



¹⁸F-FDG PET/CT total lesion glycolysis is associated with circulating tumor cell counts in patients with stage I to IIIA non-small cell lung cancer

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Background: In non-small cell lung cancer (NSCLC), ¹⁸F-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) uptake determined by PET and presence of circulating tumor cells (CTCs) in the peripheral blood independently predict outcomes. For ¹⁸F-FDG PET/CT staging interpretation, standardized uptake values ($SUV_{max/avg}$) are routinely used in clinical reporting. The goal was to investigate whether ¹⁸F-FDG uptake measured by $SUV_{max/avg}$, but also measures of metabolic tumor volume (MTV) and total lesion glycolysis (TLG) ($MTV \times SUV_{avg}$), are associated with CTCs.

Methods: Prospectively, 7.5 mL blood was drawn from NSCLC patients at the time of staging ¹⁸F-FDG PET/CT and from healthy control subjects. CTCs were identified by immunofluorescent staining (CK8/18/19^{pos}/EpCAM^{pos}/CD45^{neg}/DAPI^{pos} nucleus). ¹⁸F-FDG PET/CTs were analyzed for SUV_{max} , SUV_{avg} , MTV, and TLG.

Results: In 16 NSCLC patients with stage I–IIIA, MTV and TLG, in contrast to SUV_{max} and SUV_{avg} , were positively associated with CTCs (linear regression analysis). No CTCs were detectable in 20 healthy control subjects.

Conclusions: This pilot study demonstrates that ¹⁸F-FDG PET/CT TLG correlates with CTCs in NSCLC patients without distant metastases. TLG might be a more appropriate marker for hematogenous micrometastatic potential than SUVs.

Keywords: ¹⁸F-FDG PET/CT; SUV; total lesion glycolysis (TLG); circulating tumor cells (CTCs); non-small cell lung cancer (NSCLC)

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Introduction

Five-year survival of non-small cell lung cancer (NSCLC) patients remains low at 18% (1). ¹⁸F-fluoro-2-deoxy-D-

glucose (¹⁸F-FDG) PET/CT, representing active tumor biology based on glucose metabolism, is the standard imaging modality for NSCLC staging (2). ¹⁸F-FDG uptake

parameters are predictive of poor outcome in NSCLC and other cancer types (3,4). Increased ^{18}F -FDG uptake on PET is associated with higher tumor glucose metabolism, which correlates with metastatic spread (5). Standardized uptake values ($\text{SUV}_{\text{avg}/\text{max}}$) are parameters typically reported in staging PET imaging for NSCLC, calculated as the ratio of radioactivity per unit volume of a region of interest (ROI) to the activity per unit whole body volume (6). However, combined measures of metabolic and volumetric tumor information, such as metabolic tumor volume (MTV) and total lesion glycolysis (TLG) (calculated as $\text{MTV} \times \text{SUV}_{\text{avg}}$), may be a more appropriate predictor for micrometastatic spread than SUVs (7,8).

Circulating tumor cells (CTCs)—defined as $\text{CK}^{\text{pos}}/\text{EpCAM}^{\text{pos}}/\text{CD45}^{\text{neg}}$ with a DAPI^{pos} nucleus—are found in the blood of cancer patients and correlate negatively with survival in NSCLC (9). CTCs have been implicated in development of metastases (10). Due to their simple detection, CTC liquid biomarker research is a most active field in translational oncology. The linkage between CTCs and tumor glucose metabolism is poorly understood (11). Since both CTCs and PET activity are associated with metastatic spread, we hypothesized that metabolic PET parameters correlate with CTCs in NSCLC.

This is a pilot study that demonstrates that TLG is associated with CTCs in non-metastatic NSCLC patients, in contrast to $\text{SUV}_{\text{max}/\text{avg}}$. Results suggest that TLG may be a better marker than SUVs for micrometastatic spread of NSCLC.

Methods

Subjects

Institutional Review Board approval was obtained (IRB2010166/IRB2004401-VA). Prospectively, treatment-naïve NSCLC patients that underwent staging for surgery for stage I-IIIa (AJCC 8th ed.) between July 2016 to December 2017 and healthy never-smokers were enrolled and signed informed consents.

