11C-choline pharmacokinetics in recurrent prostate cancer

Milan Grkovski¹, Karem Gharzeddine²*, Peter Sawan²*, Heiko Schöder²,³, Laure Michaud², Wolfgang A. Weber²,³,⁴ and John L. Humm¹

¹Department of Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY, USA
²Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA
³Molecular Imaging and Therapy Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA
⁴The University Hospital Klinikum rechts der Isar, Technical University of Munich, Germany

*These Authors contributed equally.

Running title: 11C-choline dynamic PET in PCa

Keywords: 11C-choline; dynamic PET; kinetic modeling; prostate cancer; metastasis;

Financial support: This study was supported by the cancer center grant P30 CA008748 (P.I. Craig B. Thompson).

Corresponding Author:
Milan Grkovski, PhD
Department of Medical Physics
Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, NY 10065
Phone: 212-639-2480
Email: grkovskm@mkscc.org

Conflict of Interest: The authors declare no potential conflicts of interest.

Word count: 5377
ABSTRACT

The aim of this study was to investigate the value of pharmacokinetic modeling for quantifying $^{11}$C-choline uptake in patients with recurrent prostate cancer.

Methods: 194 patients with clinical suspicion of recurrent prostate cancer underwent $^{11}$C-choline dynamic positron emission tomography over the pelvic region (0-8-min), followed by 6 min static acquisition at ~25 min post-injection. Regions of interest (ROIs) were drawn over sites of disease identified by a radiologist with experience in nuclear medicine. $^{11}$C-choline uptake and pharmacokinetics were evaluated by standardized uptake value (SUV), graphical analysis (Patlak plot; $K_P$) and 1- and 2-compartment pharmacokinetic models ($K_1$, $K_1/k_2$, $k_3$, $k_4$ and macro-parameter $K_C$). 24 local recurrences, 65 metastatic lymph nodes, 19 osseous metastases and 60 inflammatory lymph nodes were included in the analysis, which was subsequently repeated for ROIs placed over gluteus maximus muscle and adipose tissue as control.

Results: Mean SUV and $K_P$ were 3.60±2.16 and 0.28±0.22 min$^{-1}$ in lesions compared to 2.11±1.33 and 0.15±0.10 min$^{-1}$ in muscle and 0.26±0.07 and 0.02±0.01 min$^{-1}$ in adipose tissue. According to Akaike Information Criterion, 2-compartment irreversible model was most appropriate in 85% of lesions, and resulted in: $K_1$=0.79±0.98 min$^{-1}$ (range, 0.11-7.17 min$^{-1}$), $K_1/k_2$=2.92±3.52 (range, 0.31-20.00), $k_3$=0.36±0.30 min$^{-1}$ (range, 0.00-1.00 min$^{-1}$) and $K_C$=0.28±0.22 min$^{-1}$ (range, 0.00-1.33 min$^{-1}$). Spearman’s $\rho$ between SUV-$K_P$, SUV-$K_C$ and $K_P$-$K_C$ was 0.94, 0.91 and 0.97, respectively. $\rho$ between SUV and $K_1$, $K_1/k_2$ and $k_3$ was 0.70, 0.44 and 0.33, respectively. Malignant lymph nodes exhibited higher SUV, $K_P$ and $K_C$ than benign lymph nodes.

Conclusion: While $^{11}$C-choline pharmacokinetic modeling has a potential to uncouple the contributions of different processes leading to intracellular entrapment of $^{11}$C-choline, high correlation between SUV and both $K_P$ and $K_C$ supports the use of simpler SUV method for evaluation of changes in choline uptake and metabolism for treatment monitoring.
INTRODUCTION

Prostate cancer (PCa) is one of the most commonly diagnosed cancers in men worldwide. It is estimated that 164,690 new cases of PCa will be diagnosed and 29,430 men will die of the disease in the United States in 2018 (1). Despite initial treatment with curative intent, biochemical recurrences, defined as an increase in prostate-specific antigen (PSA) levels, occur in >25% of cases.

