapy (*i.e*., 6 weeks after administration of the first cycle) and then CT was done after 2-3 cycles. The objective tumour response was assessed using RECIST criteria in terms of complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD) (4). Blood samples for the assessment of ctDNA were collected before the initiation of systemic therapy (termed as "Baseline ctDNA" or "P1" in the text) and 6 weeks after the first cycle of chemotherapy (termed as "Follow-up ctDNA" or "P2" in the text).

Informed consent was obtained from all the participants. The study protocol and the form of Informed consent for participants were approved by the Ethical Committee of the Faculty of Medicine and University Hospital in Pilsen, Charles University on 13th June 2016 and complied with the International Ethical Guidelines for Biomedical Research, the Declaration of Helsinki, and local laws.

^{18}F-FDG PET/CT examination. All examinations were performed on a clinical PET/CT scanner with integrated 128-row CT and 4-ring PET subsystem (Biograph mCT 128; Siemens Healthcare, Knoxville, TX, USA). The examination was initiated with a standard whole body CT scan after intravenous administration of iodine contrast agent (100 ml). The subsequent DE scan was performed in the extent of the thorax using a prototype scanning protocol consisting of two separate scans with different fixed tube voltage (140 kV and 70 kV). The PET scan was initiated 60-70 minutes after administration of ^{18}F-FDG (activity of 2.5 MBq/kg). PET dataset with attenuation correction using single-energy scan was reconstructed for subsequent analysis (8). PET dataset was analysed in consensus with two experienced radiologists (11 and 9 years' experience with hybrid imaging) using dedicated software application. Various standard parameters of FDG uptake in standard uptake values (SUV) were acquired: maximum SUV (SUVmax), peak SUV (SUVpeak, highest average 1 cm^3 equivalent), mean SUV (SUVmean). Volume metabolic parameters were calculated: metabolic tumour volume (MTV) describing real volume (ml) of the tumour tissue with defined metabolic uptake and derived parameter of total lesion glycolysis (TLG) calculated as MTV × SUVmean. Diameters in orthogonal projections and volumes were calculated in segmented lesions. DE-CT datasets were analysed using dedicated prototype software eXamine (Siemens Healthineers, Erlangen, Germany) in consensus with the same experienced radiologists. Tumours were segmented using a semiautomatic algorithm with the possibility of manual corrections of peripheral borders. The value of total iodine uptake (IU; mg) and iodine concentration related to tumour volume (IC; mg/ml) were acquired for all tumours (8).

Tumour DNA and plasma-based ctDNA extraction and mutation analyses. Tumour biopsy specimens obtained during bronchoscopy or transthoracic biopsy (cytological or formalin-fixed paraffin-embedded tissue samples) were tested for the presence of tumour-specific somatic mutations using a preselected panel of the most commonly mutated genes in NSCLC including *KRAS, TP53, EGFR,*

Table I. *Characteristics of the mutation panel.*

Gene	Exon number	Target codons	Size of PCR product [bp]	LOD [%]	DCE separation temperature [˚C]
EGFR	19	746-753	169	na	52
KRAS	2	12, 13	112	0.03	50
TP53	5	170-187	107	0.1	58
	6	187-224	169	0.5	52
	7	225-261	160	0.5	52
	8	262-307	151	0.03	56
PIK3CA	9	542	106	0.2	48
BRAF	15	600	230	0.05	48

bp: Base pair; DCE: denaturing capillary electrophoresis; LOD: limit of detection; na: not analysed.

BRAF, and *PIK3CA*. Tumour DNA was isolated from all available samples using the commercial column-based kit GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich, St. Louis, MO, USA) according to the instructions of the manufacturer for the respective tissue material. Mutation analysis based on PCR amplification of gene fragments followed by heteroduplex formation and their separation and detection by denaturing capillary electrophoresis (DCE) was performed as described previously (12-14). Details of the tested mutations are summarised in Table I. Selected tumour DNA without any detected mutation meeting the sufficient amount and concentration requirements was analysed in more detail by NGS [ArcherDx VariantPlex Solid Tumour panel on the Illumina platform (ArcherDx, Boulder, CO, USA)].

Whole blood samples were collected in stabilisation blood collection tubes (Carolina Biosystems, Prague, Czech Republic). The plasma fraction was obtained by a two-step centrifugation of the whole blood within 6-54 h after collection, and then immediately frozen at –20˚C. The assessment of Baseline ctDNA and Follow-up ctDNA was performed in patients with confirmed tumour-specific somatic mutation. CtDNA was extracted from plasma using the commercial column-based kit QIAamp Circulating Nucleic Acid Kit (Qiagen, Dusseldorf, Germany) according to the instructions of the manufacturer. Mutations in plasmatic ctDNA were detected by the abov ementioned PCR/DCE-based heteroduplex method (heteroduplex analysis after amplification of the mutated tumour-specific gene fragment). The peaks on the DCE electropherogram - homoduplex from wild-type DNA fragments (homoWT), homoduplex from mutated DNA fragments (homoMUT) and two heteroduplexes formed by one wild type and one mutated DNA fragment (hetA and hetB) – were visualised by GeneMarker software (SoftGenetics, LLC, State College, PA, USA). The equation: (homoMUT+(hetA/2)+(hetB/2))/(homoWT+hetA+hetB) ×100) was used to calculate the mutant allele frequency (MAF). The lowest MAF that can be detected and is distinguishable from background or negative control (limit of detection, LOD) was determined for each marker tested in plasma (about 0.1% depending on the mutation being detected, data not shown). CtDNA clearance was defined as undetectable ctDNA levels in the plasma during the course of systemic therapy.

