Association Between 18F-Fluoro-2-Deoxy-D-Glucose Uptake Values and Tumor Vitality: Prognostic Value of Positron Emission Tomography in Early-Stage Non-small Cell Lung Cancer

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Introduction: The prognostic value of quantitative 18F-fluoro-2-deoxy-D-glucose (FDG) uptake on positron emission tomography (PET) is controversial in unselected patients with non-small cell lung cancer (NSCLC). We assessed the in vivo FDG uptake, measured as maximum pixel standardized uptake value (SUV_{max}), in stages I and II NSCLC for its prognostic value and association with in vitro quantitative morphology of tumor vitality.

Methods: Prospective FDG-PET data were available in 91 consecutive patients operated for pathologic stages I and II NSCLC. Quantitative morphology was performed of tumor architecture, tumor cell density and immunohistochemical biomarkers for apoptosis (caspase-3), cell proliferation (Ki-67), hypoxia (HIF-1α), cellular pH regulation (carbonic anhydrase IX [CAIX]), and microvessel density (CD31).

Results: $SUV_{max} \geq$ median and SUV_{max} partial volume corrected for lesion size (PVC SUV_{max}) $\geq$ median were associated with an increased risk of death in univariable analysis. After correcting for stage, tumor size and age in multivariable analysis, only PVC $SUV_{max} \geq$ median remained significant. The strong significant association between tumor size and SUV_{max} weakened after PVC, suggesting that an important amount of SUV_{max} can be simply explained by tumor size, which is less in case for PVC SUV_{max} that associates more to the tumor cell density. In multivariable logistic regression analysis, a PVC $SUV_{max} \geq$ median could be explained by high Ki-67 and high-CAIX length density.

Conclusion: PVC SUV_{max} has a prognostic value in completely resected stages I and II NSCLC. A high-quantitative FDG uptake is associated with characteristics of tumor vitality such as high tumor cell density, high cell proliferation, and extracellular acidosis.

Key Words: FDG-PET scan, Non-small cell lung cancer, Prognosis, SUV.

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In 1930, Warburg[1] considered the shift in energy production from oxidative phosphorylation to glycolysis as a fundamental characteristic of a cancer cell. Dedicated positron emission tomography (PET) using 18F-fluoro-2-deoxy-D-glucose (FDG), a functional imaging technique taking advantage of this glycolytic shift, became an innovative noninvasive clinical cancer imaging for the diagnosis and the staging of non-small cell lung cancer (NSCLC). FDG is a glucose analog, which shows enhanced cellular uptake as a result of the elevated expression of the glucose transporter proteins (GLUTs) in most cancer cells. Once in the cancer cell, FDG is phosphorylated to FDG-6-PO_4 that cannot be further metabolized in the glycolytic pathway, thereby rendering cancer cells detectable using PET.

NSCLC is a group of carcinomas with variable biologic aggressiveness and prognosis. Clinicopathologic tumor, node, metastasis (TNM)-stage is still the most powerful prognostic factor, but even the most favorable stages, i.e., pathologic stages I and II have a 30 to 40% and 45 to 60% risk of death within 5 years after resection, respectively. Therefore, better prognostic factors are warranted. During the last decade, several retrospective analyses and one literature-based meta-analysis found that metabolic imaging relying on maximum pixel standardized uptake value (SUV_{max}) of the primary tumor on FDG-PET was a significant and independent prognostic factor in patients with NSCLC.[2–6] But recently, these findings also became controversial when a large prospective[7] and retrospective[8] study concluded that the prognostic role of FDG-PET seemed to be of

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limited value in patients with NSCLC populations. Moreover, no clear cutoff point was found as many factors may cause methodological variability in SUV measurements across different centers (such as patient preparation procedures, scan acquisition, image reconstruction, and data analysis settings), and the relationship between the cancer cell physiology and quantitative FDG uptake on PET is less understood. Many different biologic processes may affect FDG uptake by NSCLC on both the cellular and tissue level. On the cellular level, glucose uptake is mediated by the expression of GLUT[9–11] and hexokinase-II activity,[11] and glucose demand is enhanced by cellular proliferation (which is related to Ki-67 or proliferating cell nuclear antigen expression),[9,12,13] alterations of tumor suppressor genes[14] and the expression of hypoxia-inducible-factor 1α (HIF-1α).[15] On the tissue level, glucose delivery is a function of tumor perfusion that is associated with angiogenesis, anatomically reflected by microvessel density.[16] Moreover, the rate of glucose metabolism may be influenced by the presence of necrosis, reflecting that proliferation outnumbers metabolic supply, or the presence of lymphocytes and macrophages that may be confounding active metabolic cells. Additionally, tumor cells (TC) and the tumor-associated stroma (with its inflammatory interstitium, fibrocollagenous interstitium, and vasculature) should be seen as a functional domain with a metabolic cooperation between cancer cells and newly formed fibroblasts and vessels.[17]

