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The maximum uptake of ^{18}F-deoxyglucose on positron emission tomography scan correlates with survival, hypoxia inducible factor-1α and GLUT-1 in non-small cell lung cancer

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ABSTRACT

The purpose of this study was to investigate the relation between the standardised uptake value (SUV) on ^{18}F-fluoro-2-deoxy-glucose-positron emission tomography scan and hypoxia related markers (HIF-1α and CAIX), a proliferation-related marker (Ki-67) and glucose transporters (GLUT-1 and GLUT-3) in non-small cell lung cancer (NSCLC). One hundred and two patients, scheduled for complete resection, received a PET scan in Leuven or Maastricht/Aachen. The maximal SUV (SUV_{max}) was correlated with survival and immunohistochemical staining patterns. The actuarial survival was worse for patients showing a high SUV_{max}, the best discriminative value being 8.0 (Leuven, $p = 0.032$) and 11.0 (Maastricht, $p = 0.007$). Tumours with a high SUV_{max} expressed in a higher proportion HIF-1α (63.1% versus 37.9%, $p = 0.024$) and GLUT-1 (82.9% versus 62.5%, $p = 0.025$), than tumours with a low SUV_{max}. No significant difference was found in the expression of CAIX, Ki-67 and GLUT-3. This study supports preclinical data that hypoxia is associated with a higher uptake of FDG.

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1. Introduction

TNM stage is up till now the best prognostic indicator for survival after radical operation in NSCLC.[1] However, even patients with the same stage may have a very different survival. Pre-treatment characteristics that add prognostic information are therefore of interest. Non-invasive imaging modalities, like positron emission tomography (PET), are increasingly used in the staging and treatment of NSCLC. The maximal uptake of ^{18}F-fluoro-2-deoxy-glucose (FDG) in the primary tumour was consistently shown to be an independent prognostic factor for survival.[2–5] However, the actual mechanisms by which a high FDG uptake leads to a worse prognosis are not well known. Different molecular markers, representing independent pathways, like hypoxia, apoptosis and angiogenesis, have been associated with a high risk of recurrence and death in NSCLC patients.[6–8]

Hypoxia might play an important role in the uptake of FDG, since it leads via the Hypoxia Inducible Factor-1α (HIF-1α) pathway to up regulation of glucose transporters, Carbonic Anhydrase IX (CAIX) and other target genes.[9,10] Preclinical studies suggest that hypoxic conditions correspond to a higher FDG uptake.[11–14] In addition, hypoxia is an important cause of treatment failure in many tumours and both HIF-1α and CA IX have been related to a poor outcome.[6,8,15,16]

Since hypoxia leads to an increased rate of glycolysis, which in turn, increases the uptake of FDG, we hypothesised that the worse prognosis of NSCLC patients with a high FDG uptake would be related to hypoxia. Therefore, we investigated the impact of tumour hypoxia, as assessed by the expression of the endogenous hypoxia markers HIF-1α and CAIX, and glucose metabolism (GLUT-1 and GLUT-3) on the uptake of FDG on PET scan before surgery in NSCLC.

2. Patients and methods

2.1. Study population

The surgical specimens evaluated were taken from 102 patients with a histologically proven NSCLC. All patients with a clinical stage I or II, who had undergone resection of their tumour with curative intent and had had a diagnostic PET scan, were included. All patients were operated on at the University Hospital Gasthuisberg of Leuven ($n = 56$, November 1994 to October 1997) or the University Hospital of Maastricht ($n = 46$, February 1998 to September 2002). Only adenocarcinoma, squamous cell carcinoma and large cell/undifferentiated carcinoma were included. FDG-PET scanning was performed on two different types of PET scanners. All patients fasted for at least six hours before scanning, and glucose levels were checked.