Statistics. Standard frequency tables and descriptive statistics were used to characterize the patient samples. For the analysis of correlations between the dynamics of PET/CT variables and the dynamics of ctDNA levels, the dynamics of both were expressed by simple absolute differences (early follow-up - before treatment for PET/CT, P2-P1 for ctDNA, both denoted by "Δ"). For the analyses of their association with treatment response and survival, the dynamics of PET/CT variables were expressed as relative change in percent [(early follow-up - before treatment)/before treatment, denoted by "Δ… (%)"]. The RECIST treatment response was analysed as an ordinal variable with levels set as CR+PR<SD<PD. Kendall's tau non-parametric correlation was used to assess the associations among the continuous (PET/CT variables, ctDNA levels) or ordinal (treatment response) variables.

For the survival analysis, progression-free survival (PFS) was determined from the initiation of the therapy to the date of disease progression or exitus. Overall survival (OS) was determined from the initiation of the therapy to the date of exitus. Patients who had not reached the PFS/OS endpoint were censored at the date of the last follow-up. The association of quantitative variables (PET/CT variables, ctDNA levels) with OS and PFS was explored using univariable Cox proportional hazards model. Cox hazard rates (HRs) of relative changes are stated for 100% change of the variable. All of the quantitative variables were tested both as measured and after Box–Cox transformation to compensate for possibly non-normal distributions (the respective p-value being denoted p_{BC}). In order to visualise these associations with Kaplan–Meier plots, a threshold value was determined for each prognostic variable and the patients were stratified into two groups accordingly. Each threshold was found through an automated optimisation process implemented in Matlab (2021a, MathWorks Inc., Natick, MA, USA), in which the threshold value producing the smallest Cox–Mantel p-value was determined and selected. The associations of categorical variables with PFS/OS were tested by the Kaplan–Meier method with Gehan–Wilcoxon test.

The ability of PET/CT dynamics to predict objective therapy response and PFS was investigated by extending the traditional two-class receiver operating characteristic (ROC) analysis to three classes (CR+PR, SD and PD; PFS of >6 months, 3-6 months and 0-3 months) according to Nakas and Yiannoutsos (15). Each pair of classification thresholds (one for distinguishing between the first and second class, and one between the second and third class) represents a point on the ROC surface, whose coordinates are determined by the proportions of truly positively classified cases from individual classes. The further the surface arches away from the origin, *i.e.*, the greater the volume under the surface (VUS), the better the

discriminative ability of the variable is. A VUS of 1/6 (=0.167) corresponds to random guessing (contrasting with the traditional 2D ROC area under curve, where the analogous value is 0.5), while a VUS of 1 represents perfect classification. The curves in which the ROC surface intersects the zero planes are the common 2D ROC curves for binary classification into the respective category pairs. The same ROC techniques were also used to construct and optimize predictive models combining the ΔSUVmax (%) with P2 – ctDNA levels to predict treatment response and PFS (the two variables were only slightly correlated with Kendall's τ=0.229). Each of the two independent models defined a new predictive variable as a linear combination of the two standardised (*i.e.*, normalised) input variables. The linear combination coefficients were optimised to maximise the VUS in the ROC analysis. The optimization was carried out using an enumerative exploration of the whole possible range of rotation angles of the combined factor with respect to the orthogonal coordinate system defined by the input variables. All ROC-related analyses were performed using in-house written functions in MATLAB (2021a; MathWorks Inc., Natick, MA, USA).

All reported *p*-values are two-tailed and the level of statistical significance was set at α=0.05. Statistical processing and testing were performed using STATISTICA (Version 12; StatSoft, Inc., TuIsa, OK, USA), if not stated otherwise.

Results

Patient characteristics. In total, 84 patients with advanced-stage NSCLC treated with first-line chemotherapy were enrolled in the study. The baseline patient characteristics are summarised in Table II.

Correlation between ctDNA levels and PET/CT parameters before treatment initiation and their dynamics. A significant correlation was found between the baseline ctDNA levels and baseline TLG (*p*=0.001), MTV (*p*=0.001), and IC (*p*=0.003) before treatment initiation as well as between the change of ctDNA levels and TLG (*p*=0.002), MTV (*p*=0.006), and IC (*p*=0.001) after administration of two cycles of chemotherapy. No correlations were found between ctDNA levels and SUV (max, peak, mean) or IU at baseline or after two cycles of chemotherapy. The results of the correlation analysis are summarised in Table III.

