Chapter 4

Feasibility study of voxel-by-voxel analysis for assessing tumor response with FAZA PET/CT in patients with advanced non-small-cell lung cancer (NSCLC)

ABSTRACT

Purpose: Hypoxia is unevenly distributed within malignancies and is associated with resistance to chemotherapy. Characterization of changes in intratumoral hypoxic regions is possible with specially developed PET tracers such as FAZA while tumor metabolism can be measured with FDG.

Materials and Methods: PET-CT with FDG and FAZA were performed in patients with NSCLC at baseline and after the second chemotherapy cycle. FAZA and FDG scans were 3-dimensionally aligned using IMALYTICS software with elastix toolkit. The primary tumors were outlined, and on the FDG scan volumes of interest (VOI) were drawn using a 41% adaptive threshold technique. Subsequently, the VOI was transferred to the FAZA scan. Maximum tumor to background (FAZA T/B), tumor to blood (FAZA T/Bl) ratios and the fractional hypoxic volume (FHV) were assessed. Measurements were corrected for partial volume effect. Finally, a voxel-by-voxel analysis of the primary tumor was performed to assess regional uptake differences.

Results: In the primary tumor of 7 patients, median FDG SUVmax decreased significantly \(p=0.05\). There was no significant decrease in FAZA uptake as measured with T/Bmax \(p=0.40\) or T/Blmax \(p=0.40\) or the FHV \(p=0.18\).

Additionally, volumetric voxel-by-voxel analysis showed that low hypoxic tumors did not feature a significant change in hypoxic status between baseline and after two cycles of chemotherapy, whereas highly hypoxic tumors did. Voxel-by-voxel analysis was unable to establish a relationship between hypoxia (T/Bl) and metabolism (SUV) at baseline \(R^2=0.25\) or after 2 cycles of chemotherapy \(R^2=0.18\).

Conclusion: By traditional analysis a decreased glucose metabolism but no hypoxic change in primary NSCLC tumors was observed after chemotherapy. Volumetric voxel-by-voxel analysis, by contrast, showed hypoxic changes after chemotherapy in highly hypoxic tumors. Voxel-by-voxel analysis did not show a relationship between hypoxia and metabolism, indicative that FDG is not an adequate tool for measuring hypoxia in this setting.
INTRODUCTION

Hypoxia is an important feature of malignant tumors with direct influence on the efficacy of chemo- and radiotherapy. It is unevenly distributed within tumors, which can be related to an abnormal vasculature and to an elevation in interstitial pressure associated with tumor growth [1]. Hypoxic tumor cells are associated with more aggressive phenotypes and with resistance to both chemo- and radiotherapy [2].

The gold standard in assessing tumor hypoxia is the polarographic Eppendorf electrode method. Unfortunately, this technique is invasive, and therefore, it can only be applied in well accessible superficial tumors. And it can measure oxygen concentrations at the site of the electrode only. Consequently, multiple punctures are necessary to measure heterogeneity in oxygen distribution within a tumor. These shortcomings may be overcome by using PET radiopharmaceuticals aimed at visualizing and quantifying hypoxia. Different radiopharmaceuticals have been developed, most of them based upon nitroimidazole compounds. The intracellular retention of these compounds is based upon the oxygen concentration with tissue retention (in vitro) in hypoxic conditions observed up to 28 times normal uptake [3, 4]. Nitroimidazole tracers are diffused through the cell membranes and then undergo reduction to yield radical anions. Under normoxic conditions, these radicals anions are reoxidized and diffuse out of the cell. However, under hypoxic concentration, re-oxidation cannot occur and the radicals are irreversible bound to macromolecules, resulting in increasing tracer accumulation under decreasing oxygen concentration. The most widely used PET hypoxia tracer from this group is fluoromisonidazole (FMISO). Another hypoxic tracer is fluoroazomycin arabinoside (FAZA) [5-9], when compared to FMISO has a more favorable signal to noise ratio [6, 10].

Hypoxia and glucose metabolism are distinct metabolic processes, yet closely interlinked through the hypoxia inducible factor (HIF). Consistent HIF pathway activation in tumors is related to the expression of two different receptors viz. GLUT-1 and GLUT-3 [11, 12]. There is a clear relationship between FDG uptake (especially) GLUT-1 and GLUT-3 expression [13]. This suggests an interdependency of both processes and consequently FDG uptake could function as a biomarker of hypoxia. Indeed, such a relationship has been shown [14] but a number of recent publications failed to show a clear relation between the two tracers [8, 15-17].