\(^{11}\)C-choline is a positron emission tomography (PET) radiopharmaceutical for the detection and localization of recurrent PCa, with the Food and Drug Administration approval primarily based on its ability to detect disease not visualized with conventional imaging. Choline is a precursor of phospholipids, major structural components of the plasma membrane, and is as such necessary for membrane synthesis and cell division (2). It is predominantly transported into cells via high-affinity sodium dependent choline transporters, phosphorylated by choline kinase (which is overexpressed in many malignant tumors including prostate cancer) and incorporated into phospholipids such as phosphatidylcholine. Choline also plays a role in cell proliferation by modulating transmembrane signaling (3).

Meta-analysis of PCa patients with biochemical recurrence undergoing choline PET (12 studies, n = 1055 patients) by Umbehr and colleagues reported a pooled sensitivity of 85% and a pooled specificity of 88% (4). Evangelista et al. reviewed the use of choline PET in biochemical relapse of PCa (19 studies, n=1555 patients), reporting a pooled sensitivity of 86% and a pooled specificity of 93% for all sites of disease (5). More recently, Fanti et al., evaluating \(^{11}\)C-Choline in recurrent PCa (29 studies, n=2686 patients), reported 89% pooled sensitivity and 89% pooled specificity (6). On the other hand, Ren et al., performing a meta-analysis of \(^{18}\)F-Fluciclovine PET/CT (6 studies, n=251 patients) in the diagnosis of recurrent prostate carcinoma, reported 87% pooled sensitivity and 66% pooled specificity (7), however specificities for extraprostatic disease might be higher that the meta-analysis suggests (8). \(^{11}\)C-choline is relatively insensitive in patients with biochemically recurrent PCa after surgery with PSA values <2 ng/mL. A recent study comparing \(^{68}\)Ga-labelled PSMA-11 and \(^{11}\)C-choline PET for detection of PCa metastases concluded that \(^{68}\)Ga-labelled PSMA-11 PET exhibited significantly higher uptake and detection rate than \(^{11}\)C-choline PET in the subgroup of patients with PSA levels below 1 ng/mL (9). However, choline PET/CT is attractive for monitoring tumor response to therapy in patients with metastatic prostate cancer. Choline
uptake reflects a basic metabolic process of the cancer cells that is not directly regulated by androgen signaling. In contrast, PSMA expression is negatively regulated by androgens. During androgen deprivation therapy uptake of PSMA ligands may therefore increase at least temporarily in responding tumors (10). Quantitative analysis of PSMA PET scans during androgen deprivation therapy, a cornerstone of all prostate cancer therapy, may therefore be challenging.

To use choline PET/CT for monitoring tumor response it is important to provide quantitative measures of tracer uptake. The simplest approach is to assess choline uptake is the calculation of standardized uptake values (SUVs) at a fixed point in time. For FDG PET multiple studies have shown that SUVs and SUV changes during therapy are well correlated with glucose metabolic rates determined from tracer kinetic modeling (11). Therefore, SUVs are now routinely used to monitor changes in glucose metabolic activity (12). However, there are only few similar analyses for choline PET/CT.

The aim of the current study was to explore the potential added benefit of pharmacokinetic modeling for quantifying PET uptake in a large cohort of patients with recurrent prostate cancer with a radiotracer that has already been extensively used in a clinical setting. We also sought to determine how well SUVs from static PET scans correlate with choline uptake rates derived from dynamic scans. Furthermore, we investigated if pharmacokinetic modeling of 11C-choline biodistribution can facilitate better differentiation of tumor involved and inflammatory lymph nodes.
MATERIALS AND METHODS

Patient selection

All patients participated in an IRB-approved expanded access study (Protocol 15-117) and signed a written informed consent regarding the examination and use of anonymous data for research and publication purposes. Subject inclusion criteria were: (i) biopsy-proven adenocarcinoma of the prostate initially treated with curative intent; and (ii) biochemical recurrence defined as either Prostate-specific antigen (PSA) ≥ 0.2 ng/mL in at least two sequential tests for patients treated with prostatectomy, PSA ≥ 0.2 ng/mL above the post therapy nadir for patients treated with radiation therapy, brachytherapy or cryotherapy, or PSA ≥ 0.2 ng/mL above the most recent therapy nadir for patients who have received additional treatment in the recurrent setting; (iii) negative or inconclusive findings in standard-of-care imaging studies (e.g., CT, MRI, conventional bone scintigraphy or $^{111}$In capromab pendetide scintigraphy); and (iv) age ≥ 18+ years. 194 patients were included in the study between March 2016 and December 2016.

