Use of $[^{11}C]$Choline PET-CT as a Noninvasive Method for Detecting Pelvic Lymph Node Status from Prostate Cancer and Relationship with Choline Kinase Expression

Kaiyumars Contractor$^1$, Amarnath Challapalli$^1$, Tara Barwick$^2$, Mathias Winkler$^1$, Giles Hellawell$^1$, Steve Hazell$^3$, Giampaolo Tomasi$^1$, Adil Al-Nahhas$^2$, Paola Mapelli$^1$, Laura M. Kenny$^1$, Paul Tadrous$^4$, R. Charles Coombes$^1$, Eric O. Aboagye$^1$, and Stephen Mangar$^1$

Abstract

**Purpose:** To evaluate the accuracy and biological basis for $[^{11}C]$choline-PET-CT in the nodal staging of high risk localized prostate cancer patients.

**Experimental Design:** Twenty-eight patients underwent dynamic $[^{11}C]$choline-PET-CT of the pelvis and lower abdomen prior to extended laparoscopic pelvic lymph node dissection (eLPL). The sensitivity and specificity of $[^{11}C]$choline PET, $[^{11}C]$choline PET-CT, and MRI for nodal detection were calculated. Average and maximal standardized uptake values (SUV$_{\text{ave}}$, SUV$_{\text{max}}$) were compared with choline kinase alpha (CHK$\alpha$) and Ki67 immunohistochemistry scores.

**Results:** Four hundred and six lymph nodes (LN), in 26 patients, were assessable. Twenty-seven (6.7%) involved pelvic nodes at eLPL were detected in 9 patients. Seventeen of the 27 involved nodes were subcentimeter. The sensitivity and specificity on a per nodal basis were 18.5% and 98.7%, 40.7% and 98.4%, and 51.9% and 98.4% for MRI, $[^{11}C]$choline PET, and $[^{11}C]$choline PET-CT, respectively. Sensitivity was higher for $[^{11}C]$choline PET-CT compared with MRI ($P = 0.007$). A higher nodal detection rate, including subcentimeter nodes, was seen with $[^{11}C]$choline PET-CT than MRI. Malignant lesions showed CHK$\alpha$ expression in both cytoplasm and nucleus. SUV$_{\text{ave}}$ and SUV$_{\text{max}}$ strongly correlated with CHK$\alpha$ staining intensity ($r = 0.68$, $P < 0.0001$ and $r = 0.63$, $P = 0.0004$, respectively). In contrast, Ki67 expression was generally low in all tumors.

**Conclusion:** This study establishes the relationship between $[^{11}C]$choline PET-CT uptake with choline kinase expression in prostate cancer and allows it to be used as a noninvasive means of staging pelvic LNs, being highly specific and more sensitive than MRI, including the detection of subcentimeter disease.

Introduction

The evaluation of lymph nodes (LN) has important therapeutic and prognostic significance in patients diagnosed with prostate cancer. Although a curative approach can be adopted for those with organ-confined node-negative disease with modalities such as surgery, external beam radiotherapy, or brachytherapy, those with node-positive disease ultimately relapse with metastatic disease (relapse rate 30%–50% at 5 years, 90% at 10 years; refs. 1, 2). As such, the presence of LN involvement reduces the 5-year disease-free survival from 85% to approximately 50%, with a shift in focus of treatment to long-term androgen deprivation with the addition of pelvic radiotherapy to reduce loco-regional recurrence (3, 4). Pelvic LN dissection is currently the gold standard for evaluating the presence of nodal involvement (5, 6). This procedure can either be open or laparoscopic and is usually limited to the external iliac and obturator nodes, though a more extended procedure to include the internal iliac nodes is usually advocated for those with a higher risk of nodal disease (7). Either way, both these methods are invasive, associated with morbidity (8) and, importantly, may not be able to sample all potential LN areas.

It is thus important to have a sensitive and reliable noninvasive means of detecting nodal involvement. The criteria for nodal characterization using cross-sectional imaging, such as computerized tomography (CT) or...
Translational Relevance

This study establishes the feasibility of using [11C]choline-PET CT as a noninvasive means of staging pelvic lymph nodes in high-risk prostate cancer, being highly specific and more sensitive than PET alone or MRI, including the detection of subcentimeter disease. The high specificity could potentially be helpful clinically in terms of selecting out patients who may not require pelvic radiotherapy. We also showed, for the first time in prostate cancer biopsies, that tumor radio-labeled choline uptake is related to choline kinase alpha expression in prostate cancer. This relationship could be exploited to develop new drugs for prostate cancer.

Magnetic Resonance Imaging (MRI), relies primarily on morphologic assessment on the basis of size and shape, with a nodal short axis diameter of 1 cm generally accepted as an upper limit of normal. A threshold of 1 cm in the short axis diameter for oval nodes and 0.8 cm for round nodes has been recommended as criteria for diagnosis of prostate cancer nodal metastases (9). A recent meta-analysis on the diagnostic accuracy of cross-sectional imaging in the staging of pelvic LNs in prostate cancer reported a high pooled specificity for MRI of 0.82 with a low and heterogenous pooled sensitivity of 0.39 (10). The lack of sensitivity belies the fact that nodal involvement is not always correlated with enlargement and enlarged nodes may also be due to a benign etiology. Neither MRI nor lymphangiography has shown higher sensitivity than CT scanning in the detection of nodal metastases (10, 11). The use of an MR contrast agent containing ultrasmall particles of iron oxide (ferumoxtran-10-Sinerem, USPIO) has been shown to yield sensitivity and specificity above 90% in the detection of prostate cancer LN metastases (12). However, this is not widely available and its intravenous infusion is not without side effects (13). While further studies using diffusion weighted MR undoubtedly have improved intraprostatic tumor detection and localization, this method has been less satisfactory for assessing pelvic nodal disease (14).

Positron emission tomography (PET) offers functional information about tissue activity, thereby having the potential to provide superior staging information as well as the ability to monitor the response to treatment. The clinical experience with [18F]fluorodeoxyglucose (FDG) PET in prostate cancer is limited due to variable uptake of [18F]FDG in prostate cancer and the rapid excretion of FDG in urine, causing an accumulation of activity in the bladder (15–17). [11C]choline is a relatively new radiopharmaceutical for PET imaging, and its utility in visualizing and staging prostate cancer has been published (18, 19). Malignant transformation is postulated to be associated with changes in pathways of choline transport, utilization, and increased choline kinase alpha (CHKα) expression that will lead to an increased uptake of choline (20, 21). As illustrated by a number of MR Spectroscopy studies (21–23), CHKα converts choline to phosphocholine in cells that is elevated during transformation and progression. The tumor PET signal from [11C]choline, however, comprises of free [11C]choline and [11C]phosphocholine, as well as the oxidation product, [11C]betaine (24). The PET signal (tumor [11C]choline uptake), therefore, largely reflects transport and phosphorylation of [11C]choline and, to a lesser extent (given that liver and kidneys produce most of the circulating [11C]betaine), [11C]choline oxidation. Unlike [18F]FDG, it has low renal elimination and therefore, visualization of the prostate and surrounding nodes may be enhanced by the low accumulation of tracer within the bladder (16). Preliminary studies of [11C]choline-PET in pelvic nodal staging in prostate cancer patients have shown early promise (25–27). However, no study to date has established a direct relationship between CHKα expression and [11C]choline uptake in prostate tumors.

This prospective study compares the use of [11C]choline PET-CT with MRI in determining pelvic nodal status in patients with high risk localized prostate cancer undergoing surgical staging with extended laparoscopic pelvic lymph node dissection (eLPN; reference standard). We also sought to document the early kinetics of [11C]choline from dynamic imaging up to 60 minutes postradiotracer injection. In addition, the association between [11C]choline uptake [standardized uptake values (SUV)] and immunohistochemistry scores for CHKα and Ki67 expression in prostate tumors and involved nodes were compared.

Materials and Methods

Patients

Patients with histologically confirmed prostate cancer staged as either high risk localized (either prostate-specific antigen (PSA) >20 ng/mL or Gleason score 8 to 10 or TNM stage ≥ T2)/locally advanced (nodal disease on staging MRI of the pelvis) were eligible for the study. Patients with visceral or bone metastases were ineligible. Ethical approval for the study was granted by the Hospital Research Ethics Committee. All patients gave written informed consent to participate in the study, which was carried out according to the Declaration of Helsinki guidelines. The administration of radioactivity for the PET scans was approved by the Administration of Radioactive Substances Advisory Committee, United Kingdom.

Imaging protocol

[11C]choline was synthesized at Hammersmith Imanet according to the method described by Pascale and colleagues (28). To minimize post-biopsy effects, all imaging studies were done at least 6 weeks after the transrectal biopsy. Subjects were asked to fast for 6 hours prior to the procedure (as bowel choline uptake interferes with interpretation of [11C]choline images). All patients were scanned on a PET-CT (GE-Discovery RX) scanner after being
positioned such that the field of view (FOV) included the whole pelvis and the lower abdomen. This was followed by a diagnostic quality CT scan (settings were 300 mA, 120 kVp, 0.8 sec/rotation i.e., 65 mAs, 8 × 2.5-mm slices and pitch 1.35), which was used for attenuation correction and coregistration with the PET images. [11C]choline was administered by a bolus intravenous injection over 10 to 30 seconds. PET scanning (3-dimensional acquisition) was commenced over 2 bed positions (3 minutes per bed position) starting from the distal margin of the pelvic floor, covering the pelvis, and lower abdomen (axial FOV per bed position, 15.7 cm; transaxial, 70 cm) for 65 minutes. Raw PET data were corrected for scatter and attenuation and reconstructed with an iterative OSEM (ordered subset expectation maximum) algorithm comprising 8 iterations and 21 subsets. Decay corrected images were then viewed using Analyze software (Analyze Version 7; Biomedical Imaging Resource). From summed images, regions of interest (ROI) were drawn manually around visible tumors in the prostate, and any visible pelvic nodes. The [11C]choline radioactivity concentration within the ROIs was then determined and normalized for injected radioactivity and body weight to obtain SUVave and SUVmax.

MRI acquisition

All patients underwent standard noncontrast staging MRI of the pelvis from aortic bifurcation to pubic symphysis comprising of T1-weighted axial images; axial, sagittal, and coronal T2-weighted images and small FOV axial T2-weighted images through the prostate. The imaging was done on a 1.5-Tesla Philips scanner in 5 patients and a 1.5-Tesla Siemens-Magnetom scanner in 21 patients.

Extended laparoscopic extraperitoneal pelvic lymphadenectomy

This was done in a standard predefined protocol by the Urologists within an average of 22 days (2–49 days) of the [11C]choline PET-CT. Nodal status was discussed with the surgeon before lymphadenectomy using information from both MRI and the [11C]choline PET-CT images. The eLPL included nodes along the external and internal iliac vessels to the ureter proximally, obturator nerve medially, and the genitofemoral nerve laterally. All nodes removed were carefully labeled for size and anatomical location. Nodes were fixed, paraffin embedded, stained with hematoxylin and eosin, and reported as negative or positive for metastasis by a histopathologist with a specialist interest in urologic malignancy. The samples were also subjected to additional immunohistochemistry with Ki67 and CHKα (vide infra).

Image interpretation

The images of the [11C]choline PET-CT were interpreted prospectively to outline the ROIs, carry out SUV analysis, and discuss outcome with surgeons preoperatively. Furthermore, all the imaging data (MRI, [11C]choline PET and [11C]choline PET-CT) were pooled and evaluated by a dual accredited nuclear medicine radiologist, blinded to the results of the histopathology, on separate occasions to avoid reporting bias. The criteria used for assessing nodal involvement are given in Table 1.

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Criteria for nodal involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>Size ratio criteria&lt;sup&gt;a&lt;/sup&gt; (9, 12)</td>
</tr>
<tr>
<td></td>
<td>Benign: nodes less than 8 mm short axis</td>
</tr>
<tr>
<td></td>
<td>Malignant: nodes &gt;10 mm short axis and round nodes &gt;8 mm (ratio of the short to long axis &gt;0.8)</td>
</tr>
<tr>
<td>[11C]Choline PET</td>
<td>Focal uptake outside the normal physiologic distribution of tracer in locations corresponding to nodal chains</td>
</tr>
<tr>
<td>[11C]Choline PET-CT</td>
<td>Nodes with increased tracer uptake above the background, even when &lt;10 mm in short-axis diameter</td>
</tr>
</tbody>
</table>

Table 1. Criteria for nodal involvement and ROC analysis

<table>
<thead>
<tr>
<th>Scale</th>
<th>MRI</th>
<th>[11C]Choline PET/PET-CT&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nodes &lt;4 mm or not seen</td>
<td>Definitely normal</td>
</tr>
<tr>
<td>2</td>
<td>Nodes = 4–5.9 mm</td>
<td>Probably normal (more likely to be physiologic)</td>
</tr>
<tr>
<td>3</td>
<td>Nodes = 6–7.9 mm</td>
<td>Indeterminate (equally physiologic/pathologic)</td>
</tr>
<tr>
<td>4</td>
<td>Nodes &gt;8 mm but &lt;10 mm</td>
<td>Probably abnormal (more likely to be pathologic)</td>
</tr>
<tr>
<td>5</td>
<td>Nodes ≥10 mm</td>
<td>Definitely malignant</td>
</tr>
</tbody>
</table>

<sup>a</sup>Short-axis and long-axis diameters of the identifiable LNs were measured using electronic calipers on the scanner console.

<sup>b</sup>Definitely normal, probably normal, and indeterminate were considered benign and probably abnormal and definitely abnormal were considered malignant.
Immunohistochemistry

CHK immunohistochemistry was carried out using a primary polyclonal, human anti-CHKα antibody (catalogue no. HPA024153; Sigma-Aldrich), as per manufacturer’s instructions. The positive control used was bronchial tissue as per manufacturer’s specifications. Slides were then scored independently by 3 pathologists using the intensity of cytoplasmic and nuclear staining in prostate tumor cells from 1 to 3 (1+, low intensity; 2+, moderate intensity; 3+, high intensity including nuclear staining). Ki67 staining was done using anti-Ki67 antibody (NCL-Ki67-MM1; Novocastra Laboratories). Tonsil tissue was used as a positive control. Ki67 score was determined by dividing the total number of Ki67-positive tumor cells with the total number of tumor cells counted in 8 high powered fields (200× magnification) using an Olympus microscope. The final score was expressed as a percentage.

Statistical considerations

The mean, SD, medians, range, and frequencies were used as descriptive statistics. The sensitivity, specificity, and number of correctly recognized cases with MRI, \[^{11}C\]choline PET and \[^{11}C\]choline PET-CT in nodal detection were calculated for a per patient and per node analysis. The comparison of each imaging method was done using the McNemar test implemented in its uncorrected exact form, on the basis of binomial distribution (29). Receiver operating characteristic (ROC) analysis and the area under the curve (AUC) was determined by recalculating sensitivity and specificity for MRI, PET, and PET-CT along the 5-point grading scale for a per patient and a per nodal analysis using MedCalc statistical software (version 11.6.1). SUV\(_{60,\,\text{ave}}\) and SUV\(_{60,\,\text{max}}\) were compared with CHKα and Ki67 scores using Spearman’s correlation test and a P value of ≤0.05 was considered significant.

Results

Patients

Twenty-eight patients underwent \[^{11}C\]choline PET-CT after fulfilling the inclusion criteria. Two patients could not undergo surgery after \[^{11}C\]choline PET-CT as one became unwell and the other changed his mind about undergoing surgery. Thus 26 patients underwent \[^{11}C\]choline PET-CT followed by ePL/sampling (1 had LN sampling rather than dissection due to fibrotic and calcified LNs). All patients subsequently had neoadjuvant androgen deprivation followed by radical radiotherapy to the prostate and the pelvis. The median (mean; range) age of subjects was 67 years (67.7; 51 to 83 years), Gleason score of primary prostate biopsies was 7 (7.6; 6–9) and the pretreatment PSA levels were 26.25 (44.25; 8.1–209).

The \[^{11}C\]choline PET-CT was well tolerated with no immediate or delayed complications observed.

Table 2. Characteristics of patients with histologically positive nodes (9/26)

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Age (y)</th>
<th>GS</th>
<th>iPSA</th>
<th>cT</th>
<th>pN</th>
<th>No of + LN</th>
<th>Site of + LN</th>
<th>MRI Size (mm)</th>
<th>PET Size (mm)</th>
<th>PET-CT Size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73</td>
<td>7</td>
<td>8.54</td>
<td>T3a</td>
<td>N1</td>
<td>1</td>
<td>1-R Obt</td>
<td>R Obt 11</td>
<td>1-R Obt</td>
<td>R Obt 11</td>
</tr>
<tr>
<td>10</td>
<td>82</td>
<td>8</td>
<td>13.5</td>
<td>T3</td>
<td>N1</td>
<td>5</td>
<td>3-R Obt, 1-R II</td>
<td>2-R EI 21.8</td>
<td>3-R EI</td>
<td>3-R EI 20,11,7</td>
</tr>
<tr>
<td>13</td>
<td>56</td>
<td>7</td>
<td>50</td>
<td>T1c</td>
<td>N1</td>
<td>1</td>
<td>1-L Obt</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
</tr>
<tr>
<td>15</td>
<td>65</td>
<td>9</td>
<td>209</td>
<td>T2b</td>
<td>N1</td>
<td>7</td>
<td>4-L EI, 3-R II</td>
<td>R II 19</td>
<td>1-L EI, 3-R II</td>
<td>1-L EI, 9,18,11,5</td>
</tr>
<tr>
<td>17</td>
<td>76</td>
<td>7</td>
<td>169</td>
<td>T4</td>
<td>N1</td>
<td>1</td>
<td>1-R Obt</td>
<td>L Obt 9</td>
<td>R Obt</td>
<td>R Obt 9</td>
</tr>
<tr>
<td>20</td>
<td>76</td>
<td>7</td>
<td>21</td>
<td>T2b</td>
<td>N1</td>
<td>1</td>
<td>1-R Obt</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
</tr>
<tr>
<td>24</td>
<td>61</td>
<td>9</td>
<td>45</td>
<td>T3</td>
<td>N1</td>
<td>8</td>
<td>1-R II, 2-R Obt, 3-L EI, 2-L Obt</td>
<td>FN 1-R Obt 6,6,4</td>
<td>FN 1-R Obt, 6,6,4</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>76</td>
<td>9</td>
<td>24.5</td>
<td>T2b</td>
<td>N1</td>
<td>2</td>
<td>2-R EI</td>
<td>FN</td>
<td>FN</td>
<td>FN</td>
</tr>
<tr>
<td>27</td>
<td>51</td>
<td>7</td>
<td>44.8</td>
<td>T3b</td>
<td>N1</td>
<td>1</td>
<td>1-L II</td>
<td>L II 10</td>
<td>FN</td>
<td>FN L II 10</td>
</tr>
<tr>
<td>Mean</td>
<td>68.4</td>
<td>7.8</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13.8</td>
<td></td>
<td>9.4</td>
</tr>
<tr>
<td>Median</td>
<td>73</td>
<td>7</td>
<td>44.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

GS—Gleason score; iPSA, initial prostate-specific antigen; cT, clinical tumor stage; pN, pathologic nodal stage; LN, lymph node; R, right; L, left; Obt, obturator; II, internal iliac; GF, genitofemoral; +, positive.
Time points for SUV measurement

The average and maximum SUV at 60 minutes (SUV$_{60,\text{ave}}$ and SUV$_{60,\text{max}}$) were determined. Due to the rapid systemic metabolism of $[^{11}\text{C}]$choline (30), SUV has also been determined at an earlier time point. The time versus radioactivity curves (TAC) achieve a steady state after approximately 15 minutes (Supplementary Figs. S1 and S2). Hence, SUV$_{15,\text{ave}}$ and SUV$_{15,\text{max}}$ were reported (Supplementary Fig. S3).

$[^{11}\text{C}]$choline uptake within the malignant prostate and pelvic nodes

In addition to visualization of nodal uptake, primary prostate tumors in all 26 patients were well visualized with good tumor-to-background ratios (Fig. 1 and Supplementary Fig. S4). The median (mean ± SD; range) SUV$_{60,\text{ave}}$ and SUV$_{60,\text{max}}$ were 4.85 (4.92 ± 1.75; 2.19–9.28) and 9.97 (11.05 ± 3.72; 4.73–20.54), respectively (median SUV$_{15,\text{ave}}$ and SUV$_{15,\text{max}}$ were 4.82 and 8.80, respectively). Dynamic TACs for $[^{11}\text{C}]$choline in primary prostate tumors and the nodal metastases showed good retention of radioactivity after plateauing at approximately 15 minutes until 60 minutes with SUV$_{\text{ave}}$ (Supplementary Fig. S1 and S2). However, with SUV$_{\text{max}}$ there was a suggestion of increasing activity at 60 minutes, which may be due to the contribution of $[^{11}\text{C}]$betaine.

Diagnostic performance of MRI, $[^{11}\text{C}]$choline PET, and $[^{11}\text{C}]$choline PET-CT in detection of nodal disease

On a per patient basis, the sensitivity and specificity were 50% and 72.2%; 66.7% and 76.4%, and 77.8% and 82.4%, respectively, for MRI, $[^{11}\text{C}]$choline PET, and $[^{11}\text{C}]$choline PET-CT. On a per nodal basis, the sensitivity and specificity were 18.5% and 98.7%; 40.7% and 98.4%; and 51.9% and 98.4%, respectively, for MRI, $[^{11}\text{C}]$choline PET, and $[^{11}\text{C}]$choline PET-CT (Supplementary Table S1). No statistical difference between any 2 modalities was detected in the patient analysis, mainly owing to the relatively low number.

Figure 1. A, T1 weighted MRI (i), axial $[^{11}\text{C}]$choline PET (ii), CT (iii), and PET-CT fused (iv) shows focal uptake in a 4-mm right obturator node (arrowed) clearly separate to the ureter on the PET only (ii); in retrospect visible on MRI (i) but not called as well below size criteria. B, coronal T2 weighted MRI (i), $[^{11}\text{C}]$choline PET maximum intensity projection (MIP) (ii), CT (iii), and PET-CT fused (iv) shows focal uptake in cluster of right external iliac nodes (arrowed). Note uptake in prostate extending to seminal vesicle (green arrow on coronal MIP). C, T1 weighted MRI (i) axial $[^{11}\text{C}]$choline PET (ii), CT (iii), and PET-CT fused (iv) shows focal uptake in 10-mm left obturator node (arrowed) which was false positive. B; Bladder activity.
of subjects. In the per nodal analysis, the sensitivity was significantly improved with the use of $[11C]$choline PET-CT ($P = 0.007$) and $[11C]$choline PET ($P = 0.07$) compared with MRI imaging, without a decrease in the specificity ($P = 1, 1, \text{and } 0.48$ for $[11C]$choline PET vs. MRI, $[11C]$choline PET-CT vs. MRI and $[11C]$choline PET-CT vs. $[11C]$choline PET comparisons, respectively).

ROC analysis (Fig. 2) showed the overall diagnostic performance improved in the following order: MRI < $[11C]$choline PET < $[11C]$choline PET-CT.

Table 3 shows the detection rate of MRI, $[11C]$choline PET, and $[11C]$choline PET-CT for nodal metastases according to the diameter of the involved LNs. A higher LN detection rate, including the detection of subcentimeter nodes, was seen with $[11C]$choline PET-CT than MRI. The mean diameter of the positive LNs on histopathology was 9.8 mm and that of the true positive (TP) LNs was 13.8 and 9.4 mm, respectively, on MRI and $[11C]$choline PET-CT (using CT component for size definition).

Sites of nodal involvement

The majority of the nodes were detected within the standard surgical template. However, 4 of 26 patients (15.4%) had focal increased uptake above the region of eLPL (common iliac (CI) region and lower para aortic region—median SUV$_{60}$ ave and SUV$_{60}$ max of 1.12 and 3.91; median SUV$_{15}$ ave and SUV$_{15}$ max of 2.61 and 6.51, respectively) as detected on imaging and therefore were not

<p>| Table 3. Detection rate of the three imaging modalities by the size of the node |
|-------------------------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Size of infiltrated nodes (mm)</th>
<th>No. of LN</th>
<th>MRI + (%)</th>
<th>$[11C]$choline PET + (%)</th>
<th>$[11C]$choline PET-CT + (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1–1.9</td>
<td>1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2–4.9</td>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>5–9.9</td>
<td>12</td>
<td>0 (0)</td>
<td>4 (33)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>≥10</td>
<td>10</td>
<td>5 (60)</td>
<td>7 (70)</td>
<td>9 (90)</td>
</tr>
</tbody>
</table>

+, positive.
sampled. Eight of 26 (31%) patients had nodes detected below the surgical template, of which 3 patients had discrete unilateral uptake in the inguinal LNs (median SUV60 ave and SUV60 max of 1.21 and 2.50; median SUV15 ave and SUV15 max of 1.54 and 2.63, respectively); significantly lower as compared with TP pelvic nodes (P values of 0.002, 0.0002, 0.004, and 0.0002, respectively, for SUV15 ave, SUV15 max, SUV60 ave and SUV60 max in the 2-sided t-test) which was interpreted as probably reactive uptake and therefore were considered nonmetastatic (Supplementary Fig. S5). One patient had a 5-mm tumor focus in a genitofemoral node, which was outside the FOV.

Nodal analysis on MRI

In 4 of 9 patients, MRI was positive for 5 malignant true positive (TP) nodes with a median maximum diameter of 11 mm (range: 9–21 mm; mean: 13.8 mm).

In 22 malignant nodes using size criteria, MRI was false negative (FN). Eighteen of 22 (82%) nodes were subcentimeter and were reported as normal. Four nodes more than 10 mm were missed. This was due to a cluster of 3 nodes reported as one (Fig. 1), lateral extension of tumor obscuring the obturator node and 2 nodes measuring 12 and 15 mm on histology, which measured 4 and 5 mm on MRI, highlighting the pitfall of gross nodal measurements which may include surrounding perinodal fat and soft tissue.

In 4 patients, MRI was false positive (FP; a total of 5 nodes). This was due to a probable sampling error in 2 patients (Fig. 1), reactive external iliac (EI) nodes in 1 patient and a positive round reactive obturator node which was negative on PET-CT.

Nodal analysis on [11C]choline PET-CT

In 7 of 9 patients, [11C]choline PET-CT was TP for 14 malignant LNs (Fig. 1). The median maximum diameter of the malignant LNs detected was 9 mm (range: 4–20 mm; mean: 9.4 mm).

In 13 malignant nodes, [11C]choline PET-CT was FN as explained in the preceding paragraph. In 3 patients, [11C]choline PET-CT was FP in 6 nodes. In one patient, a further FP node close to the saturation band was called on PET-CT but not PET only. The other 5 nodes in 2 patients were FP on both PET only and combined PET-CT as explained above.

Ki67 and CHKα expression in prostate tumors and nodal metastases

Biopsy samples from 20 prostate cores and 7 metastatic nodes were available for immunostaining (Supplementary Table S2). There was cytoplasmic CHKα staining in all prostate tumor cells that varied in intensity from 1 to 3 (Fig. 3A) compared with a positive control (Supplementary Fig. S6). In one section, some benign glandular areas were also weakly stained (Fig. 3B). In one section, an increased nuclear staining for CHKα with increasing Gleason scores was also observed, especially between Gleason 3 and 5 (Fig. 3C) visually differentiating the 2 grades. There was no relationship between cytoplasmic intensity and nuclear staining of CHKα. In fact, in one tumor, an area of prostatic intraepithelial neoplasia (PIN) showed cytoplasmic as well as nuclear staining (Fig. 3D). In pelvic nodes, benign nodes showed no CHKα staining (Fig. 3E), whereas malignant nodes showed moderate cytoplasmic staining (Fig. 3F). Ki67 staining revealed (Fig. 3G and H) that most primary and nodal prostate tumors had a low proliferation index (median 3%, range: 1%–17%).

Spearman’s correlation test was used to test the association between [11C]choline SUV and tumor immunohistochemistry, PSA and Gleason scores (Supplementary Table S3). There was a positive correlation between SUV60 ave or SUV60 max (Supplementary Fig. S7), with cytoplasmic CHKα intensity in prostate tumors (r = 0.68, P = 0.0001 and r = 0.63, P = 0.0004, respectively). This positive correlation was seen even at early time points (SUV15 ave, SUV15 max: r = 0.55, P = 0.003, and r = 0.46, P = 0.02, respectively). [11C]choline SUV also weakly correlated with serum PSA levels at diagnosis. There was no correlation of [11C]choline SUV with either Ki67 or Gleason scores. The association between immunohistochemistry scores for CHKα and Ki67 with Gleason’s scores or PSA was assessed. Only Gleason scores and Ki67 indices showed a positive correlation (r = 0.55, P = 0.01).

Discussion

This study supports the feasibility of using [11C]choline PET-CT in determining pelvic LN status in patients with high-risk prostate cancer. This method is specific and shows early promise in yielding a greater diagnostic accuracy than either MRI or PET only scanning. This is especially evident in
the detection of subcentimeter disease, although the sensitivity is not sufficient to exclude lymphadenectomy, as metastases less than 6 mm in particular may be missed. However, it has the potential to highlight nodal uptake outside the surgical template for LN dissection, especially in the CI and para-aortic area as shown in this study, which can have significant consequences in terms of patient management.

Figure 3. CHK immunostaining showing (A) cytoplasmic staining (B) CHKα expression in benign (B) and malignant (M) acini (C) differential CHKα in Gleason stage 3 and 5, (D) CHKα in PIN, (E) no staining in a benign node, (F) malignant focus in node, (G) low Ki67 staining in prostate tumor and, (H) low Ki67 staining in metastatic node. Magnifications of 200×.
The somewhat disappointing performance of [18F]FDG PET in the setting of prostate cancer has prompted interest in newer PET tracers, such as [18F] and [11C]choline, for the detection of primary tumor within the prostate and the staging of pelvic nodal disease. For the detection of the primary tumor, some authors have reported 100% sensitivity (19, 31, 32), whereas others report lower detection rates ranging from 19% to 58%, depending on whether results were reported on a per patient or per lesion basis (33–36). Supplementary Table S4 summarizes the published studies assessing LN stage and shows varied and conflicting results (25–27, 37–39). Likewise for staging of pelvic nodal disease, the reported sensitivity and specificity ranged from 50% to 80% and 90% to 96%, respectively, in studies that employed PET alone based on a per patient analysis (25, 26). The variation in sensitivity may be in part due to patient selection.

In this study, we have assessed the value of MRI, [11C]choline PET, and [11C]choline PET-CT imaging in the preoperative staging of high-risk prostate cancer patients. We have shown an overall sensitivity and specificity of [11C]choline PET-CT on a per patient basis, of 77.7% and 82.4%, respectively, in the detection of nodal metastases. These results were superior than both MRI (50% and 72.2%) and [11C]choline PET (66.6% and 76.4%), although were not significantly different probably due to relatively low patient numbers. For MRI, the sensitivity and specificity achieved in our study are in keeping with previously reported data (12, 40). Dynamic contrast MRI may help with tumor localization within the prostate, but there are no specific reports on the additional benefit in nodal staging. More importantly, on a per nodal basis (27/406), the sensitivity was significantly higher for [11C]choline PET-CT (51.9%) compared with MRI (18.5%; P = 0.007) with a greater confidence for identifying subcentimeter involved LNs, which occurred in 30% of the cases. However, in this study apart from one 4-mm node, we were unable to detect low volume metastases of less than 5 mm in diameter, probably reflecting the limited spatial resolution of the current generation of scanners.

In one of the first published series, De Jong and colleagues obtained promising results with [11C]choline PET in the preoperative nodal staging of 67 patients, with a sensitivity of 80% in a per patient-based analysis. Metastatic LNs ranging from 0.5 to 3 cm in size with a mean SUV of 4.7 (2.9–9.1) were reported. FP activity in 2 patients was attributed to inflammatory change and focal bowel activity. However, in their study, about 50% of the node positive patients had a PSA of >50 ng/mL (range: 3–500), compared with our mean PSA value of 44.25 ng/mL (range: 8.1–209), which may have contributed to a selection bias and may underrepresent the cohort of high risk localized prostate cancer patients for which radiotherapy to the pelvis would be indicated (25). Conversely, Hacker and colleagues (38) reported a very low sensitivity of 10% in a study of 20 patients assessed with F-18 fluorocholine. The mean diameter of metastatic LNs in their study was 3.8 mm, which is well below the resolution of PET.

In more recent studies utilizing [11C]choline PET-CT, Schiavina and colleagues (27) evaluated 57 intermediate or high-risk prostate cancer patients prior to surgical treatment. They reported a sensitivity of 60% and a specificity of 98% for the detection of nodal metastases. Husarik and colleagues (39) evaluated 111 patients with prostate cancer in a [18F]choline PET-CT study, 43 of whom had staging for assessment of primary disease. The PET-CT findings were correlated to the histopathologic findings of 115 sampled LNs in 25 patients, with sensitivity and specificity on a per patient basis of 33% and 100%, respectively. Beheshti and colleagues (37) evaluated 130 patients with intermediate or high-risk prostate cancer with [18F]fluorocholine (FCH) PET-CT prior to extended pelvic node dissection with sensitivity and specificity in the detection of malignant nodes of 45% and 96%, respectively. Furthermore, they reported a change in management in 15% of cases. The authors also noted discrete FCH uptake in inguinal LNs which was interpreted as probable reactive uptake and therefore excluded from data analysis. This was similarly observed in our study cohort (8 of 26 patients), although the visible inguinal nodes had significantly lower SUVs than both the metastatic LNs and the malignant prostate. As nodal dissection does not routinely remove inguinal nodes as part of standard practice, it may be difficult to ascertain whether these were involved. The underlying assumption is that inguinal nodes were all within physiologic limits of less than 10 mm in dimension based on the fact that prostate tumors normally do not spread to inguinal nodes (41).