Association between follow-up ctDNA levels and relative change of PET/CT parameters with objective treatment response. We found that the objective treatment response according to the RECIST criteria (CR+PR *vs*. SD *vs*. PD) was significantly associated with follow-up ctDNA levels (*p*<0.001) and relative change of all PET/CT parameters assessed including SUVmax (*p*<0.001), SUVpeak (*p*<0.001), SUVmean (*p*<0.001), TLG (*p*<0.001), MTV (*p*<0.001), IU (*p*=0.008), and IC (*p*<0.001) (Table IV, Figure 1).

Association between follow-up ctDNA levels and relative change of PET/CT parameters with survival. The univariable Cox proportional hazards model revealed that PFS was significantly associated with follow-up ctDNA levels (HR=1.237, *p*<0.001), relative change of SUVmax (HR=5.040, *p*=0.002), TLG (HR=1.171, *p*=0.042) and IU (HR=3.022, *p*=0.039); OS was significantly associated with

Table II. *Baseline characteristics of patients.*

Characteristic	n (%) or value
Gender	
Male	56 (66.7)
Female	28 (33.3)
Age	
Median [range]	65 [40-81]
Histology	
Adenocarcinoma	82 (97.6)
Squamous	2 (2.4)
TNM stage	
III	11 (13.1)
IV	73 (86.9)
Distant metastatic sites#	
Pleura	12 (14.3)
Liver	11 (13.1)
Bone	24 (28.6)
Brain	22 (26.2)
Adrenal gland	15 (17.9)
Lung	22 (26.2)
Other	23 (27.4)
None	11 (13.1)
ECOG PS	
0	1 (1.2)
1	69 (82.1)
2	14 (16.7)
Smoking	
Never-smoker	10 (11.9)
Smoker	50 (59.5)
Former smoker	24 (28.6)
Objective treatment response (RECIST)	
CR	1 (1.3)
PR	17 (21.3)
SD	39 (48.8)
PD	23 (28.8)
Unknown	4 (–)
Gene mutations analysed in ctDNA	
None	19 (25.0)
Detected	63 (75.0)
KRAS	35 (55.6)
TP53	20 (31.7)
BRAF	2 (3.2)
PIK3CA	1 (1.6)
EGFR	1 (1.6)
GNAQ	1 (1.6)
MET	1 (1.6)
NOTCH2	1 (1.6)
STK11	1 (1.6)

ECOG PS: Eastern Cooperative Oncology Group performance status; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; ctDNA: circulating tumour DNA; #One patient could have more metastatic sites.

Table III. *Correlation between ctDNA levels and PET/CT parameters before the treatment initiation and their dynamics after administration of two cycles of chemotherapy.*

Variables	n	Kendall's τ	*p*-Value
Baseline ctDNA (MAF)			
Baseline SUVmax	47	0.117	0.247
Baseline SUVpeak	47	0.164	0.105
Baseline SUVmean	47	0.069	0.491
Baseline TLG	47	0.329	**0.001**
Baseline MTV	47	0.346	**0.001**
Baseline IU	47	–0.105	0.299
Baseline IC	47	0.303	**0.003**
ΔctDNA (MAF)			
ΔSUVmax	31	0.036	0.774
ΔSUVpeak	31	0.102	0.422
ΔSUVmean	31	0.061	0.633
ΔTLG	31	0.396	**0.002**
ΔMTV	31	0.348	**0.006**
ΔIU	31	0.082	0.516
ΔIC	31	0.435	**0.001**

n: Number of included patients; ctDNA: circulating tumour DNA; MAF: mutated allele frequency; Δ: change (absolute); SUV: standard uptake value; TLG: total lesion glycolysis; MTV: metabolic tumour volume; IU: total iodine uptake; IC: iodine concentration related to tumour volume. Significant *p*-Values are shown in bold.

Table IV. *Association between ctDNA levels and relative change of PET/CT parameters with objective treatment response according to the RECIST criteria.*

Variable	n	Kendall's τ	*p*-Value
ΔSUVmax (%)	50	0.514	**<0.001**
ΔSUVpeak (%)	50	0.493	**<0.001**
ΔSUVmean (%)	50	0.42	**<0.001**
ΔTLG (%)	50	0.493	**<0.001**
ΔMTV (%)	50	0.404	**<0.001**
ΔIU (%)	50	0.258	**0.008**
ΔIC (%)	50	0.388	**<0.001**
Baseline ctDNA (MAF)	55	0.024	0.794
Follow-up ctDNA (MAF)	53	0.361	**<0.001**

n: Number of included patients; ctDNA: circulating tumour DNA; MAF: mutated allele frequency; Δ… (%): relative change; SUV: standard uptake value; TLG: total lesion glycolysis; MTV: metabolic tumour volume; IU: total iodine uptake; IC: iodine concentration related to tumour volume. Significant *p*-Values are shown in bold.

follow-up ctDNA levels (HR=1.100, p=0.021) and relative change of TLG (HR=1.195, p=0.023) (Table V).

The median PFS and OS for patients who achieved ctDNA clearance after two cycles of chemotherapy was 7.35 (95%CI=5.51-9.44) and 22.07 (95%CI=9.28-34.48) months compared to 1.89 (95%CI=1.29-4.46) and 5.72 (95%CI=3.39-12.80) months for those who did not (p<0.001 and p=0.0067, respectively) (Figure 2).