PET/CT imaging

$^{11}$C-choline was synthesized on-site by N-$^{11}$C-methylation of dimethylethanolamine in ethanol with $^{11}$C-methyl iodide and isolated by solid-phase extraction on a cation-exchange cartridge. Residual dimethylethanolamine and $^{11}$C-methyl iodide were removed from the cartridge by rinsing it with ethanol and water, and the remaining purified product eluted with sterile isotonic saline through a sterilizing filter into the final product vial.

Patients fasted for at least 4 hours before the scan. All patients were encouraged to void before and after imaging. Concurrent with an intravenous bolus injection of 370-740 MBq dose of $^{11}$C-choline, patients underwent dynamic PET on the General Electric Discovery 690 or Discovery 710 PET/CT (GE Healthcare Inc.) over the pelvic region (0-8-min; binned into 6×10-sec, 4×30-sec and 5×60-sec). This was followed by a whole-body scan lasting ~18 min (not used for the purpose of pharmacokinetic modeling), and a 6-min static acquisition at ~26 min post-injection, focused over the pelvic region. The x-ray computed tomography (CT) images acquired immediately prior to the PET scan were performed with the following settings: 120 kVp, 80 mAs, and 3.8-mm slice thickness. All PET emission data was
corrected for attenuation, scatter, and random events, and then iteratively reconstructed into a
128×128×47 matrix (voxel dimensions: 5.46×5.46×3.27 mm³) using the ordered subset expectation
maximization algorithm provided by the manufacturer.

**Image analysis**

Image analysis was performed in PMOD v3.604 (PMOD Technologies GmbH). Regions of interest
(ROIs) were manually drawn over sites of disease and inflammatory lymph nodes that were identified by a
radiologist with experience in PET/CT imaging and nuclear medicine, on a late time-point 6 min static
PET. For this purpose, PET scans were fused with their corresponding CT scans to provide anatomical
information. Uptake in inguinal lymph nodes was considered as inflammatory, because the inguinal nodes
are not in the lymphatic drainage system of the prostate (13).

¹¹C-choline uptake was measured by the mean standardized uptake value (SUV) within the ROI.
Graphical analysis was performed to calculate the Patlak slope (KP). In addition, irreversible and
reversible 1- compartment (1T1K and 1T2K, respectively) and 2-compartment (2T3K and 2T4K,
respectively) pharmacokinetic models were investigated to calculate kinetic rate constants K₁, Kᵢ/K₂, k₃,
k₄. In all 4 compartment models, blood volume fraction vb was also fitted. Additionally, macro-parameter
derived from compartmental analysis (KC) was calculated, and compared to the KP. KC is an
unidirectional uptake rate constant defined as

\[ KC = \frac{K_1 k_3}{k_2 + k_3}. \]  

(1)

¹¹C-choline equilibration time, T*, was calculated as

\[ T^* = 7 \cdot \frac{\ln(2)}{(k_2 + k_3)} \]  

(2)

and represents the time after which unbound ¹¹C-choline has reached >99% of its final ratio relative to
blood. The input function was derived from the dynamic PET images, segmenting the femoral artery on
the early frame with the highest image intensity by selecting ~200 hottest voxels. Image-derived input
function time activity curves (TACs) were defined using the available temporal data (i.e., the first 8 min.
dynamic acquisition and the last 6 min static frame) and fitted with a triphasic exponential function. The target activity concentration at each time frame was weighed by

\[ w_i = \frac{1}{\sigma_i^2}, \quad \sigma_i = c \sqrt{\frac{AC(t_i)}{\Delta t_i \times e^{-\lambda t_i}}}, \]

where \( c \) is the scaling factor, \( \Delta t_i \) is the frame duration, \( AC(t_i) \) is the decay-corrected activity concentration measured at the mid-frame time \( t_i \), and \( \lambda = \ln 2 / T_{1/2} \) is the isotope decay constant. Since metabolite counts were not acquired on a patient-by-patient basis, population-wise metabolite correction was applied using previously reported values \((14,15)\). Akaike Information Criterion as implemented in PMOD was used to determine the most appropriate compartmental model \((16)\). The correlation between investigated metrics was analyzed using Spearman’s \( \rho \). Comparisons of metric values between different groups was performed with unpaired 2-tailed Student \( t \) test. \( p<0.05 \) was assumed to represent statistical significance.
RESULTS

194 patients were included in the study. Pre-treatment risk categories according to National Comprehensive Cancer Network (NCCN) guidelines were I (n=1 patient), IIA (n=28 patients), IIB (n=23 patients), III (n=71 patients) and IV (n=58 patients). In n=13 patients, risk category could not be calculated. In total, 48% of patients (94 out of 194) had positive findings on the whole-body $^{11}$C-choline scan, indicating local recurrence in 12% of patients (24 out of 194), lymph node metastasis in 32% of patients (62 out of 194) and bone metastasis in 14% of patients (27 out of 194). 4 out of 27 patients with bone metastases also exhibited lymph node metastases. Median PSA and Gleason score were 2.5 ng/mL (range, 0.3-82.9 ng/mL) and 7 (range, 6-10), respectively. Detection rate for local recurrence and/or metastatic disease varied depending on the PSA values: 12% (6 out of 49) for patients with PSA <0.5 ng/mL, 34% (11 out of 32) for patients with PSA between 0.5-1.0 ng/mL, 68% (21 out of 31) for patients with PSA between 1.0-2.0 ng/mL and 68% (56 out of 82) for patients with PSA >2.0 ng/mL. Detection according to the NCCN risk category was: 100% (1 out of 1; I), 64% (18 out of 28; IIA), 30% (7 out of 23; IIB), 48% (34 out of 71; III), 47% (27 out of 58; IV) and 54% (7 out of 13; unknown).

Several patients had lesions outside the 15.7 cm axial field of view of the dynamic scan (n=21). Pharmacokinetic modeling was performed in the remaining n=73 patients, with 108 malignant lesions (24 local recurrences, 65 lymph node metastases and 19 osseous metastases) being included in the analysis. For these patients, the pharmacokinetic modeling was also conducted for ROIs placed over gluteus maximus muscle and adipose tissue. Additionally, 60 probably inflammatory inguinal lymph nodes were analyzed in n=26 patients. Biopsies were performed in 33 out of 73 patients for which pharmacokinetic modeling was performed. In all 33 patients, the results were positive. Biopsy results were negative in all 3 patients for which biopsies were performed in inguinal lymph nodes.

Mean SUV and $K^P$ were $3.60\pm2.16$ and $0.26\pm0.17$ min$^{-1}$ in malignant lesions compared to $1.63\pm0.70$ and $0.11\pm0.04$ min$^{-1}$ in inflammatory lymph nodes, $1.02\pm0.40$ and $0.07\pm0.03$ min$^{-1}$ in muscle and $0.26\pm0.07$ and $0.02\pm0.01$ min$^{-1}$ in adipose tissue (Table 1). Malignant lymph nodes were found to have significantly higher SUV, $K^P$ and $K^C$ than inflammatory lymph nodes (Table 1, p<0.01). $^{11}$C-choline
equilibration was sufficiently fast relative to the total scan duration in all lesions and inflammatory lymph nodes.

According to Akaike Information Criterion as implemented in PMOD, 2T3K model was the most appropriate in 85% of lesions, with 1T1K and 1T2K models being the most appropriate in the remaining 15% of lesions. Akaike Information Criterion values for 1T1K, 1T2K, 2T3K and 2T4K models were 147±16, 140±15, 127±12 and 132±18, respectively. Pharmacokinetic modeling results for all analyzed tissues, as calculated with 2T3K model, are summarized in Table 1. Significantly higher SUV, $K_P^P$, $K_C^C$, $K_1$, $K_1/k_2$, and $k_3$ values were observed in malignant lesions compared to inflammatory lymph nodes, with values for muscle and adipose tissue being lower still. $^{11}$C-choline distribution volume (approximated by $K_1/k_2$) was around unity in inflammatory lymph nodes and muscle tissue; however, it was slightly lower in adipose tissue, indicating the dependence of radiotracer distribution volume on tissue composition. Osseous and lymph node metastases were significantly better perfused than local recurrences or reactive lymph nodes. Adipose tissue was the least well perfused, with $k_3$ values close to zero. Sub-analysis of lesions divided into local recurrences, lymph nodes and osseous metastases is also included in Table 1. Despite having similar SUV values (4.00±2.25 and 3.86±1.24, respectively), significant difference was observed in $K_1$ between local recurrences and osseous metastases ($K_1=0.49±0.36 \text{ min}^{-1}$ and $K_1=0.99±0.92 \text{ min}^{-1}$, respectively; $p=0.02$).

Spearman’s $\rho$ between lesion SUV-$K_P^P$ was 0.94 (Fig. 1A), whereas between lesion SUV-$K_C^C$ it was 0.91. High correlation has been observed between $K_P^P$ and $K_C^C$; $\rho=0.97$ (Fig. 1B). Slight discrepancy between lesion $K_P^P$ and $K_C^C$ values was mediated by $K_1$: $\rho$ between $\Delta(K_P^P-K_C^C)$ and $K_1$ was found to be -0.67. The discrepancy becomes more apparent for $K_1\geq0.4 \text{ min}^{-1}$ (corresponding to SUV$\geq$~6), indicating that the compartment model is not robust in these scenarios. Among the investigated kinetic rate constants, the correlation with SUV was the strongest for $K_1$ in all tissues, $\rho=0.71$, whereas for $K_1/k_2$ and $k_3$ it was $\rho=0.44$ and $\rho=0.33$ (Table 2). No correlation was found between lesion $K_1$ and $k_3$ ($p=-0.16; \rho=0.09$), however $\rho$ between $K_1/k_2$ and $k_3$ was -0.36 ($p<0.01$). No significant correlation was observed between Gleason nor NCCN score and any of the imaging metrics (i.e., SUV and kinetic rate constants).
Spearman’s ρ reached significance for correlations between PSA and SUV, $K_P$ and $K_{IC}$ ($\rho=0.28$, $\rho=0.26$ and $\rho=0.21$, respectively).

In 15% of lesions where 1-compartment models resulted in a lower Akaike Information Criterion value, fitting of time activity curves was sub-optimal regardless of model used. This was primarily due to continuing gradual increase of tumor signal after an initial plateau was reached and the sub-optimal metabolite correction which likely underestimated the radiotracer available for intracellular trapping (resulting in $k_3 \geq 0.4 \text{ min}^{-1}$). When the analysis in all 108 lesions was repeated using input functions not corrected for metabolites, the quality of the fits improved substantially in the aforementioned 15% of lesions, and $2T3K$ model was assessed as most appropriate in almost all cases. SUV-$K_P$ and SUV-$K_{IC}$ correlations were lower however, $\rho=0.83$ and $\rho=0.80$ respectively, while the corresponding correlations for $K_1$, $K_1/k_2$ and $k_3$ were $\rho=0.72$, $\rho=0.67$ and $\rho=0.31$, respectively.

An example of both concordance and discordance between SUV and $^{11}$C-choline pharmacokinetics is presented in Fig. 2. In Patient #1 (PSA = 8.6 ng/mL, Gleason score = 7), metastatic lymph node with a higher SUV also exhibits higher $K_1$ and $K_1/k_2$ (as well as higher $K_{IC}$; Fig. 2A). On the other hand, the two osseous metastases in Patient #2 (PSA = 9.8 ng/mL, Gleason score = 8) exhibit markedly different kinetic rate constants despite having similar SUV (Fig. 2B).
DISCUSSION

Compared to dynamic imaging, static PET scans are clinically more feasible, shorter and fit more readily into a busy clinical schedule. However, our study indicates that similar SUVs can be produced by lesions exhibiting very different pharmacokinetics that reflect differences in tumor differentiation and perfusion. Nevertheless, high correlation was observed between SUV and uptake rate constant $K_1$ as determined either by graphical Patlak analysis or pharmacokinetic modeling, supporting the value of using the simpler static measure in place of dynamic PET scans for quantifying $^{11}$C-choline uptake in these patients for treatment monitoring.

Intracellular choline levels are determined both by the rate of choline uptake as well as by the rate of phosphatidylcholine synthesis and degradation. Experimental studies have indicated that a large fraction of the intracellular choline still represents non-metabolized choline, suggesting that choline transport and not phosphorylation is the key factor for choline uptake of cancer cells (17,18). Henriksen and colleagues demonstrated that while tumor uptake of the choline derivative $^{18}$F-deshydroxycholine that cannot be phosphorylated by choline kinase has been shown to be similar to $^{11}$C-choline at early time points (<5 minutes post-injection), as radiolabel internalization was largely dependent on the choline transport rate, at longer incubation times (>20 minutes post-injection), uptake of $^{11}$C-choline was significantly higher as choline phosphorylation became the dominant step in cellular enrichment (19).

The accumulation of $^{11}$C-choline in tumors was rapid, as observed previously (15,20,21). In many cases, a plateau was already reached within 1-3 dynamic 10-sec frames. Among all investigated kinetic rate constants, $K_1$ was most correlated with SUV. These results are consistent with the foregoing observations if $K_1$ is taken to not only reflect tumor perfusion and vascular permeability, but also the rate of choline transporter activity, while $k_3$ is assumed to reflect the irreversible and slower process of phosphorylation by choline kinase that increases intracellular trapping of choline. In our study, $K_1$ and $k_3$ were not significantly correlated with each other. The preference of an irreversible 2-compartment model over other investigated models by Akaike Information Criterion indicates that the inclusion of the kinetic rate constant $k_3$ is preferential for describing the $^{11}$C pharmacokinetics.
Inaba has measured prostate cancer blood flow using $^{15}$O-water PET and reported that it averaged 15.7 mL/min/100 g (i.e., 0.157 min⁻¹, assuming unit density tissue), compared to 29.4 mL/min/100 g in prostate cancer tissue (22). Subsequent studies confirmed that the average blood flow rate in prostate cancer is approximately 2-3 times higher than in normal prostate (23). In the current study, comparison with normal prostate tissue was not possible since all patients underwent prostatectomy prior to $^{11}$C-choline PET scans, although $K_1$ was about 3x higher than values previously reported for normal prostate (Table 1; 19,20). Osseous and lymph node metastases were found to have still higher $K_1$ values compared to local recurrences (Table 1). Prostate cancer has a high propensity to metastasize to bone (24), specifically to the most heavily vascularized parts of the skeleton (25), and stimulate osteoblast activity (26). This is facilitated by the presence of adhesive molecules on tumor cells that bind them to marrow stromal cells, in turn increasing the production of angiogenic factors and bone-resorbing factors that enhance tumor growth (27). High perfusion of osteoblastic metastases has also been described using $^{18}$F-NaF PET (28).

Analysis of time activity curves revealed that in several cases, activity concentration continued to gradually increase after an initial plateau was reached, in agreement with previous observations (29). Apart from free $^{11}$C-choline and $^{11}$C-phosphocholine, tumor PET signal from $^{11}$C-choline scan comprises also of the metabolite $^{11}$C-betaine (an organic osmolyte produced in liver and kidneys), which contributes to the total uptake through donation of the radiolabeled methyl group to L-homocysteine, producing radiolabeled L-methionine (2). Both $^{11}$C-choline and $^{11}$C-betaine enter the cells, albeit with different rates that can conceivably vary between lesions. This process could in principle be modeled as a combined 4-Tissue compartment model, where tissue kinetics of both $^{11}$C-choline and $^{11}$C-betaine are described by two interlinked 2-Tissue compartment models, however such a model requires more parameters that can be reliably fitted. There is also evidence that small amounts of $^{11}$C-choline or its radioactive metabolites are slowly accumulated into blood cells (30,31), introducing additional variability regarding how much of the measured $^{11}$C activity concentration in blood is available for transport and intracellular trapping.

To date, a single study of $^{11}$C-choline pharmacokinetics in PCa focused on 14 patients with newly diagnosed disease (20). The Authors reported very high correlation between SUV and $K_1$ values as derived from graphical analysis (Pearson’s $r=0.96$, p<0.01), in agreement with our results. On the other
hand, Verwer and colleagues, investigating $^{18}$F-fluoromethylcholine dynamic PET in 8 PCa patients, concluded that commonly used SUV metrics cannot be applied to quantify $^{18}$F-fluoromethylcholine uptake reliably (32). The Authors calculated $K_t$ from 1T1K model (thus reflecting $K_i$), and reported a SUV-$K_t$ $R^2$ of 0.34 (in our study, corresponding $R^2$ was comparable, at 0.38). However, we found that 1T1K was not the most appropriate model, and that in most cases, 2T3K model resulted in better-quality fits. The Authors also report SUV-$K_t^C$ $R^2$ of 0.58 (we observed a corresponding $R^2$ of 0.75), while results of graphical analysis are not included (32). In another study of $^{18}$F-fluoroethylcholine dynamic PET in PCa patients (33), the Authors constrained $K_1$ to $\leq$1 ml/min$^{-1}$·g$^{-1}$ (Fig. 7A in ref. 33), however since $K_1$ does not have same units as $k_2$ or $k_3$ (which represent fractions of mass transferred per unit time), physiological values of $K_1$ can theoretically be above 1 ml·min$^{-1}$·g$^{-1}$. This likely deteriorated the reported low correlation between SUV and $K_1$ ($R^2$ of 0.08).

$^{11}$C-choline uptake in inguinal lymph nodes was considered to be likely inflammatory (13), however a limitation of our study is that the disease recurrence could be confirmed for 33 out of 73 patients only, as biopsies were not performed in the remaining patients. Another limitation is the absence of $^{11}$C-choline metabolite analysis on a patient-by-patient basis, since arterial blood was not collected. Instead, population-wise metabolite correction function, derived using previously published data (14,15), was multiplied with the patient’s image-derived input function. In the foregoing studies, metabolization of $^{11}$C-choline resulted in the ~15% of radioactivity present at 60 minutes post-injection being due to $^{11}$C-choline, with $^{11}$C-betaine the only observed radioactive metabolite. Patient-wise variability in metabolite fraction probably impacted the quality of fitting; however, it is not expected to have altered the interpretation of the results.
CONCLUSION

The data presented in this paper is the result of, to best of our knowledge, the largest pharmacokinetic analysis of dynamic $^{11}$C-choline PET scans performed in recurrent metastatic prostate cancer. Our analysis shows that $^{11}$C-choline pharmacokinetics in this setting is best described by the irreversible 2-compartment model. Malignant lymph nodes exhibit higher SUV, as well as $K_p$ and $K_c$, when compared to benign lymph nodes. Osseous metastases exhibit higher blood flow compared to local recurrences, despite having similar SUV values. While pharmacokinetic modeling has a potential to uncouple the contributions of different processes leading to entrapment of $^{11}$C-choline within cancer cells, high correlation between SUV and both $K_p$ and $K_c$ supports the use of simpler SUV method for evaluation of changes in choline uptake and metabolism for treatment monitoring.

**Funding:** This study was supported by the cancer center grant P30 CA008748 (P.I. Craig B. Thompson).

**Conflict of Interest:** The authors declare no potential conflicts of interest.
REFERENCES


Figure 1. Population-wise comparison of $^{11}$C-choline metrics derived from static and dynamic PET acquisitions. (A) Scatterplot of Patlak slope $K^P$ (x-axis) vs. SUV (y-axis) for all lesions (black, n=108...
ρ=0.94) and inflammatory lymph nodes (cyan, n=60, ρ=0.93). Regression slope = 11.5, regression intercept = 0.6. (B) Scatterplot of influx rate constant $K_i$ as derived from graphical Patlak analysis ($K_{iP}$; $x$-axis) and compartmental modeling ($K_{iC}$; $y$-axis) for all lesions (black, n=108, ρ=0.97) and inflammatory lymph nodes (cyan, n=60; ρ=0.96). Second degree polynomial fit and line of identity are shown as solid red and dashed black lines, respectively.
Figure 2. Relationship between SUV and $^{11}$C-choline pharmacokinetics in two patients with biopsy proven disease recurrence. