Clinical Study
18F-fluoro-L-thymidine Positron Emission Tomography for Mucosal Head and Neck Squamous Cell Carcinoma Treated with Definitive Chemoradiation: A Pilot Study of Nodal Assessment and Tracer Safety

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We aim to assess the utility and safety of 18F-fluoro-L-thymidine- positron emission tomography (FLT-PET), in reference to 18F-2-fluoro-2-deoxy-D-glucose (FDG-PET) in the assessment of nodal involvement for mucosal head and neck SCC (HNSCC).

Methods. Ten patients with HNSCC receiving definitive chemoradiation (CRT) were enrolled. Baseline FLT-PET and FDG-PET were obtained. The total number of involved lymph nodes and ultimate nodal staging by the baseline FDG-PET and FLT-PET was compared. Receiver Operating Characteristics (ROC) analysis for the matched nodes was performed to identify an optimal maximal standardized uptake value (SUVmax) cutpoint. Results. The tracer uptake by the involved nodes on FDG-PET was higher than those judged to be involved by FLT-PET (mean SUVmax: 5.9 versus 3.4; 𝑃< 0.001). More abnormal lymph nodes were detected by FLT-PET than FDG-PET (Odds ratio = 3.67; 𝑃= 0.004). The optimal SUVmax cutpoint for FLT-PET to correspond with positive FDG-PET for the matched lymph nodes was 3.25 (range 3.1–3.4). Conclusions. It is unlikely that FLT-PET will be a more accurate staging investigation than FDG-PET. A SUVmax of 3.25 may be considered as a reference cut-off in determining if a cervical lymph node is involved for HNSCC. Validation in a surgical cohort with pathological correlation is warranted.

1. Introduction

Positron emission tomography using 18F-2-fluoro-2-deoxy-D-glucose (FDG-PET), generally now performed in conjunction with Computed Tomography (FDG PET/CT), has become a valuable part of modern oncological practice for a wide range of roles in a variety of malignancies [1, 2]. In the setting of mucosal head and neck squamous cell carcinoma (HNSCC), FDG-PET/CT is superior to conventional CT scanning for the detection of cervical lymph node metastases [3–5]. Other radiotracers offer the potential to biologically target various aspects of tumour behaviour. F-18 fluorothymidine (FLT) is a novel PET radiotracer that images cellular proliferation [6]. FLT is a radioisotopically labelled analogue of thymidine which is retained in proliferating tissues through the enzyme thymidine kinase 1 (TK1) that phosphorylates FLT to FLT-5 phosphate. This unique feature makes FLT a promising tracer with the potential to play a valuable role in oncology practice. In contrast to FDG, which is preferentially taken up by cells consuming glucose, FLT is predominantly taken up by...
cells that undergo increased cellular proliferation. The degree of proliferative activity can be quantified by the maximal standardized uptake value (SUVmax).

The role of FLT-PET in tumour staging was evaluated by several authors in the setting of lung malignancy, showing lesser tumour uptake compared with FDG [7–10]. In addition to diagnosis and staging, the role of FLT-PET in prognosis of malignancy has also been investigated in the setting of lymphoma and breast cancer [11–13]. In the setting of HNSCC, FLT-PET is reported to be an effective tool in detecting laryngeal cancer [14]. It has a diagnostic sensitivity similar to that of FDG-PET, although there is considerable physiologic background uptake in tissues such as bone marrow, and generally lower uptake by tumours. It is also suggested that FLT-PET may not effectively differentiate reactive from metastatic cervical lymph nodes due to the presence of proliferating cells in the germinal centre [15].

In terms of the safety of FLT as a diagnostic tracer, there has been minimal prospective report on this aspect although liver and bone marrow toxicities have been reported in the therapeutic use of FLT as an antiviral agent [16].

This pilot study describes our initial experience of FLT-PET in 10 patients with HNSCC treated with definitive CRT. More specifically, we examined the utility of FLT-PET in nodal staging compared with FDG-PET. The potential haematological and hepatic toxicities associated with FLT as a tracer were also examined. We also aim to correlate locoregional control with FLT uptake at the primary tumour.