After automated optimisation of the stratification threshold, relative change in SUVmax, TLG and IU showed strong difference in PFS at threshold values of decrease of at least 24.3%, 28% and 28%, respectively (Figure 3).

ROC analyses for prediction of objective treatment response and PFS using a combination of follow-up ctDNA levels and relative change of PET/CT parameters. ROC analyses showed that VUS for prediction of objective treatment response using relative change of SUVmax was 0.597 (Figure 4A), and VUS for prediction of objective treatment response using a combination of follow-up ctDNA levels with relative change in SUVmax showed an improvement to the value of 0.648 (Figure 4B). ROC analyses showed that VUS for prediction of PFS using relative change of SUVmax was 0.367 (Figure 4C) and VUS for prediction of PFS using a combination of follow-up ctDNA levels with relative change of SUVmax was 0.431 (Figure 4D).

Discussion

The results of the present prospective study demonstrate significant correlations between ctDNA levels and PET/CT parameters at baseline as well as during the course of the first-line chemotherapy. Further, we found significant association of ctDNA dynamics and relative change of PET/CT parameters with objective response and survival. We suggest that the combination of ctDNA assessment with PET/CT is a promising approach for the follow-up monitoring of therapy response and prognosis estimation of patients with advanced NSCLC.

The strong correlations between plasma ctDNA levels with MTV and TLG assessed by ^{18}F-FDG PET/CT, found in our study, support the hypothesis that ctDNA levels are related to the overall tumour burden, which has been proposed previously (16). The data from previous studies show higher ctDNA levels in advanced-stage patients compared to those with early-stage (17-19), as well as correlations between the dynamics of ctDNA levels and treatment response or cancer relapse (19-22). The correlation between ctDNA levels and tumour volume assessed by CT scan in NSCLC patients has been recently reported by Pécuchet *et al*. and by Newman *et al*. (9, 19). PET/CT represents an emerging nuclear imaging method used for TNM staging and follow-up monitoring of cancer patients. MTV and TLG are novel volume-based PET/CT parameters capable of measuring the metabolic tumour burden by incorporating both volumetric data and metabolic activity (23). The correlation between metabolic tumour burden defined as MTV or TLG with cfDNA or ctDNA levels has been shown in several studies on various cancer types