Two studies have reported on a per-nodal analysis. Husarik and colleagues in their study including 25 patients staged with [18F]fluorocholine reported a low sensitivity of 20% (1 of 5 involved nodes) and a specificity of 100%. All the FN nodes had tumor foci of less than 5 mm. The mean SUV\textsubscript{max} of the detected LNs was 5.04; range: 4.9–5.2. Notably, only obturator nodes were removed rather than a more extensive lymphadenectomy, and the authors did not comment on FCH positive nodes outside the obturator region. Schiavina and colleagues, in the study mentioned earlier, reported a sensitivity of 41.4% and a specificity of 99.8% on a per nodal analysis. The mean diameter (in mm) of the metastatic deposit of TP nodes was significantly higher than that of FN nodes (9.2 vs. 4.2; P = 0.001). Our per-nodal results of sensitivity and specificity with [11C]choline were similar at 51.9% and 98.4%. A limitation of our study was the technical difficulties encountered with the interpretation of findings on the PET scans in the region of the saturation band (where there was an overlap when the 2 bed positions were fused), which accounted for some of the FP results on the PET alone. In the 2 patients in whom MRI and PET-CT were FP for a 26-mm and 10-mm node, there is the possibility, despite careful use of surgical templates, that these nodes were not sampled. The median SUV\textsubscript{60} of FP LNs was 6.51, compared with 7.77 for the TP nodes.

This study is one of the first to evaluate the time-dependent uptake of [11C]choline in prostate tumors up to 60 minutes. Dynamic TACs for [11C]choline in primary
contrary to the high CHKα expression in prostate cancer. Both semiquantitative parameters of choline uptake in tumors correlated well with CHKα scores (best with SUV_{60,ave} τ = 0.68, P < 0.0001, Spearman’s test). It was observed that benign prostatic tissue as well as PIN in the malignant cores showed cytoplasmic and nuclear staining. This may represent the range of CHKα expression in normal and premalignant tissues. In certain tissue sections, nuclear staining was observed, particularly in PIN and in certain high Gleason grade tumors and although we do not fully understand this phenomena, a possible reason is that, as with other cellular proteins such as ERK1/2, phosphorylated CHKα may translocate to the nucleus. This hypothesis needs further evaluation. This study also showed that proliferation in prostate tumors was low, as reflected by the low Ki67 index in most tumors. This was contrary to the high CHKα expression. For this precise reason, there was no correlation between [11C]choline SUV and Ki67 scores in tumors. A possible explanation for this is that for prostate malignancies, CHKα expression is a proliferation-independent marker of the prostate tumor phenotype. This is contrary to the evidence in other cell/tumor types linking CHKα or choline metabolites and proliferation (42–44). Of note, one study has reported an association between choline uptake and Ki67 scores in prostate tumors (45). Piert and colleagues showed that tumor-to-benign prostate background ratio was significantly high in tumors with a Ki67 score of more than 5% (P < 0.01). In our study, Ki67 indices were in the range of 1% to 17%. Seven cores had a Ki67 index of more than 5% with a mean SUV_{60, ave} of 4.7 which is higher than that reported by Piert and colleagues. Ki67 did, however, correlate with Gleason score (r = 0.55, P = 0.01, Spearman’s test).

The main drawback to [11C]choline is the relatively short half-life (20.9 minutes) and thus the compound needs to be used close to where it is manufactured. Newer more stable and specific choline compounds are in development (24).

To conclude, this detailed study establishes the feasibility of [11C]choline PET-CT as a noninvasive means of staging pelvic LN in prostate cancer, being highly specific (98.4%) and more sensitive than PET alone or MRI. The high specificity is potentially helpful clinically in terms of selecting out those patients with high-risk prostate cancer who may not need pelvic radiotherapy. Although it cannot currently replace MRI as a staging tool, its ability to detect subcentimeter nodes and a differential SUV value between involved and physiologic LNs allows for this functional imaging methodology to assess the radiation response to involved nodes. The relationship between CHKα expression and [11C]choline uptake, together with the avid intratumoral uptake of choline shown in this study, merits further investigation in a larger patient population and in patients with other risk profiles.

Disclose of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Use of $[^{11}\text{C}]$Choline PET-CT as a Noninvasive Method for Detecting Pelvic Lymph Node Status from Prostate Cancer and Relationship with Choline Kinase Expression

Kaiyumars Contractor, Amarnath Challapalli, Tara Barwick, et al.