2. Methods

This was a single arm phase 2 pilot study. Patients with stage III-IV HNSCC suitable for definitive radiotherapy to 70 Gy with concurrent cisplatin were eligible. All 10 patients received curative three dimensional radiotherapy. The gross tumour received a total dose of 70 Gy in 35 fractions over 7 weeks while bilateral nodal regions at risk received 50 Gy in 25 fractions over 5 weeks. All patients received concurrent Cisplatin at either 40 mg/m² (weekly) or 100 mg/m² (three weekly). Patients underwent baseline FDG-PET/CT and FLT-PET/CT imaging on separate days prior to commencing CRT. The diagnostic dose of FLT administered is 260 MBq ±5% for each scan. FDG was administered at a dose of 5.28 MBq/kg ±5%, according to actual patient weight. This study had institutional human research ethics committee approval. All 10 patients gave their informed consent prior to their inclusion in the study.

Inclusion criteria were previously untreated biopsy proven HNSCC of the oral cavity, oropharynx, hypopharynx, and larynx; stage III or IV (TNM staging, version 7) [17] suitable for definitive CRT; age ≥18 years; performance status ≤2; absolute neutrophil count ≥1.5×10⁹/L, platelet count ≥100×10⁹/L, and haemoglobin ≥9 g/dL; serum bilirubin <1.25 times upper limit of normal range (ULN) and Aspartate Aminotransferase/Alanine aminotransferase ≤2.5 times ULN; calculated creatinine clearance (Cockcroft-Gault) ≥55 mL/min; signed informed consent and available for followup for a minimum of 2 years after treatment.

Exclusion criteria were a diagnosis of nasopharyngeal carcinoma or paranasal cavity tumours and the presence of any distant metastases on conventional CT scan and/or FDG-PET; a history of previous malignancy or other synchronous malignancies excluding superficial basal cell carcinoma and squamous cell carcinoma; pregnant or lactating women; previous radiotherapy to the head and neck region; high risk for poor compliance with therapy or follow-up as assessed by investigator; unable to undergo PET studies.

Baseline blood tests including full blood count (FBC), liver function test (LFT), and coagulation profile were performed prior to the administration of FLT. Progress blood tests were repeated at 1 week after baseline FLT-PET, prior to commencing CRT. Hepatic and myelotoxicities were recorded using Common Terminology Criteria for Acute Toxicity Version 4.

Patients were fasted for four hours prior to the scan. For FDG scans, diabetic patients were asked to withhold insulin and oral hypoglycaemic drugs during this period, and a blood sugar level >11.1 mmol/L was considered a contraindication for the scan. Both FLT and FDG scans were performed following an uptake period of approximately one hour. The recorded uptake time for the first scan was then used for follow-up studies for the same patient. Injected activities were 5.3 MBq/Kg (min 250 MBq and max 550 MBq) for FDG and 250 MBq (without adjustment for weight) for FLT. All scans were performed on a Philips Gemini GXL 16 PET/CT scanner (Philips Medical Systems, Cleveland, Ohio, USA). Emission images were acquired for 2 minutes per bed position and reconstructed using the ordered subset expectation maximisation (OSEM) algorithm. Low dose CT (20–50 mA, 140 kV) was used for attenuation correction, and intravenous contrast materials were not used.

The specific activity of FLT was 294 (61–116) GBq/micro mole; mean (min–max). There was a QC limit of less than 1.54 micrograms/mL. FLT at the lowest acceptable specific activity of 7.4 GBq/mmol. The FLT dose to patients would be 35 nmol with an administered activity of around 250 MBq. This dose is 100 times lower than the dose used in therapeutic studies of nonradiolabelled FLT.

2.1. PET/CT Reading Plan. Each PET scan was anonymised and read by 3 separate nuclear medicine physicians blinded to each other. Each reader placed standard regions of interest (ROIs) at the location of what they considered to be the primary site and pathologically involved nodes. The adjudication was performed by an independent (4th) nuclear medicine physician, who had access to all the ROIs placed by the three blinded readers. The majority opinion (2/3) was considered as the final report. SUVmax was calculated by the independent expert based on the majority-accepted ROIs. Lymph node size was also measured for these sites on the CT dataset by an experienced head and neck radiologist. All PET scan reads were performed using Hermes multimodality viewing workstations.