^{18}F -FDG PET/CT

Imaging was performed with Biograph 32-Slice PET/CT scanners (Siemens, Germany), approximately one hour after ^{18}F -FDG intravenous injection using commercially available Medrad® Intego PET Infusion System. Dose of ^{18}F -FDG injected was based on body weight and equaled 0.04 mCi/pound body weight. The acquisition time per bed ranged

from 1–4 minutes, based on BMI (with longest times applied for patients with severe morbid obesity). Patients fasted overnight and underwent blood glucose testing prior to scanning [median 95 mg/dL (range, 72–130)]. Attenuation correction of images was performed with low-dose CT without intravenous contrast. MIM Software (Cleveland, OH) was used to determine SUV_{max} , SUV_{avg} , MTV was calculated by 3D region isocontour-based VOIs (12). TLG was calculated as $\text{SUV}_{\text{avg}} \times \text{MTV}$.

CTC detection

Seven point five milliliter of blood were drawn in CellSave® tubes (Menarini, Huntingdon Valley, PA), and CTCs were enriched by size with CellSieve™ microfilter (Creatv MicroTech, Rockville, MD), as described (13,14). In alignment with the FDA-approved definition, CTCs were identified by immunofluorescence as $\text{CK}^{\text{pos}}/\text{EpCAM}^{\text{pos}}/\text{CD45}^{\text{neg}}$ with a DAPI^{pos} nucleus.

Statistical analysis

Research personnel were blinded to study-specific outcomes by separation from the recruiting staff. Statistical significance refers to a $P < 0.05$. Analyses were performed using Prism 5.00 (GraphPad, La Jolla, CA).

Results

Characteristics of subjects and CTC detection

In total, 36 subjects were prospectively recruited: 16 NSCLC patients and 20 healthy never-smokers (Table 1). All NSCLC patients carried CTCs, however none of the 20 healthy controls had CTCs detectable (Figure 1).

^{18}F -FDG-PET/CT uptake parameter associations with CTCs

To associate metabolic parameters with CTCs, linear regression analyses were performed (Figure 2). We found a significant positive association between MTV and CTCs [slope= 0.13, 95% CI, (0.03, 0.23), $F(1,15)=7.60$, $P=0.016$, $r^2=0.35$], and TLG and CTCs [slope =0.03, 95% CI, (0.01, 0.05), $F(1,15)=9.81$, $P=0.007$, $r^2=0.41$]. No significant correlations between SUV_{max} and CTCs [slope= -0.21, 95% CI, (-0.93, 0.51), $F(1,15)=0.38$, $P=0.546$, $r^2=0.03$] or SUV_{avg} and CTCs [slope =-0.42, 95% CI, (-1.79, 0.95), $F(1,15)=0.43$, $P=0.522$, $r^2=0.03$] were found. There was no

Table 1 Subjects' clinicopathological characteristics, ¹⁸F-FDG PET/CT and CTC results

Characteristic	N	Median (range)
Total subjects analyzed	36	
NSCLC	16	
Age		63 [48–79]
Gender		
Females	8 (50%)	
Males	8 (50%)	
AJCC stages (8th ed.)		
I	11 (68.8%)	
II	3 (18.8%)	
IIIA	2 (12.4%)	
pT stage		
pT1	8 (50%)	
pT2	6 (37.5%)	
pT3	1 (6.3%)	
pT4	1 (6.2%)	
pN stage		
pN0	13 (82.2%)	
pN1	2 (12.5%)	
pN2	1 (6.3%)	
Histology		
Adenocarcinoma	8 (50%)	
Squamous cell	7 (43.8%)	
Large-cell neuroendocrine	1 (6.2%)	
Treatments received		
No treatment	9 (56.3%)	
Adjuvant chemotherapy	6 (37.5%)	
Adjuvant chemotherapy and radiation	1 (6.2%)	
CTCs/7.5 mL of blood	16/16 (100%)	19 [13–48]
¹⁸ F-FDG PET/CT parameters		
SUV _{max}		12.37 (2.37–27.39)
SUV _{avg}		7.50 (1.62–14.37)
TLG		64.73 (2.61–798.0)
MTV		10.26 (1.10–164.2)
Healthy never-smoker controls	20	
Age	44 (28–69)	
Gender		
Females	10 (50%)	
Males	10 (50%)	
CTCs/7.5 mL of blood	0/20	0

CTC, circulating tumor cell; NSCLC, non-small cell lung cancer; TLG, total lesion glycolysis; MTV, metabolic tumor volume.