Traditionally in most patient studies, measurement of the maximum and/or mean standard uptake value (SUV), tumor to background mean ratio (T/B) and/or tumor to blood mean (T/Bl) ratio are used. Nowadays, total lesion glycolysis (TLG) and metabolic tumor volume (MTV) have also become popular [18-21]. Despite their widespread use, they all have a drawback in the sense that heterogeneity of
tumor metabolism is ignored. Alternatively, assessing localized treatment effects in the individual tumor areas is possible by using volumetric voxel-by-voxel techniques. Using these techniques, each individual PET voxel representing a predefined volume is anatomically aligned on CT and comparisons are made between subsequent PET/CT scans. This technique is already used clinically in other imaging modalities such as magnetic resonance imaging (parametric response mapping) [22, 23].

The purpose of this paper is to apply a voxel-by-voxel approach in a clinical setting and to compare the techniques with traditionally measured SUVmax, tumor to background (T/B) and tumor to blood (T/B) calculations. As the model for this comparison we chose to use patients with NSCLC, and to test it with 2 PET modalities: FDG and FAZA.

**MATERIALS AND METHODS**

**Patients**

Treatment naive patients with advanced NSCLC were selected. All patients underwent PET-scans with both FAZA and FDG at baseline and after 2 cycles of chemotherapy. The study was approved by the Institutional Review Board of the University Medical Center Groningen according to the ICH/GCP principles as is outlined under EU and Dutch law. All patients gave written informed consent prior to study participation.

**FDG-PET/CT**

FDG-PET was performed on a [Siemens, Germany] Biograph mCT 64. FDG images were reconstructed according to EANM guidelines [24]. Blood samples were taken before tracer injection to confirm acceptable blood glucose level (<11 mmol/l) after a minimal 4 hours fasting period. Patients were injected with 3 MBq/kg bodyweight of FDG intravenously. After 60 min, a scan was made from the mid-thigh to the brain. Scan times per bed position were dependent on patient weight; 1 minute per bed position if less than 60 kg, 2 minutes if between 60-90 kg and 3 minutes if above 90 kg[25].

Standard Uptake Values (SUV) was obtained by delineating the volume of interest comprising the entire tumor volume using the IMALYTICS Research Workstation (Philips Technologie GmbH Innovative Technologie, Aachen, Germany). SUVs were normalized for blood glucose levels. If the tumor size in the shortest axis was smaller than 30 mm, the SUVmax was corrected for partial volume based on historical data obtained with a NEMA phantom [15]. Tumor response was assessed according to the 1999 EORTC recommendations [26].
Contrast enhanced CT was performed on the Somatom CT which is part of the mCT in the same session. Scan time was 8 seconds, craniocaudally at inspiration. Effective mAs was 80, 120 kV with the care dose setting active. Slice thickness was 0.5 mm, pitch was 14 with a rotation of 0.5 seconds. Patients were injected with 55 ml of Iomeron contrast 350 mg/ml (Bracco Imaging Deutschland GmbH, Konstanz, Germany) at a speed of 2.5 ml/sec. The tumor size of the primary tumor and response was measured according to RECIST 1.1 criteria [27].

The baseline scan was performed before any therapy was given as part of the diagnostic workup. The scan after 2 cycles of chemotherapy was performed within 2 days after the second cycle of chemotherapy.

**FAZA-PET/CT**

FAZA-PET scans were made on the same mCT machine as described previously [15]. Patients received 370 MBq of FAZA intravenously. After 120 min, a scan was made from the mid-thigh to the brain. The same FDG scan patient position and contention system were used in order to improve reproducibility. FAZA T/Bgmax and T/Blmax were estimated in the same way as FDG SUV, using the FDG volume of interest (VOI) as will be discussed below. Partial volume correction was applied using the same method as for the FDG PET. Baseline scan was performed as soon as possible after inclusion (median 2 days after FDG PET). The scan after 2 cycles of chemotherapy was performed 2-3 days after the second cycle of chemotherapy (median 1 day after FDG PET).