2.1.1. Read 1 (Baseline FLT-PET/CT). Each reader identified and marked the primary tumour and all involved lymph
Table 1: Baseline characteristics of the study cohort. M: Male; F: Female.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Primary site</th>
<th>TNM stage on FDG/PET CT†</th>
<th>N stage on FLT/PET CT‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>73</td>
<td>Oral cavity</td>
<td>T4N0M0</td>
<td>2b</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>60</td>
<td>Glottis</td>
<td>T3N0M0</td>
<td>2c</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>69</td>
<td>Glottis</td>
<td>T4N2cM0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>56</td>
<td>Oropharynx</td>
<td>T2N2bM0</td>
<td>2b</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>55</td>
<td>Glottis</td>
<td>T4N1M0</td>
<td>2b</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>64</td>
<td>Hypopharynx</td>
<td>T3N2bM0</td>
<td>2c</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>49</td>
<td>Oropharynx</td>
<td>T2N2bM0</td>
<td>2b</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>59</td>
<td>Oropharynx</td>
<td>T1N1M0</td>
<td>2c</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>52</td>
<td>Oropharynx</td>
<td>T2N2bM0</td>
<td>2b</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>39</td>
<td>Oropharynx</td>
<td>T4N0M0</td>
<td>2c</td>
</tr>
</tbody>
</table>

†18F-2-fluoro-2-deoxy-D-glucose positron emission tomography with computed tomography.
‡18F-fluoro-L-thymidine positron emission tomography with computed tomography.

nodes (if greater than 10 then only the 10 nodes with highest SUVmax were used). Readers were provided with clinical information and anonymised CT/MRI reports.

2.1.2. Read 2 (Baseline FDG-PET/CT). Readers were blinded to the baseline FLT-PET scan. To minimise intraobserver bias from recall of previous scans, a 4-week gap was applied between read 1 and read 2. Each reader identified and marked primary and all involved lymph nodes (if greater than 10 then only the 10 nodes with highest SUVmax were used). Readers were provided with clinical information and anonymised CT/MRI reports.

2.1.3. Independent Expert. After read 1 and read 2 interpretations by the 3 readers, the independent expert performed the following.

1. Obtained the SUVmax of the primary tumour.
2. Checked all reads and on a node by node basis determined (on a majority of reader basis) whether a node is involved.
3. Ensured all involved nodes had Region of Interest (ROI) drawn and uniquely identified. This unique identification number remained associated with this node throughout the course of all studies (and was common across FLT and FDG studies before, during and after treatment).
4. Determined and recorded SUVmax of involved nodes for both FLT and FDG studies.
5. Indicated to a diagnostic head and neck radiologist which nodes were involved. The radiologist then determined the node size. FDG and FLT N-staging was then able to be assigned using AJCC version 7 [17].

2.2. Statistical Analysis. The mean SUVmax of the involved nodes was calculated for the baseline FDG-PET/CT and baseline FLT-PET/CT. The total numbers of involved lymph nodes on the two baseline scans were counted. The significance level of the difference mentioned above was calculated using paired t-test and McNemar’s test for matched proportions. A two-sided P value of ≤0.05 was considered significant.

The sensitivity and positive predictive value of FLT-PET/CT on the detection of potentially involved lymph nodes relative to FDG-PET/CT were calculated.

The Receiver Operating Characteristics (ROCs) analysis was based on the subset of matched nodes so we have positive/negative pairings by FLT versus FDG. The ROC analysis used FDG positive/negative as the gold standard and aligned these with SUVmax values for FLT to define an optimal cutpoint of SUVmax for FLT. This allowed the prediction of FDG positivity/negativity based on combined sensitivity and specificity.

The rate of locoregional control was calculated actuarially using Kaplan Meier’s method.

3. Results

Ten patients were enrolled over a 9-month period between April 2008 and January 2009. The baseline characteristics of the ten patients are shown in Table 1. The median followup (from the date of consent to the date last seen by clinicians) was 40 months (range: 33–45 months).

There was no locoregional failure observed so the rate of locoregional control was 100%. Patient 5 developed a metachronous solitary speculated non-small cell lung cancer (SCC) who later on developed bone metastasis and died. His larynx and neck remained free of disease. The solitary nature and the speculated appearance on CT were highly suggestive of a primary lung cancer rather than a pulmonary metastasis. Patient 10 developed a right sided apical lung tumour below the clavicle, just below the initial head and neck radiotherapy. He was treated with surgery followed by postoperative chemoradiation. Unfortunately, he now developed drop metastasis to the right diaphragm and may have that surgically excised. As there was recurrence in the head and neck region for the cohort, we were not able to correlate FLT uptake to locoregional control.
Of all the blood tests collected pre- and postbaseline FLT-PET/CT, patient 7 developed grade 2 decreased lymphocyte count (dropped from 2.35 to 0.75 x 10⁹/L) and patient 3 had grade 1 gamma-glutamyl transpeptidase rise (increased from 48 to 72; reference <55). These findings were not clinically significant and did not affect the administration of chemotherapy. Patient 3’s increase in gamma-glutamyl transpeptidase resolved by day 20 after FLT. Patient 7’s lymphopenia resolved by day 9 after FLT.

3.1. Nodal Staging. On consensus expert visual interpretation, FDG-PET/CT identified 17 positive lymph nodes at baseline, while FLT-PET/CT detected 33, with 11 of these identified by both methods. The difference in unique identification was significantly higher for FLT with an odds ratio of 3.7 and 95% confidence interval of 1.5–9.0 (P = 0.004). The nodal stage was determined for all 10 patients on both FDG-PET/CT and FLT-PET/CT (Table 1). Overall, FLT agreed with FDG in 3 cases (30% concordance) but “upstaged” nodal disease in 6 cases (60%) and one case was “downstaged” (10%). Examples of FLT disagreed with FDG and FLT were illustrated in Figure 1.

The "sensitivity" and the “positive predictive value” of FLT-PET/CT on nodal staging relative to FDG-PET/CT were 65% and 33%. The specificity and negative predictive value could not be calculated for the general nodal status as the absolute number of negative lymph nodes in the neck could not be quantified. Figure 1 illustrated mismatches of nodes between FDG-PET/CT and FLT-PET/CT.

In general, lymph nodes identified by the scan readers to be involved by FDG-PET/CT had significantly higher uptake (mean SUVmax = 5.9, range: 2.4–10.2) than those by FLT-PET/CT (mean SUVmax = 3.4, range: 1.7–9.9) with a P value <0.001. Among nodes judged positive by FLT-PET/CT, the mean SUVmax values for nodes also positive on FDG-PET/CT were higher than for the nodes negative by FDG (4.8 v 2.6; P = 0.003).

ROC analysis for the SUVmax values for 33 FLT-PET positive nodes was performed and classified by whether these nodes were positive (II) or negative (22) for FDG-PET, which was taken as the gold standard. The ROC curve had an area under the curve of 0.80 (95%CI 0.63,0.97). The optimal cut point for FLT to correspond with positive FDG was 3.25 (range 3.1–3.4). This point maximises the sum of sensitivity and specificity (0.64 and 0.91). Hence using only SUVmax values >3.25 for FLT as positive, there are only 9 FLT positives and 7 of these correspond with FDG positives. The difference in unique identification is now significantly higher for FDG with odds ratio of 5.0 (95% CI 1.0–25.8) and P = 0.039. At this cutpoint, sensitivity of FLT to predict FDG was lowered from 65% to 41% but positive predictive value was increased from 33% to 78%.

4. Discussion

FLT has previously been used as an antiviral therapy for patients with immunodeficiencies. When administered at therapeutic doses, it has been reported that FLT can cause haematological toxicities within 4 weeks of treatment [16]. There has been no objective evidence to suggest such toxicities when FLT is administered at a diagnostic dose. Juweid et al. indicated that diagnostic use of FLT at a tracer dose is unlikely to cause hepatorenal and haematological toxicities [18]. Two of our patients were recorded to have mild hepatic and haematological toxicities at one week after FLT but recovered later on. Our study supported the notion that intravenous administration of FLT at a diagnostic dose does not result in clinically relevant hepatic and/or haematological toxicities at 1 week after the tracer injection. Delayed toxicity measurements would not be interpretable as all 10 patients were commenced on concurrent Cisplatin.

We adopted a meticulous reading plan which involved four experienced nuclear medicine physicians and a head and neck radiologist. Time lapses were introduced between reads to minimise intraobserver bias from recall of previous scans. Thus, we felt confident that the data generated is minimally biased and has internal validity.

The ultimate gold standard in nodal staging of mucosal head and neck patients is surgical neck dissection. As our patients were all treated by definitive curative CRT, surgical confirmation on the number of positive and negative lymph nodes was not possible. For practical reasons, we did not subject our patients to routine fine needle aspirations for each positive lymph node on PET scans as this would add to patients’ already intense pretreatment schedule. Often the lymph nodes in question were small and close to carotid arteries which were technically difficult to sample. The next best modality to stage the nodal status was FDG-PET/CT which was used as a surrogate. The sensitivity and specificity of FDG-PET/CT in nodal staging of mucosal head and neck cancer have been reported to range between 81.1%–100% and 77%–98.2%, respectively [19–23]. In reference to surgical neck dissection, FDG-PET is generally found to be slightly superior to CT and/or MRI [19–23].

The sensitivity of FLT-PET/CT for detection of nodal involvement relative to FDG-PET/CT was 65% while the positive predictive value was 33%. We were not able to determine the specificity and negative predictive value as the number of negative lymph nodes cannot be quantified without neck dissection. Significantly more positive lymph nodes were identified on FLT-PET/CT than on FDG-PET/CT which led to the low positive predictive value. Our finding is consistent with other series suggesting low positive predictive value (37.5%) of FLT-PET/CT in nodal staging of mucosal head and neck SCC using pathology findings from neck dissections as the gold standard [15]. Another possible explanation is that our nuclear medicine physicians were overly sensitive in FLT scan interpretation of the lymph nodes, as this tracer is not in routine clinical use in our institution. In contrast, FDG-PET/CT has been used for staging of HNSCC for many years, so most nuclear medicine physicians can recognise mildly FDG-avid reactive lymph nodes that may not contain tumour.

We used SUVmax for semiquantitative assessment of FDG uptake. Using a SUVmax of 3.25 for FLT-PET/CT as the cutpoint yielded the highest sum of sensitivity and specificity
in reference to FDG-PET/CT. We suggest using this value as a reference cut-off in future interpretation of HNSCC with caution. While SUVmax is widely used and quoted in the literature, there are well-known limitations and pitfalls. These include variables related to the tracer uptake time, scanner and image reconstruction factors, and normalisation factors. The use of SUVmax rather than average SUV somewhat mitigates against errors due to location and size of ROI’s used for image analysis. While it is possible to compare SUVmax results within and between different scanners and institutions, this should only be undertaken with caution and when due diligence is given to quality assurance and calibration procedures. Further prospective larger surgical series with pathological verification of neck dissection will be required to validate this value.

As there was no locoregional failure in the head and neck region, we were not able to correlate treatment outcome to tumour uptake on FLT-PET. Our hypothesis was that tumour with higher FLT uptake would respond less effectively to radiation due to more rapid proliferation. While the
absence of in-field locoregional failure was a great outcome for patients involved in the study, we were not able to identify a group of patients on the basis of FLT-PET whom were destined to fail chemoradiation.

5. Conclusion

Given the potential for reactive benign nodes to exhibit FLT uptake at baseline, we conclude that it is unlikely that FLT-PET will prove to be a more accurate staging investigation than FDG-PET. A SUVmax of 3.25 may be considered as a reference cut-off in determining if a cervical lymph node is involved for HNSCC. FLT used as a diagnostic tracer was safe and did not cause clinically significant hepatic and/or haematological toxicities. The prognostic value of FLT-PET could not be evaluated in our study due to the lack of locoregional failure.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References