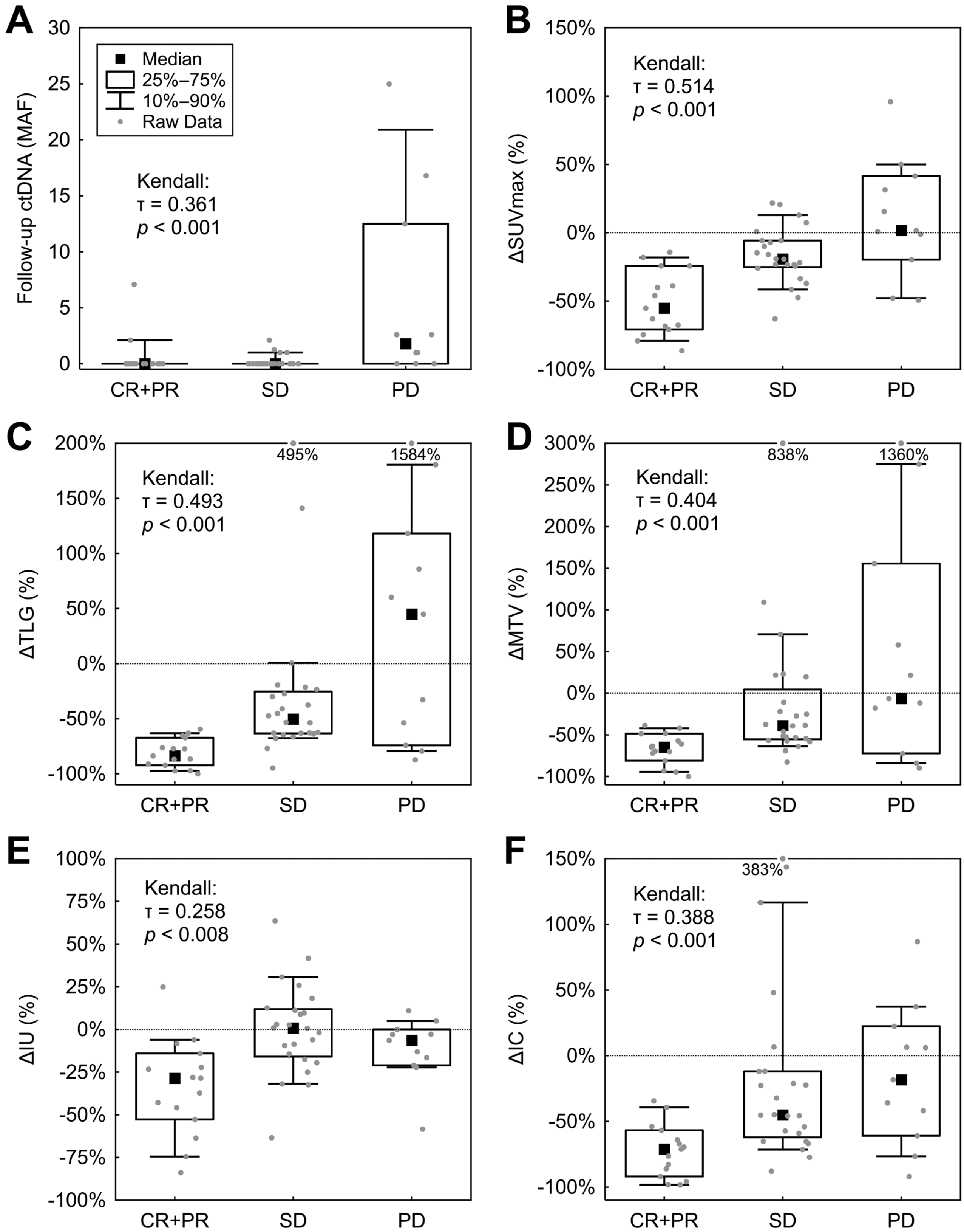

Figure 1. *Association between follow-up ctDNA levels (A) and relative change of PET/CT parameters (B, C, D, E, F) after two cycles of chemotherapy with objective treatment response.*

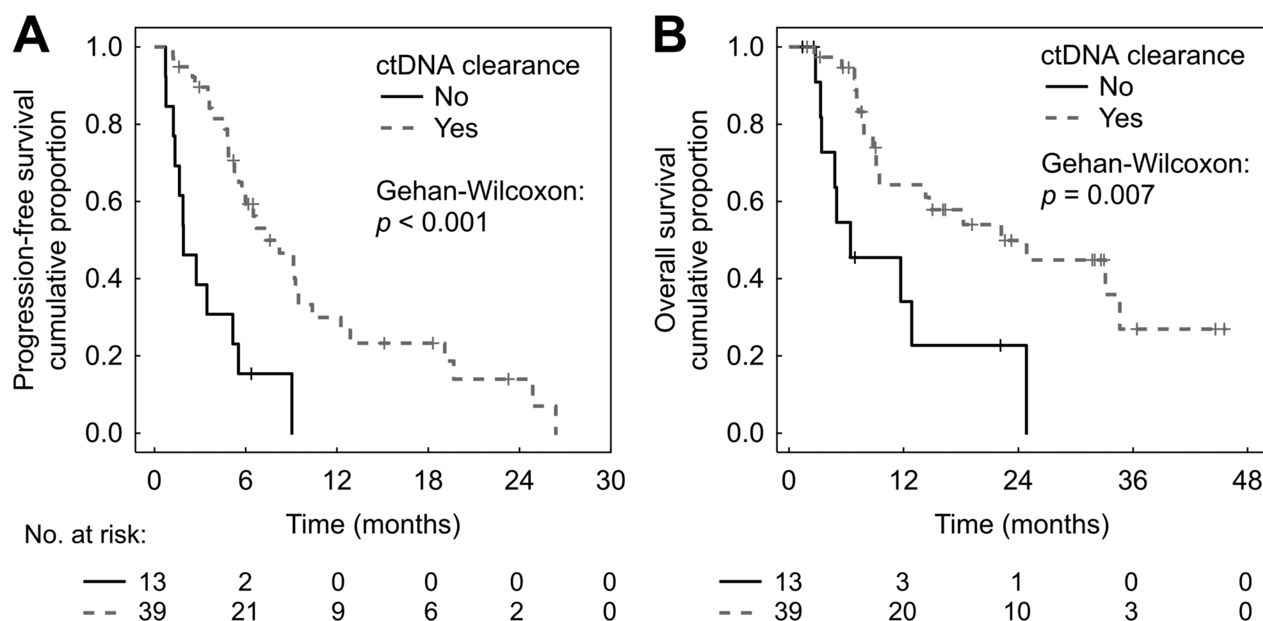

Figure 2. *Progression-free survival (PFS) (A) and overall survival (OS) (B) according to ctDNA clearance after two cycles of chemotherapy.*

Table V. *Association between ctDNA levels and relative change of PET/CT parameters with survival.*

Variable	PFS			OS		
	p-Value	p_{BC}	HR (95%CI)	*p*-Value	p_{BC}	HR (95%CI)
ΔSUVmax (%)	**0.002**	**0.006**	5.040 (1.785-14.228)	0.361	0.427	1.627 (0.573-4.620)
ΔSUVpeak (%)	0.152	0.062	1.470 (0.868-2.490)	0.460	0.519	1.411 (0.566-3.513)
ΔSUVmean (%)	0.923	0.643	1.026 (0.607-1.735)	0.719	0.617	1.131 (0.578-2.211)
ΔTLG (%)	**0.042**	**0.084**	1.171 (1.005-1.363)	**0.023**	**0.269**	1.195 (1.025-1.394)
ΔMTV (%)	0.361	0.273	1.060 (0.935-1.202)	0.110	0.400	1.125 (0.974-1.300)
ΔIU (%)	**0.039**	**0.043**	3.022 (1.055-8.654)	0.794	0.956	1.209 (0.291-5.015)
ΔIC (%)	0.927	0.534	1.014 (0.758-1.355)	0.697	0.741	1.079 (0.736-1.582)
Baseline ctDNA (MAF)	0.362	0.483	1.015 (0.983-1.049)	0.193	0.061	1.028 (0.986-1.072)
Follow-up ctDNA (MAF)	**<0.001**	**<0.001**	1.237 (1.124-1.362)	**0.021**	**0.020**	1.100 (1.015-1.193)