The first group of patients from Leuven ($n = 56$) was scanned with a CTI-Siemens (Iselin, NJ) 931/08/12 PET scanner with an axial field of view (FOV) of 10.1 cm and a spatial resolution of 8 mm. After injection (6.5 MBq/kg, maximal 555 MBq), a 60 min dynamic emission study was followed by a 10 min static acquisition. Images were reconstructed using filtered back projection. The patients from Maastricht ($n = 46$) were scanned with an ECAT EXACT 922 (Siemens-CTI, Knoxville, TN) in Aachen with an axial FOV of 162 mm and a spatial resolution of 6 mm. After a median time of 60 min (range 45–120 min) after injection (3.5 MBq/kg) 2-D whole body emission images were acquired. Images were reconstructed using an iterative reconstruction algorithm. For the determination of the standardised uptake value (SUV) a region of interest (ROI) was drawn by the nuclear medicine physician on the transaxial images around the primary tumour. SUV was then automatically calculated as activity concentration of FDG uptake divided by injected dose/body weight.[17] To avoid partial volume effects as much as possible, the maximal SUV (SUV_{max}) within this ROI was calculated.

Since the two PET scanners used in this study have different characteristics, a potential concern was different measurements of SUV_{max} on the two machines. Phantom measurements to compare the two machines could not be performed, since the CTI-Siemens was no longer used. Since a direct comparison of the results of the two groups was not possible, we determined the best discriminating factor for survival for both groups scanned on the two separate PET scanners, as has been described by Vansteenkiste and colleagues and used by other authors.[4,5] Different cut-off levels, within an in the literature most often mentioned range between 5 and 13, were used for this purpose.

The final staging was based on the findings at pathologic examination (TNM classification, 6th edition, 2002). Follow-up data from all patients were collected until August 2005, using the patients' files. If necessary, the patient's general practitioner or referring specialist was contacted to complete the follow-up. Since data were collected retrospectively, only overall survival was estimated.

2.2. Materials

The specimens studied were routinely processed, formalin fixed and paraffin embedded. Representative histological sections of the tumour specimen were taken to cut tissue sections of 4 μm thicknesses and stained with haematoxylin and eosin. For immunohistochemical staining sections were dewaxed in xylene and rehydrated by passing through graded alcohols. Endogenous peroxidase was blocked by applying 0.03% hydrogen peroxidase (20 min). If necessary, antigen retrieval was achieved using a citrate or TE buffer and sections were pretreated with blocking normal rabbit (CAIX) or calf (HIF-1α) serum. The following primary antibodies were used: mouse monoclonal antihuman CA IX antibody M75 (Slovak Academy of Sciences, Bratislava, 1:50, 45 min), mouse monoclonal antihuman HIF-1α antibody (BD Biosciences Pharmingen, 1:50, overnight), rabbit anti-GLUT-1 and anti-GLUT-3, (AB1341 and AB1345 polyclonal antiserum, Chemicon, Temecula, 1:500, 2 h) and monoclonal mouse antihuman MIB-1 antibody (Ki-67 antigen DAKO M7240, 1:100, 45 min).

As second layer Dako Envision+ (CAIX and HIF-1α) or biotinylated antibody and biotinylated horse-radish peroxidase complex (GLUT-1, GLUT-3 and Ki-67) were used. Finally, all slides were developed with diaminobenzidine (DAB) and counterstained with haematoxylin, dehydrated and mounted. Positive and negative (primary antibody omitted) controls were used to check the procedures.

Sections were assessed using a light microscope in a blinded fashion by at least two observers (A.vB, DR and RJvS). If discrepancies were found, a consensus was reached using a conference microscope. For Ki-67 at least 500 tumour cells were counted and the percentage of Ki-67 positive cells was noted. Tissue samples showing no or just weak membranous staining for GLUT-1 and GLUT-3 were considered negative, whereas strong membranous staining was considered positive. For CA IX membranous (m)CA IX staining and nuclear staining of HIF-1α a semi-quantitative scoring method was used: 0 = <5%, 1 = 5–25%, 2 = 25–50%, 3 = 50–75% and 4 = >75% of cells positive. Finally, the results were dichotomised. Tumours with no or low positive staining were considered negative and tumours with ⩾25% positivity were considered positive.