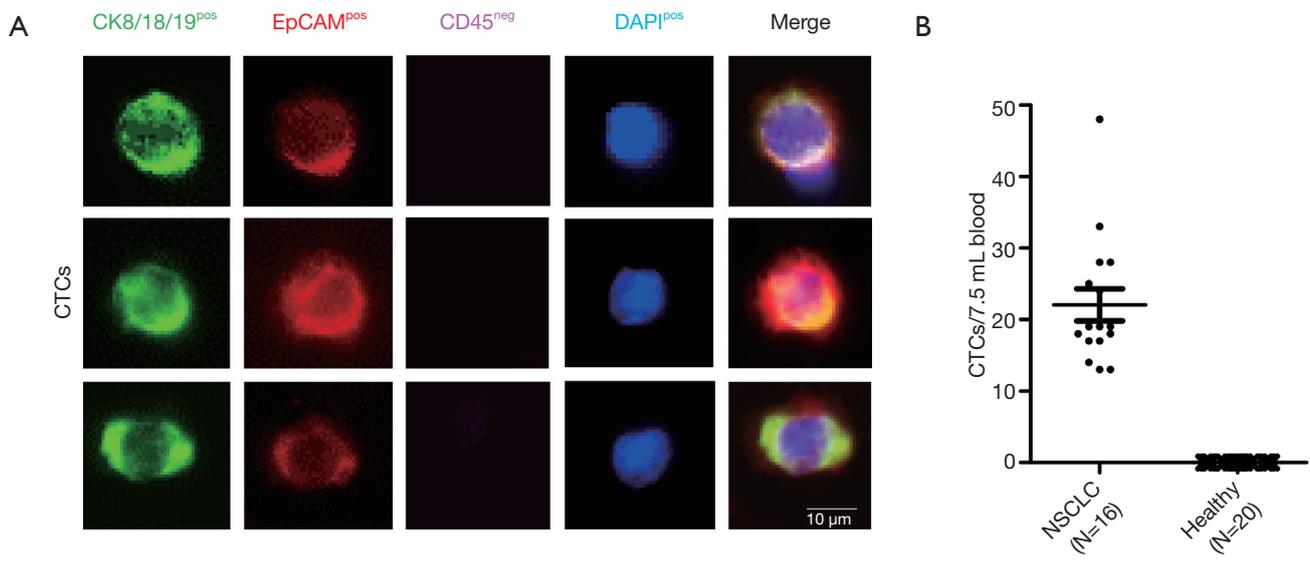


Figure 1 CTC analysis. (A) At the time of staging, CTCs were identified in 7.5 mL of blood by immunofluorescent staining. Presented are staining patterns of three CTCs from different NSCLC patients. (B) CTC counts in NSCLC patients and healthy never-smoking controls (bars: mean \pm standard error of the mean). CTC, circulating tumor cell; NSCLC, non-small cell lung cancer.

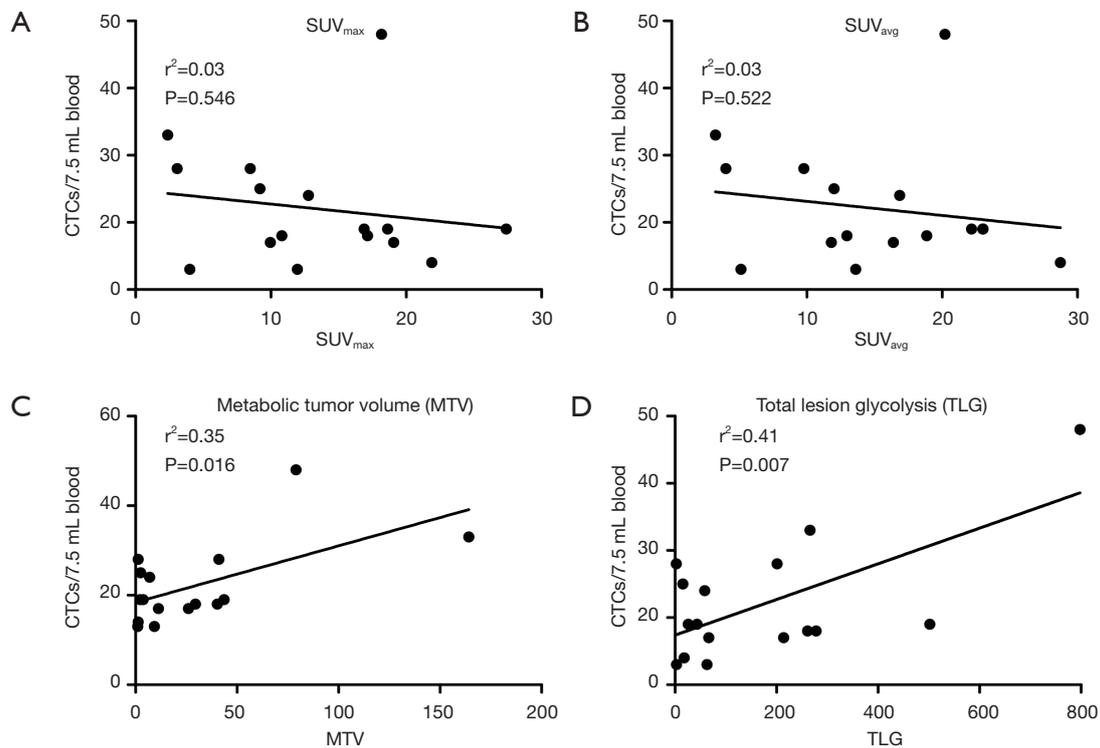


Figure 2 Relationship between ^{18}F -FDG PET/CT metabolic uptake parameters and CTCs in NSCLC. Linear regression graphs are shown. MTV and TLG were positively associated with CTCs. CTC, circulating tumor cell; NSCLC, non-small cell lung cancer.

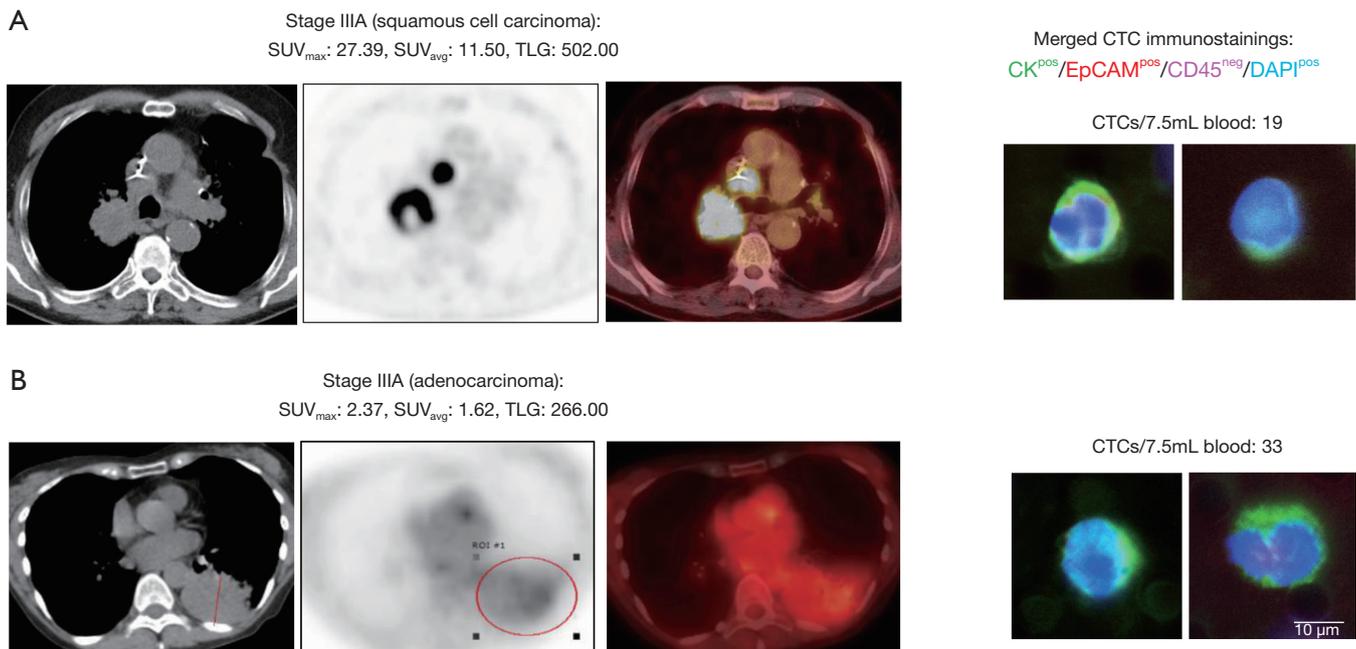


Figure 3 ^{18}F -FDG PET/CT metabolic analysis and CTCs in two NSCLC patients. (A) Stage IIIA NSCLC tumor with necrotic center (arrow) that is not ^{18}F -FDG-avid, contributing to reduction of TLG. CTC count was 19/7.5 mL of blood; (B) stage IIIA NSCLC, large and pathologically confirmed tumor size of 11.2 cm (no atelectasis) with low FDG uptake, but a high TLG due to homogeneous ^{18}F -FDG distribution, with high CTC count of 33/7.5 mL of blood. CTC, circulating tumor cell; NSCLC, non-small cell lung cancer; TLG, total lesion glycolysis.

significant association between pathologic tumor size (pT) and CTCs, or pT and metabolic parameters.

A multivariate linear regression analysis predicting CTCs from MTV, controlling for AJCC stage, was performed. This overall model was not significant [$F(2,13)=2.20$, $P=0.061$]—due to a non-significant slope for stage. The intercept was 18.06 [95% CI, (8.23, 27.89)]. MTV was a significant predictor for CTCs [slope= 0.13, 95% CI, (0.02, 0.23), $P=0.008$]. AJCC stage was not a significant predictor for CTCs [slope= -0.25, 95% CI, (-5.74, 6.24), $P=0.930$]. Residuals for MTV were normally distributed and homoscedastic. The r^2 was 0.35 in this multivariate regression model. Similarly, we did a multivariate linear regression predicting CTCs from TLG, controlling for stage. The regression model was significant ($F(2,13)=5.01$, $P=0.024$). The intercept was 14.50 [95% CI, (4.57, 24.44)]. TLG was a significant predictor for CTCs (slope= 0.03, 95% CI, (0.01, 0.05), $P=0.008$). Stage was not a significant predictor for CTCs [slope= 1.91, 95% CI, (-3.72, 7.53), $P=0.477$]. Residuals for TLG were normally distributed and homoscedastic. The r^2 was 0.44 in this multivariate linear

regression.

This pilot study results suggest that MTV and TLG, in contrast to SUV_{max/avg}, are potential predictors for micrometastatic hematogenous spread measured by CTCs in NSCLC patients (Figure 3).

Discussion

SUV_{max} is the standard metabolic parameter applied for clinical NSCLC staging (15). However the TLG, a measure that takes the metabolic tumor volume burden into account, may provide a better reflection of cancer biology (7). Our findings suggest that in addition to MTV, TLG is a more accurate marker than SUVs for micrometastatic NSCLC spread represented by CTCs. Correlation of SUV_{max} with poor outcome in NSCLC has been suggested, but conflicting data exist (3,5,16). Independently, ^{18}F -FDG PET/CT TLG and CTCs in the blood predict increased risk of recurrence in NSCLC, but the association of ^{18}F -FDG uptake parameters with CTCs is poorly understood (11,17-19). We performed metabolic

^{18}F -FDG PET/CT analysis in primary lung tumors, but not in loco-regional lymph node metastases (if present). Although we did not include NSCLC patients with distant metastases, the study is limited by population heterogeneity. The majority of patients had lung tumors with no loco-regional nodal metastases, but three patients had ipsilateral intrapulmonary/hilar and/or mediastinal lymph node metastases.

In metastatic NSCLC patients undergoing chemotherapy, a correlation between a decrease in CTCs and SUV_{max} has been demonstrated (19). In another cohort of metastatic NSCLC patients that often have multiple tumor sites with different metabolic uptake, no correlation between CTCs and metabolic parameters, including TLG, was noted (20). In contrast, our study focused on non-metastatic NSCLC patients that might be less diverse than metastatic patients, which may explain why an association between TLG and CTCs was observed. In a comparable group of NSCLC patients undergoing surgery, presence of CTCs one month after resection correlated with SUV_{max} , but TLG was not analyzed in this study (17). In a heterogeneous group of NSCLC patients of all stages, CTCs detected by a non-EpCAM-based method neither correlated with SUV_{max} , nor TLG (18). Interestingly, presence of CTC clusters (≥ 2 CTCs in aggregate) was associated with TLG, but it needs to be taken into consideration that clusters are more prevalent in metastatic NSCLC (21). EpCAM-independent detection technologies yield higher CTC counts due to down-regulation of EpCAM during epithelial-mesenchymal transition of cancer cells (18). However, we chose to identify CTCs as $\text{CK}^{\text{pos}}/\text{EpCAM}^{\text{pos}}$ —in alignment with the FDA-approved definition (22).

This pilot study links TLG to CTCs in non-metastatic NSCLC patients. Although we exclusively studied patients with local tumors or loco-regional nodal disease (without distant metastases), the extent of tumor burden varied amongst patients which may affect glucose metabolism and CTC shedding. Interpretation of our results are limited by small sample size and should be confirmed in larger cohorts.

Conclusions

^{18}F -FDG PET/CT TLG, in contrast to SUVs, is associated with presence of CTCs in stage I-III NSCLC patients. TLG might be an appropriate marker than SUVs for micrometastatic spread of NSCLC measured by CTCs in the blood.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tlcr.2020.04.10>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Institutional Review Board of University of Missouri (IRB2010166/IRB2004401-VA) and informed consent was taken from all individual participants.

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