PFS: Progression-free survival; OS: overall survival; HR: hazard rate; CI: confidence interval; ctDNA: circulating tumour DNA; SUV: standard uptake value; TLG: total lesion glycolysis; MTV: metabolic tumour volume; IU: total iodine uptake; IC: iodine concentration related to tumour volume; PET/CT: positron emission tomography/computed tomography. Significant *p*-Values are shown in bold.

including NSCLC (24-30). However, this knowledge has been still limited, mostly based on results from retrospective studies involving relatively small number of patients. Notably, the reports correlating ctDNA with PET/CT parameters during the treatment of NSCLC patients have yield inconsistent data. Our results are in agreement with those reported by Winther-Larsen *et al*. (29). Their retrospective study including 46 patients with advanced-stage NSCLC showed significant correlations between ctDNA levels and TLG ($p=0.001$) and tumour metabolic burden defined as the sum of TLG for all evaluable lesions ($p=0.001$) (29). Similar findings were obtained from a prospective study conducted by Hyun *et al*. (30). Their study enrolled 101 patients with advanced-stage NSCLC and the results showed significant correlations of cfDNA levels with TLG ($p<0.001$) and MTV ($p<0.001$) (30). On the other hand, however, there are two prospective studies showing the opposite. No correlations between total cfDNA (not limited to the tumour-originating fractions) and TLG or MTV in advanced-stage NSCLC were found in the studies of