2.3. Statistics

The SPSS software (version 14.0, SPSS Inc., Chicago, IL) was used to perform statistical analysis. Results are expressed as the mean ± standard deviation (SD) and range, unless otherwise indicated.

Survival curves were analysed using the Kaplan–Meier method and the log-rank test. The χ^2 test, Pearson correlation coefficient and the Mann–Whitney *U* test were used to analyse the association between the different categorical variables. *p*-Values less than 0.05 were considered statistically significant.

2.4. Ethics

According to Dutch and Belgian law and regulations of the Medical Ethical Committees, no informed consent was required for this study.

3. Results

3.1. Patient and tumour characteristics

In total, 102 patients, 83 males and 19 females, were included in the study. The mean follow-up was 42 months (range: 1–96 months) and the 2-year actuarial survival was 68.6%. One patient (1.0%) died within 30 days postoperatively due to cardiac problems. Most tumours were squamous cell carcinomas (*n* = 58, 56.9%), while 30.4% (*n* = 31) consisted of adenocarcinomas and 12.7% (*n* = 13) of large cell carcinomas. The patient and tumour characteristics are depicted in Table 1.

3.2. PET data and survival

For the first group of patients from Leuven, a median of SUV_{max} of 10.0 (mean: 10.8, SD: 5.2, range: 1.7–25.4) was observed. The most discriminative cut-off point for survival was found at an SUV_{max} of 8.0 (Fig. 1). A statistically significant worse survival was noted in patients having a tumour with a $SUV_{max} \geqslant 8$, compared to patients showing a tumour with an $SUV_{max} < 8$, a 2-year survival of 56.8% and 89.5%, respectively ($p = 0.032$) (Fig. 2a).

For the second group of patients from Maastricht, a median of SUV_{max} of 12.7 (mean: 14.1, SD: 6.5, range: 4.2–31.5) was found. The best discriminative cut-off point for this group was observed at an SUV_{max} of 11.0. Patients with a tumour with an $SUV_{max} \geqslant 11$ showed a 2-year survival of 60.6%,

Table 1 – overview of patient characteristics and tumour characteristics

	Mean	SD	Range
Age (years)	64.3	9.3	(37–85)
	Number	(%)	
Gender			
Male	83	(81.4)	
Female	19	(18.6)	
Pathology			
Adenocarcinoma	31	(30.4)	
Squamous cell carcinoma	58	(56.9)	
Large cell carcinoma	13	(12.7)	
Pathologic staging group			
IA	26	(25.5)	
IB	40	(39.2)	
IIA	0	(0)	
IIB	24	(23.5)	
IIIA	10	(9.8)	
IIIB	2	(2.0)	