**Image registration**

The IMALYTICS Research Workstation was used for deformable image transformations with the elastix toolbox (version 4.6) [28]. FDG and FAZA scans for each patient were matched at both baseline and after 2 chemotherapy cycles. The elastix non-rigid PET to PET transformation has been previously investigated and was found to have a good performance in a test-retest setting with no absolute differences in SUVmax or SUVmean [29]. The voxel-by-voxel analysis was also validated by our group in NSCLC patients (data submitted for publication). This validation was done by using the low-dose CT based images, the follow-up FDG images were aligned to the baseline FDG images and then aligned back to the follow-up FDG images in order to generate a realigned image. This was done in 39 patients. We found in this validation study that 94% of the voxels fell within the 95% range of the difference between original and realigned PET images.
Defining volumes of interest

In order to provide operator independent VOIs, calculations were performed on the FDG images using an adaptive threshold technique with a setting of 41% based on the study by Cheebsumon et al. [30]. For the background ratio, a VOI was manually defined in a tumor-free area in the mediastinum, not including the trachea, heart or vertebrae. These VOI were later transferred to the FAZA-PET/CT aligned images and was used as the reference background. Similarly, a VOI was drawn inside the thoracic aorta in order to calculate the T/Bl ratio. The fractional hypoxic tumor volume was defined as the fraction of the tumor exceeding a T/B ratio of 1.2.

Voxel-by-voxel analysis

After the alignment of the images, each voxel in a VOI is normalized to the mean background or mean blood value (obtained from corresponding VOIs) in order to calculate a T/B or T/Bl. This enables a regional comparison between FDG SUV and FAZA T/B or FAZA T/Bl respectively.

For regional comparison between baseline and follow up scans, the tumor VOI calculated from the baseline FDG was chosen and transported to the FAZA after image registration. Per patient 4 image reconstructions were made: between baseline and scans performed after 2 chemotherapy cycles of both FDG and FAZA, between FDG and FAZA at baseline, and between FDG and FAZA after 2 cycles.

Statistics

Pre and post chemotherapy FDG and FAZA scans using the maximum uptake values were analyzed with the Wilcoxon signed rank test. The voxel-by-voxel relationship between the FDG SUV and the FAZA T/B and T/Bl at baseline and after 2 cycles of chemotherapy was calculated using simple linear regression. All calculations were performed using SPSS 20.0 (International Business Machines Corp, Armonk, NY, USA).

RESULTS

Patient characteristics

Seven patients with advanced stage IV NSCLC were included. Six patients had adenocarcinoma and one had large cell lung carcinoma. Patient details are given in Table 1. Chemotherapy consisted of platinum containing doublets. Partial response according to RECIST was observed in 1 patient, stable disease in 4 patients, and progressive disease in 2 patients.
Pre and post chemotherapy differences in FAZA and FDG uptake using traditional (max and mean uptake) methods

FDG uptake (SUVmax) between baseline and after 2 cycles of chemotherapy decreased significantly (p=0.05) (Table 1). No difference in T/Bmax (p=0.40), T/Blmax (p=0.40) was observed when comparing FAZA uptake at baseline and after 2 cycles of chemotherapy.

Pre and post chemotherapy uptake analyzed voxel-by-voxel.

There was a no relationship between the baseline FDG SUV and FAZA T/B ($R^2=0.08$), and a weak relationship between the baseline FDG SUV and FAZA T/Bl ($R^2=0.25$) (figure 1A).

After 2 cycles of chemotherapy, no relationship was found between FDG SUV and FAZA T/B ($R^2<0.01$) and a weak relationship between FDG SUV and FAZA T/Bl ($R^2=0.18$) (figure 1B). Tumor response and regional hypoxic changes could not be analyzed due to small numbers.

In areas with low FAZA uptake at baseline a range of FAZA responses were seen with overall stable activity pre and post chemotherapy. In areas with high FAZA uptake reoxygenation became evident after chemotherapy. The decrease was more pronounced with the T/Bl ratio than T/B. A more evenly distributed decrease of FDG activity was observed after 2 cycles of chemotherapy (images not shown).

DISCUSSION

In this proof-of-concept study we analyzed the use of a voxel-by-voxel technique and compared it with traditional SUV, T/B and T/Bl calculations. Our results show that the technique can be used robustly in a clinical setting. Heterogeneity could be assessed and the effects of therapy varied with the metabolic status of the tumor. We established a weak relationship between FDG SUV and FAZA T/Bl in this study.

Our study is novel because it is to our knowledge the first study in which the effects of chemotherapy alone is studied on hypoxia in patients with NSCLC with FAZA using voxel-by-voxel analysis besides traditional maximum intensity methods. Our study also utilized both T/B and T/Bl and only found a weak relationship between metabolism and hypoxia when measured using T/Bl.
PET-based analysis of tumor glucose metabolism and tumor hypoxia before and during anti-neoplastic treatment

Hypoxia and metabolism: chemotherapeutic effects

Voxel-by-voxel analysis confirmed a weak relationship between these two tracers, and added information on tracer uptake heterogeneity. It showed heterogeneous responses on chemotherapy between the hypoxic and [high] metabolic tumor areas. We observed little to no overlap between hypoxia and metabolic activity at baseline. After treatment, the overall metabolic activity decreased, yet the distribution of hypoxic areas changed in the primary tumor independently of metabolism. Examination by volumetric voxel-by-voxel analysis revealed that tumor regions with initial high FAZA uptake tended to have a larger decrease in the FAZA uptake then regions with an initial low FAZA uptake, where a random distribution around the overall baseline activity was observed. For this reason, on figure 1B no decrease is seen in FAZA uptake, while a decrease in FDG is visible. A weak relationship only measurable using voxel-by-voxel analysis between hypoxia and metabolic tracers was found at baseline and after 2 cycles of chemotherapy, confirming the results of prior publications using traditional analytic methods [8, 15-17].

How to explain the lack of an association between the two tracers? Consistent HIF pathway activation is related to both GLUT-1 [11] and GLUT-3 [12] receptor expression in tumors, the same receptors of which the expression is directly related to FDG uptake [13]. Hypoxia causes increased HIF activation which is related to GLUT-1 and subsequently to FDG uptake [14]. Kaira et al. [14] investigated 140 patients and found a strong relationship between HIF and FDG uptake.

Different prior studies have shown a lack of a relationship between nitroimidazole PET tracers and metabolism. First, Gagel et al. [17] similarly to our study but using pre and post chemotherapy in NSCLC patients showed no relationship between tumor hypoxia and glucose metabolism measured with FDG PET. They furthermore showed that a decrease of FMISO uptake occurred after two cycles of chemotherapy and that unchanged or increased FMISO uptake corresponded to worse local tumor outcomes [17]. A second study showed response to chemoradiotherapy measured with FDG PET yet no response with FMISO PET [31]. A third study assessed the effects of chemoradiotherapy in NSCLC and after treatment the hypoxic lesions (measured with FAZA) resolved in the majority of patients [32]. Recently, our group did not find a relationship between FAZA and FDG in 11 untreated NSCLC patients [15]. Taken together, these four studies combined with this study indicate a lack of a relationship between metabolism and hypoxia in NSCLC, yet these studies did not perform a voxel-by-voxel analysis as we did.

Nitroimidazole compounds cannot differentiate between chronic and acute hypoxia. Acute (transient) hypoxia and chronic hypoxia can both co-exist within the same tumor [33]. Interestingly, an in vivo
study by Lin et al. [34] found that acute or chronic hypoxia had differential effects on the distinct HIF protein [34]. Furthermore, high and low levels of GLUT-1 expression were observed during acute and chronic hypoxia at 2% O₂ respectively whereas the level of GLUT-1 expression was similar at 1% O₂. The different effects of acute and chronic hypoxia on HIF proteins and subsequently on GLUT-1 expression provides an explanation why metabolism and hypoxia, while closely interlinked, are not necessarily simultaneously measurable using FDG and FAZA. This suggests that the hypoxic information gained with voxel-by-voxel analysis of FAZA PET could be seen as complimentary to the metabolic information provided by FDG PET.

Calculating hypoxia: blood, background ratio or voxel-by-voxel analysis

We used both the tumor to background as well as the tumor to blood ratios for FAZA, as there is yet no consensus which ratio better reflects hypoxia. Using traditional maximum intensity uptake methods, no relationship was observed between SUV and T/B, or between SUV and T/Bl. However, this study shows that volumetric analysis instead of single hypoxic spot analysis brings more understanding of tumor biology and characterizes the heterogeneity response towards treatment. This may have practical implications for using local treatments such as radiotherapy.

The drawback of T/Bl ratio is that metabolites which do not necessarily defuse into the “regular” background tissue are present and these metabolites still have activity. In a mouse model, T/Bl showed higher uptake ratio after 60 and 180 minutes compared with tumor to muscle [6]. The same study also showed rapid elimination via renal excretion and hepatic metabolism of the FAZA and its metabolites and after 60 minutes, approximately 73% of activity was due to unchanged FAZA [6]. One recent kinetic modeling study in humans showed that an image derived plasma input function (IDIF) correlated well with blood sampler-based plasma input function, indicative that an IDIF could be used without losing accuracy [i.e. T/Bl ratio] [35]. The authors showed that FAZA metabolism in humans is very slow, with less than a 10% decrease in 70 minutes [35]. For this reason, and because we observed only with T/Bl a weak relationship between hypoxia and SUV, we decided to only show images calculated with T/Bl ratio.

Limitations with FAZA imaging

At the start of this protocol, our group chose to use 3 minutes per bed position in order to perform whole body imaging as described previously. Only scanning the primary tumor (as is necessary for planning PET/CT for radiotherapy) opens up possibilities for full quantitative dynamic scanning and/or the use of 4D mode for future research. This limits the use of FAZA in whole body imaging for patients with metastatic disease.
No use was made in our study of 4D mode, because the choice was made to perform whole body scans. The extent of breathing motion in our study was minimal, as in 5/7 patients the tumor was in the upper and middle lobe respectively and 1 patient had a metastasis of the clavicle only.

CONCLUSION

No early changes during chemotherapy were observed with traditional hypoxic and metabolic imaging parameters in primary NSCLC tumors, except SUVmax. Using a volumetric analysis, a weak relationship between FAZA T/Bl and FDG SUV prior to and after chemotherapy was observed. Between baseline and 2 cycles of therapy using FDG the biggest decrease was seen in the primary tumor area with highest initial uptake, while in areas with initially low uptake stable to increased FDG was observed. Although to a lesser extent, a shift in hypoxic regions was observed within the primary tumor affecting high tumor hypoxia regions most, which was independently of glucose metabolism.

Therefore, hypoxia behaves in a way that is different to glucose metabolism during first-line treatment with chemotherapy in patients with stage III/IV NSCLC. FAZA PET offers additive tumor information to FDG PET. Further investigation in larger trials is needed to determine the complimentary clinical value of hypoxia imaging with FAZA in the setting of targeted treatment against hypoxic tumor cells.
**TABLE 1**: Comparison of the effect of chemotherapy on the primary tumor measured with FAZA and FDG PET including tumor response in non-small-cell lung cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>FAZA (T/B ratio)*</th>
<th>FAZA (T/Bl ratio) †</th>
<th>FDG (SUV&lt;sub&gt;max&lt;/sub&gt;) ‡</th>
<th>Fractional hypoxic volume</th>
<th>CT-scan</th>
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<tr>
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<td>Baseline 2 cycles</td>
<td>Baseline 2 cycles</td>
<td>Baseline 2 cycles</td>
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<td>27.3 18.2</td>
<td>78% 93% PD</td>
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<tr>
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<td>2.15 1.80</td>
<td>5.8 •</td>
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<td>1.01 1.64</td>
<td>8.9 6.0</td>
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</table>

* Uptake for the whole primary tumor was corrected for partial volume effect. It was then divided by the mean of the background activity and this is reported as a tumor to background (T/B) value.

† Uptake for the whole primary tumor was corrected for partial volume effect. It was then divided by the mean of the aorta activity and this is reported as a tumor to blood (T/Bl) value.

‡ Uptake for the whole primary tumor, expressed in SUV, was corrected for partial volume effect.

• Due to a dosimeter error, not usable for qualitative comparison.

CR=Complete response; PR=partial response; SD=stable disease; PD=progressive disease; NE=Not evaluable.
FIGURE 1: Comparison of regional voxel distribution in the primary tumor of FAZA (T/Bl) versus FDG [SUV] at baseline (A) and after 2 cycles of chemotherapy (B). Each tumor has a distribution lacking an association between hypoxia and glucose metabolism.

\[ R^2 = 0.25 \] for all primary tumor voxels. Each color represents one primary tumor at baseline; each point represents a tumor area of 38.4 mm³. No clear relationship is visible between hypoxia and metabolism.

\[ R^2 = 0.18 \] for all primary tumor voxels. Each color represents the same primary tumor as in 1A, but now after 2 cycles of chemotherapy. A decrease is seen in FDG, although no decrease is seen in FAZA activity.
ACKNOWLEDGEMENTS:

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REFERENCES