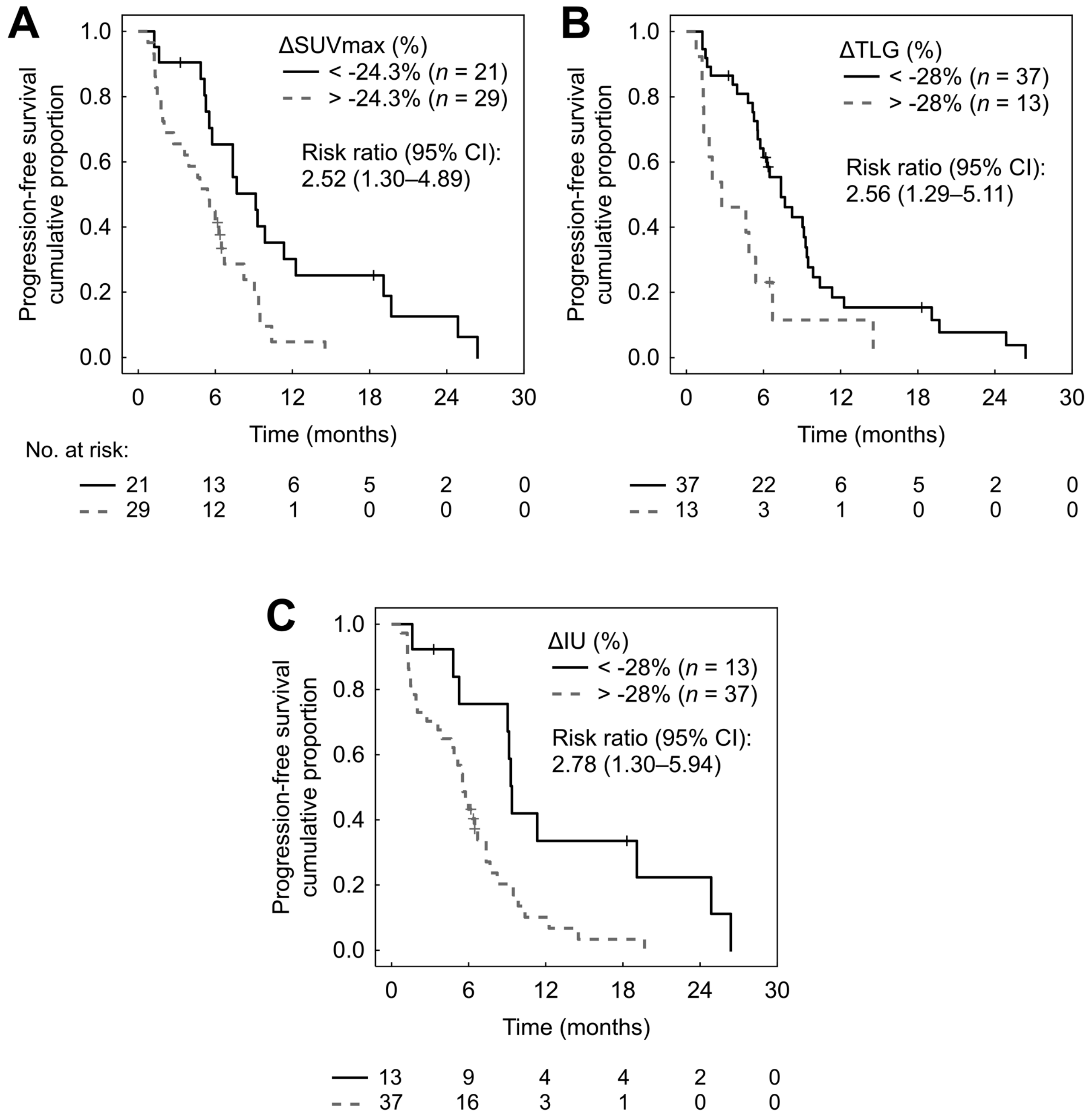

Figure 3. *Progression-free survival (PFS) according to the change (%) in PET/CT parameters after two cycles of chemotherapy using automated optimisation of stratification threshold: SUVmax (A), TLG (B) and IU (C).*

Nygaard *et al*. and Morbelli *et al*. that included 37 and 53 patients, respectively (31, 32). Both studies were limited by the small number of patients enrolled. Moreover, cfDNA has only a limited specificity as a cancer biomarker as the majority does not come from malignant cells. As mentioned above, while several studies examined the validity of the combination of cfDNA or ctDNA with PET/CT for the estimation of tumour burden or baseline staging, there still is a lack of similar data on the role of such combination in the follow-up monitoring and evaluation of treatment response in advanced-stage cancer patients undergoing systemic therapy. In our study, we found significant correlations between the dynamics of ctDNA and MTV and TLG during the course of chemotherapy, which is in

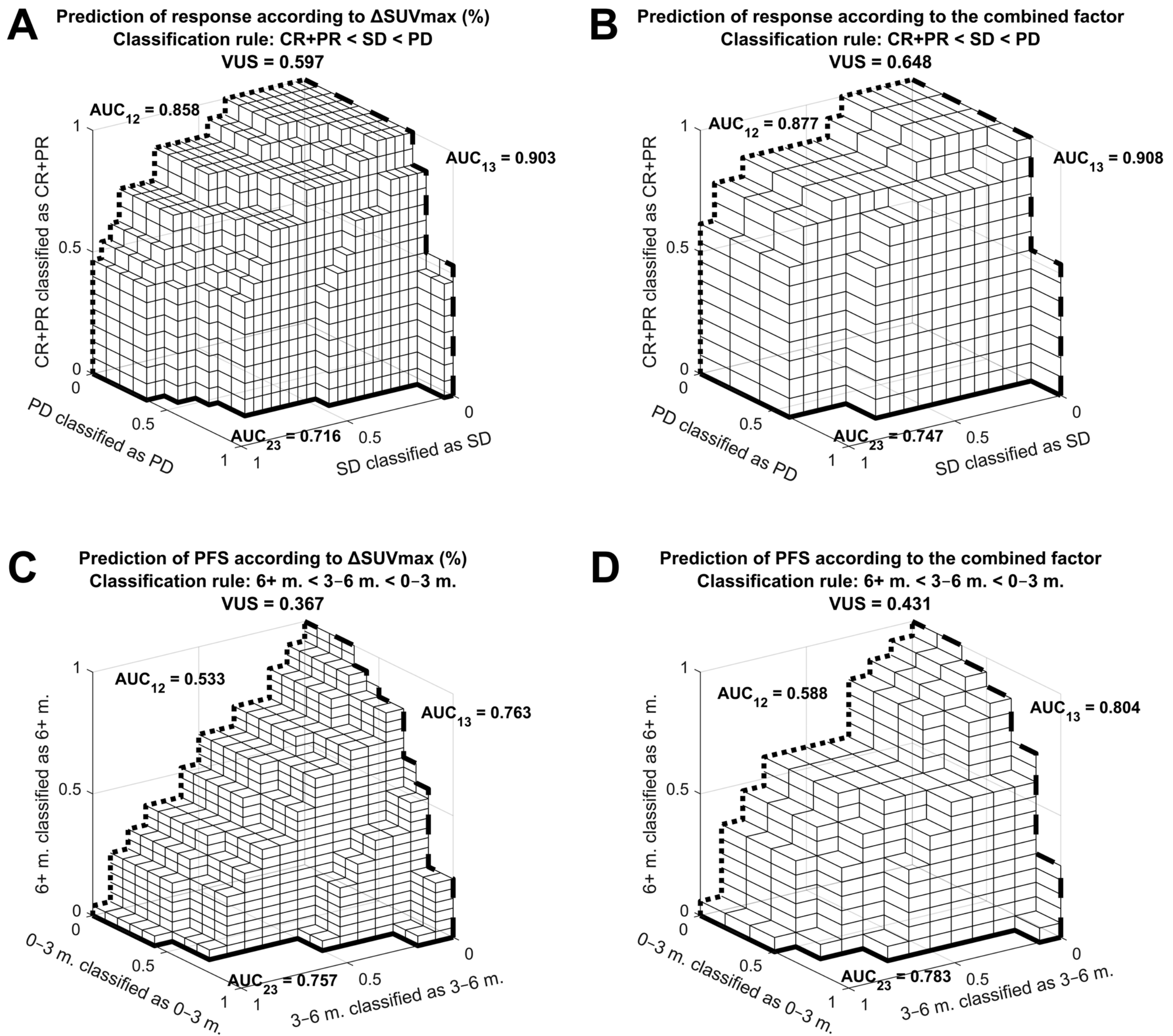

Figure 4. *Receiver operating characteristic (ROC) analyses for prediction of objective treatment response (A, B) and PFS (C, D) using relative change of SUVmax and combination of relative change of SUVmax with follow-up ctDNA levels.*

agreement with a study recently reported by Schmidkonz *et al*., however their study was focused on Ewing sarcoma (33).