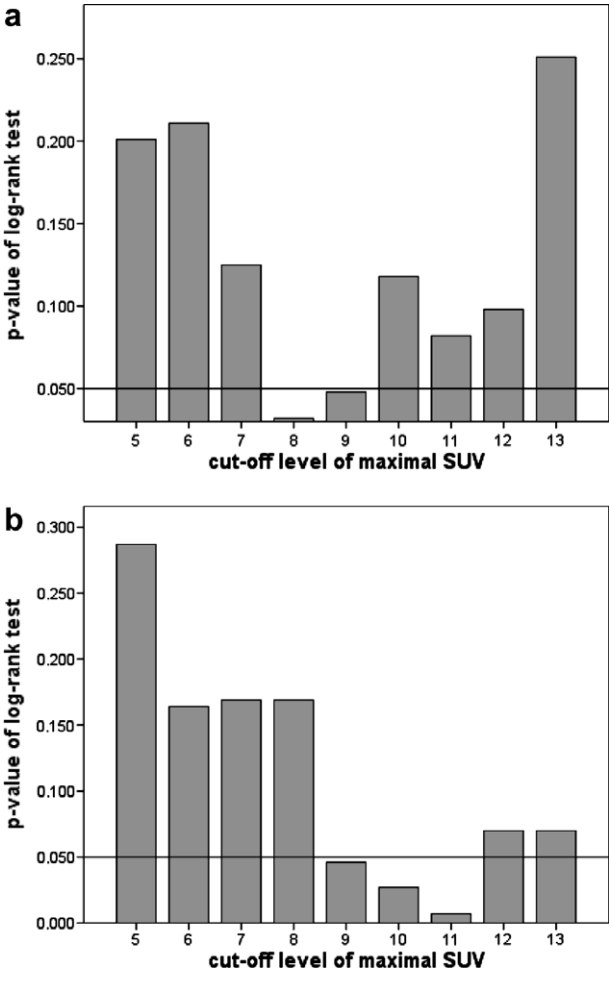

Fig. 1 – *p*-Values of log rank test for actuarial survival using different cut-off levels for SUV_{max} for the group of patients from Leuven (a) and from Maastricht (b).

while patients with a tumour showing an $SUV_{max} < 11$ showed a 2-year survival of 92.3% ($p = 0.007$) (Fig. 2b). The two groups of the different PET scanners showing a high uptake of FDG were combined and were referred to as high SUV_{max} group further on. The combination of the groups with an SUV <8 and <11, respectively, were referred to as low SUV_{max}. The combining of the groups showed a 2-year survival of 90.6% for the patients with a low SUV_{max} and of 58.6% for the patients with a high SUV_{max} ($p = 0.001$).

3.3. *PET data and immunohistochemical staining*

All immunohistochemical staining results were associated with SUV_{max} levels above and below the above-mentioned cut off points. Fig. 3 shows the different IHC staining patterns for the markers.