As the evidence for the prognostic value of FDG uptake remains limited when all stages of NSCLC are considered, we conducted this translational analysis within surgically treated stages I and II NSCLC to explore the mechanisms why quantitative FDG uptake on PET may stratify patients for prognosis. We aimed to explore the potential of FDG uptake as a prognostic factor for outcome and a predictive factor for phenotyping tumor vitality. Unlike most other studies, we performed quantitative morphology of tumor architecture and quantitative immunostaining for cell proliferation (Ki-67 expression), tumor hypoxia (nuclear HIF-1α expression), tumor pH regulation (membranous carbonic anhydrase IX [CAIX]), cellular apoptosis (caspase-3 expression), and neoangiogenesis (CD31 microvessel density) in this study. Other factors possibly influencing FDG uptake such as tumor cell density and tumor size were also investigated.

PATIENTS AND METHODS

Patients

The surgical specimens of 91 consecutive patients with NSCLC (78 males, 13 females) undergoing baseline state of the art preoperative staging including FDG-PET scan and subsequently a thoracotomy with complete resection and systematic mediastinal lymph node dissection for pathologic stages I or II at University Hospitals Leuven ($n = 56$) and Maastricht ($n = 35$). The UICC classification (TNM, 6th edition, 2002) was used for pathologic staging. Only patients with pure NSCLC histology (adenocarcinoma, squamous cell carcinoma, and large cell carcinoma) were included. No adjuvant chemotherapy was given. Overall survival follow-up was obtained by patient records or by phone contact with the referring physicians.

Dedicated FDG-PET Scan

FDG-PET scans in Leuven were performed on a CTI-Siemens (Iselin, NJ) 931/08/12 PET scanner, having an axial field of view of 10.1 cm and spatial resolution of 8 mm. The patient was positioned with the primary tumor in the filed of view of the rectilinear PET camera. Then, a transmission scan was performed before (cold transmission) to FDG injection (6.5 MBq/kg, max. 555 MBq). After FDG injection, the patient remained positioned into the PET camera, and a 60-minute dynamic study of the thorax was started, followed by a 10-minute static acquisition over the primary tumor. The standardized uptake values (SUV) of the primary tumor was calculated 60 minutes postinjection. Patients from Maastricht were scanned with an ECAT EXACT 922 CTI-Siemens (Knoxville, TN) PET scanner in Aachen, having an axial field of view of 16.2 cm and spatial resolution of 6 mm. A transmission scan was also performed before FDG injection (3.5 MBq/kg) and after a median time of 60 minutes after injection 2-D whole body emission images were acquired.

For the determination of the SUV, a volumetric Region of Interest was drawn around the primary tumor on the transaxial images. The SUV was then automatically calculated as activity concentration of FDG uptake divided by the injected dose/body weight, and the pixel with the maximum SUV was considered the SUV_{max}. Partial volume effects, caused by the limited sampling (at the level of a pixel size) and resolution of the PET scanner, result in underestimation of the regional FDG uptake, predominantly in tumors smaller than twice the resolution of the PET scanner. For appropriate partial volume correction (PVC), the necessary recovery coefficients were determined from a software phantom based on the spatial resolution and pixel size of the PET scanner used. The spatial resolution depends on the full width at half maximum (FWHM) of the PET device (for Leuven FWHM 13 mm and for Aachen FWHM of 7 mm). We took the largest diameter of the macroscopic lesion on resection to determine the tumor size. Finally, we used the PVC algorithm used by Vesselle et al.[12] to correct the SUV_{max} of lesions <40 mm.

$$\text{PVC } SUV_{max} = \left(\frac{(SUV_{max} \text{ measured} - 0.25)}{\text{Recovery Coefficient}}\right) + 0.25 \text{ (ref. 12)}$$

Because of technical differences in methodology for PET scanning between Leuven and Maastricht, we did not combine the absolute SUV_{max} values of both data sets. Instead we dichotomized the patient groups from Leuven and Maastricht based on their respective median SUV_{max} value. Thereafter, we combined the group of patients with "$SUV_{max} <$ median" from Leuven and those with $SUV_{max} <$ median from Maastricht and considered this group as the "low SUV_{max} group." The same was done for patients with a "$SUV_{max} \geq$ median," which were considered as the "high SUV_{max} group."

Quantitative Morphology of Tumor Architecture and Immunohistochemistry

Tumor analysis was performed on histologic sections stained with hematoxylin and eosin (H&E) from resection