The role of ctDNA as a promising blood-based cancer biomarker in NSCLC has been intensively investigated in recent years. The data obtained from a recent multicentre prospective cohort study performed by Song *et al*. demonstrated that ctDNA clearance during the course of systemic therapy was significantly associated with longer PFS (HR=0.28, p<0.001) and OS (HR=0.19, p<0.001) across a wide spectrum of treatment regimens (10). Similar results showing prolonged survival for patients with undetectable ctDNA during the course of systemic therapy as compared to those with persistent detectable levels of plasma ctDNA have been reported by others (9, 34, 35). In the present study, we found that follow-up ctDNA levels were significantly associated with objective treatment response according to the RECIST criteria and also with survival of patients. We found significantly longer PFS and OS for patients who achieved ctDNA clearance as compared to those with persistent detectable ctDNA during the course of systemic therapy. Thus, our results suggesting ctDNA as a valuable predictive and prognostic cancer-specific biomarker are consistent with the data from the previous studies mentioned above.

The use of ^{18}F-FDG PET/CT for staging and monitoring of therapy response in NSCLC has been well-established in the recent years. This is in line with our findings, showing significant association between the relative change of all PET/CT assessed parameters, including MTV, TLG and SUV, and objective response according to the RECIST criteria (5-7). Furthermore, the use of the DE-CT scanning technique on a single-source equipment, allowed us to accurately quantify the iodine content. Our results demonstrate significant association between the relative change of iodine-related parameters, including IC and IU, and objective response, which confirmed our previous data (7). The prognostic role of PET/CT-based parameters and their association with survival of NSCLC patients have been investigated in several studies. SUV is among the most widely studied PET/CT parameters. The association between high SUVmax and poor prognosis of NSCLC patients has been demonstrated in large meta-analyses conducted by Paesmans *et al*. and by Liu *et al*. (36, 37). Volumetric PET/CT parameters, represented by MTV and TLG, have been also suggested as valuable prognostic factors reflecting tumour burden and aggressiveness (37). In our study, we found that relative change of SUVmax was significantly associated with PFS and relative change of TLG was significantly associated with both PFS and OS.

Finally, ROC analyses suggested that the combination of follow-up ctDNA levels and relative change of SUVmax may be a promising approach for the prediction of objective treatment response and also PFS.

Although the overall size of the patient group enrolled is comparable to similar studies on ctDNA or PET/CT monitoring, the limitation remains in the relatively low number of patients where both approaches could be evaluated. For the assessment of ctDNA levels we chose to detect only the most frequent gene mutations in NSCLC. Clearly extending the spectrum of detected genes/mutations would increase the number of patients suitable for monitoring. Nevertheless, this is the first study focusing on the combination of serial assessment of ctDNA with PET/CT for the follow-up monitoring of treatment response in patients with advanced-stage NSCLC. It is strengthened by the prospective design and also by the use of the same PET/CT equipment, protocol for acquisition and reconstruction software which were used in all patients.

In conclusion, the results of our prospective study show significant correlation of ctDNA levels with MTV, TLG and IC at the baseline as well as their dynamics after two cycles of chemotherapy. The follow-up ctDNA levels and relative change of SUVmax, TLG and IU were associated with the objective treatment response and also with PFS. Finally, our data show a relevant performance of the combination of follow-up ctDNA levels with relative change of SUVmax for the assessment of objective treatment response and estimation of PFS in patients with advanced NSCLC treated with chemotherapy. Such a unique concept should be further investigated and its clinical utility validated in the future studies.

Conflicts of Interest

OF received honoraria from Novartis, Janssen, Merck and Pfizer for consultations and lectures unrelated to this project. MS received honoraria from Roche for consultations and lectures unrelated to this project. MM is currently an acting CEO of Elphogene, a diagnostic company currently involved in commercionalization of the ctDNA heteroduplex assay. J. Finek has received honoraria from Astra Zeneca, Roche, and Novartis for consultations and lectures unrelated to this project. MP, JB, LB, RP, TH, PH, MB and J. Ferda declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

Authors' Contributions

MP, OF and TH designed the study; PH performed statistical analyses; LB, RP, TH and MM performed planning and execution of molecular analyses; JB and J. Ferda analysed PET/CT data; OF, JB, RP, PH wrote the manuscript with support from MP, MS, LB, TH, MM, MB, J. Fínek and J. Ferda.

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