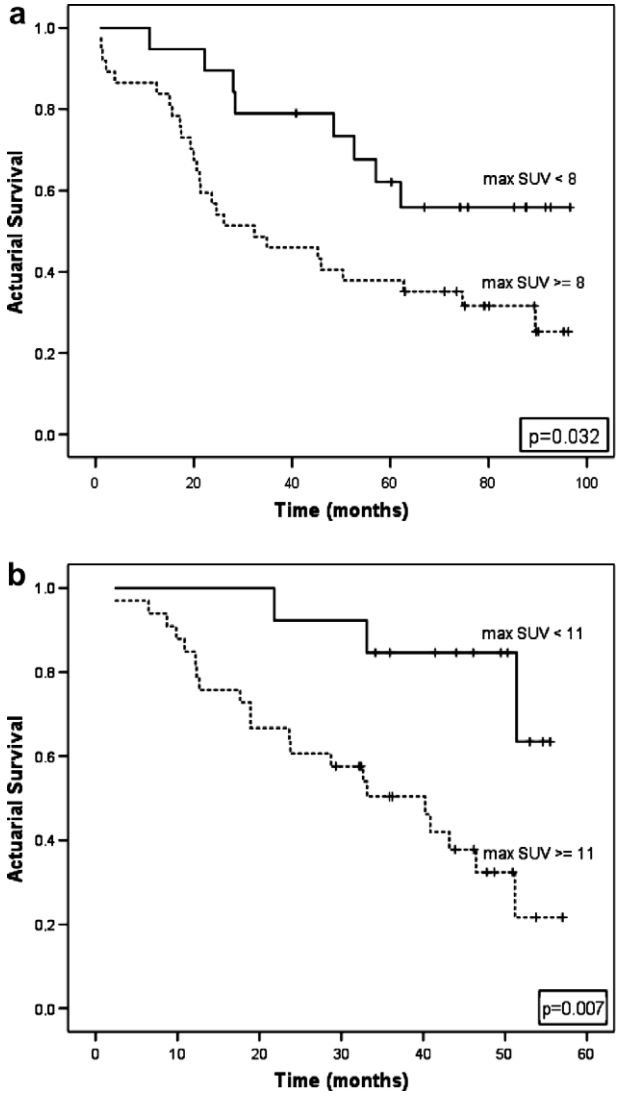

Fig. 2 – Kaplan–Meier survival curve for actuarial survival for maximal SUV (tumours with a high SUV_{max} versus tumours with a low SUV_{max}). The best discriminative cut-off level for Leuven was an SUV_{max} of 8.0 (a) and for Maastricht an SUV_{max} of 11.0 (b).

In total, 55% of the tumours showed positive nuclear staining of HIF-1α. Tumour samples with a high SUV_{max} showed positivity for HIF-1α in 63.1%, while only 37.9% of the samples with a low SUV_{max} showed nuclear staining ($p = 0.024$). Of all samples positive for HIF-1α 57% were positive for CAIX, whereas 77% of cases being positive for CAIX showed nuclear staining for HIF-1α. Although a correlation between HIF-1α and CAIX staining was observed (correlation coefficient: 0.363, $p = 0.001$), no significant difference in CAIX staining was observed between the high and low SUV_{max} groups (resp. 45.3% and 30.0% positive samples, $p = 0.16$).

For the proliferation marker Ki-67, the mean percentage of tumour cells being positive was 42.7% (SD: 21.7, range: 2.7–95.2%). In tumours with a high uptake of FDG 44.4% of cells were positive for Ki-67, while tumours with a low SUV_{max} showed a mean percentage 39.1% ($p = 0.20$).

For the glucose transporters, in total 76.5% of the tumours stained positive for GLUT-1 and 44.1% for GLUT-3. The tumours with a high SUV_{max} showed in 82.9% of cases membranous staining of GLUT-1, whereas tumours with a low uptake only in 62.5% ($p = 0.025$). For GLUT-3, there was no statistically significant difference ($p = 0.21$).

The association between SUV_{max} and the expression of the different markers and the correlation coefficients of the markers are depicted in Tables 2 and 3.

4. Discussion

Although the high FDG uptake in malignant tumours is due to an increased glucose metabolism, the exact mechanism by which FDG accumulates in malignant cells has not fully been unravelled.[17] Nevertheless, it was repeatedly shown that a high SUV_{max} is related to an inferior overall survival.[2–5,18]

This report studied the molecular mechanisms that might be involved in the prognostic role of SUV_{max} in stage I or II NSCLC surgical patients. As the patients were scanned in two departments, we used the best discriminative cut-off level for the SUV_{max} of each PET camera to create a high and low SUV_{max} group.[4,5] In this study, we confirmed a worse survival for patients with a high FDG uptake compared to a low uptake (2-year survival of resp. 58.6% and 90.6%, $p = 0.001$). Furthermore, we investigated the biological characteristics of the tumours with a high and low FDG uptake. We observed an association between the amount of FDG uptake and the endogenous marker of hypoxia (HIF-1α) and the glucose transporter GLUT-1. Although it would be of interest to asses the amount of variance in FDG uptake explained by the expression of hypoxia related markers, this was not feasible since SUV_{max} could not be used as a continue variable due to the use of different scanners. Nevertheless, to the best of our knowledge, we are the first to report an association between hypoxia related markers and SUV_{max} in lung cancer patients.[11,13,14] Indeed, Bos and colleagues investigated the correlation between different markers and the uptake of FDG in 55 breast tumours, but found no correlation between the HIF-1α and FDG uptake.[19] However, in their study a different cut-off point for HIF-1α expression (<1% or >1%) was chosen.

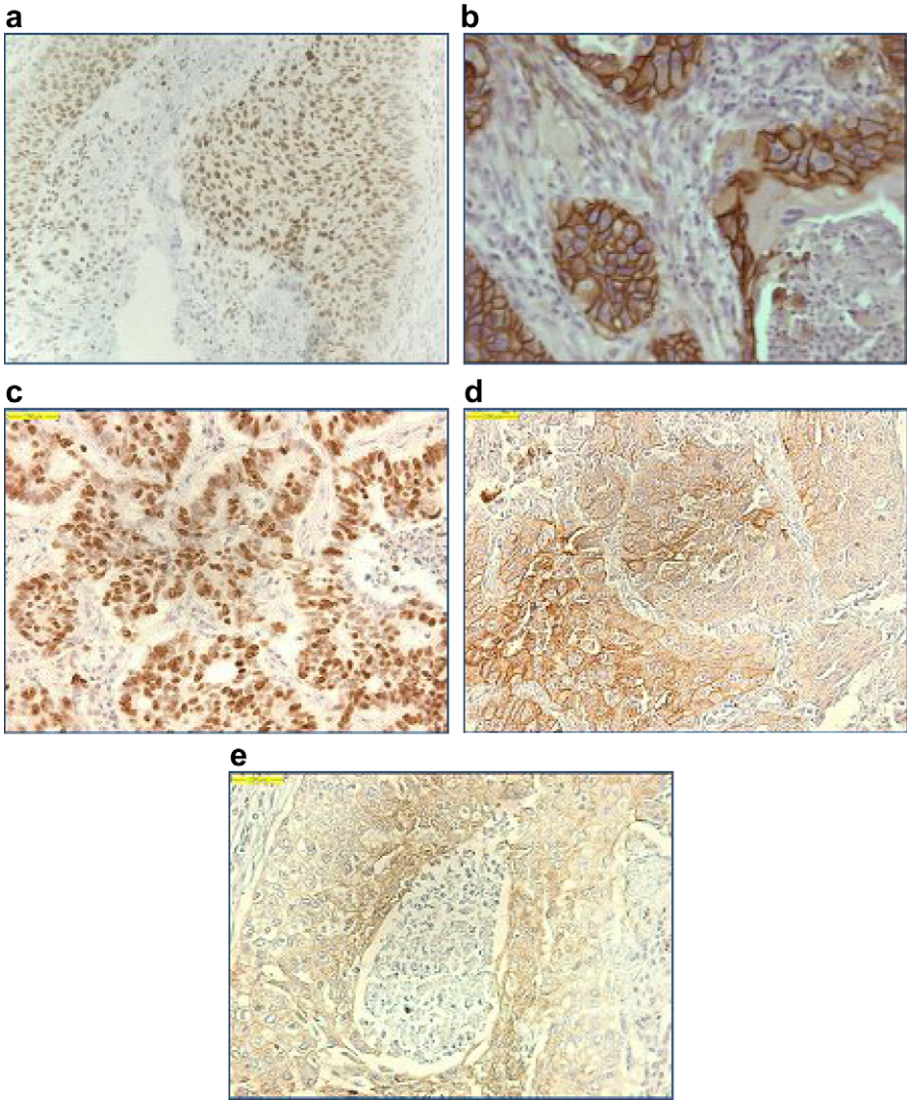

Fig. 3 – Immunohistochemical staining. Positive nuclear staining for HIF-1α (a), positive membranous staining for CAIX (b), nuclear staining of Ki-67 (c) and membranous staining positive for GLUT-1 (d) and GLUT-3 (e).

Table 2 – Relation between different immunohistochemical staining scores and the maximal SUV (SUV_{max}) of the tumour

IHC staining of surgical specimen	Low SUV_{max} ($n = 32$)	High SUV_{max} ($n = 70$)	(p-Value)
HIF-1α (% of tumours positive)	37.9%	63.1%	0.024[a]
CAIX (% of tumours positive)	30.0%	45.3%	0.16[a]
GLUT-1 (% of tumours positive)	62.5%	82.9%	0.025[a]
GLUT-3 (% of tumours positive)	53.1%	40.0%	0.21[a]
Ki-67 (MIB) (mean % of cells positive)	39.1%	44.4%	0.20[b]

a χ^2 test.
b Mann–Whitney U test.

Table 3 – Pearson correlation coefficients and p-value (between brackets) of immunohistochemical staining scores

	HIF-1α	CAIX	GLUT-1	GLUT-3
CAIX	0.36 (<0.01)			
GLUT-1	0.14 (0.19)	0.13 (0.21)		
GLUT-3	0.03 (0.79)	0.03 (0.80)	−0.19 (0.85)	
Ki-67 (MIB)	0.33 (<0.01)	0.36 (<0.01)	0.20 (0.04)	0.17 (0.10)

In relation to the staining for HIF-1α and CAIX, we observed similar results as Giatromanolaki and colleagues showing 80% of CAIX positive cells also staining positive for HIF-1α.[6] Hypoxia upregulates different genes, like CAIX,

GLUT-1 and GLUT-3, through the HIF-1α pathway.[20] However, different oxygen concentrations are required for induction of GLUT-1 and CAIX, which might explain the lack of association between SUV and CAIX, while finding a positive association between SUV and GLUT-1.[21]

For proliferative tumour activity, Veselle and colleagues showed in a study with 39 NSCLC patients a correlation between Ki-67 expression and SUV_{max}.[22] In contrast, we did not observe a correlation between the proliferation marker Ki-67 and the SUV_{max}. Although the tumours with a high uptake showed a somewhat higher percentage of Ki-67 positivity, this difference did not reach significance ($p = 0.20$). In line with our results, Chung and colleagues found no correlation of PET findings and the proliferation index, measured by flowcytometry, in NSCLC.[23] Moreover, an animal model for prostate cancer showed that a higher FDG uptake was indicative of tumour hypoxia but not for cellular proliferation.[14]

Although the expression of GLUT is not necessarily directly related to transport activity, hypoxia can increase GLUT-1 levels and glucose uptake.[24] In several tumour sites, for instance cervix, oesophagus and breast, a relation between expression of glucose transporters and FDG uptake has been observed. In NSCLC, contradictory findings have been observed. While the groups of Higashi and Mamede observed an association between the FDG uptake and GLUT-1, others did not find this correlation.[23,25–27] We could confirm a higher expression of GLUT-1 in the high SUV_{max} tumours (83%) compared to tumours with a low SUV_{max} (63%) ($p = 0.025$). The use of a different antibodies and/or different scoring methods may account for the difference in outcome of the studies.

In this study, a cut-off level of 8 and 11, respectively, were found as best discriminative cut-off levels. Findings of different cut-off levels can be explained by the usage of different PET machines and techniques.[2–5] We stress that the abovementioned cut-off levels are not generally applicable. To compare results between different scanners directly, calibrating is necessary. However, cut-off levels only represent a way to simplify a sliding scale in which a higher FDG uptake is associated with a worse survival.

The goal of this study was to investigate the biological background of a high uptake of FDG, which is correlated with a worse outcome. Our results show that a high uptake of FDG is associated with a higher HIF-1α and GLUT-1 expression. This finding might have therapeutic implications. Both clinical and preclinical studies have described the role of HIF-1α in therapy resistance.[28] Potential hypoxia-targeted therapies, like blockade of HIF-1α, are under investigation.[20,28,29] In the future, FDG-PET scans might be used for both selection of patients for different therapies and monitoring tumour response on these therapies.

In conclusion, the amount of uptake of FDG on the PET scan, as measured by the SUV_{max}, is associated with the expression of HIF-1α and GLUT-1, both upregulated under hypoxic conditions. This study provides evidence that not only *in vitro* but also *in vivo* hypoxia is associated with an increase in FDG uptake.

Conflict of interest statement

None declared.

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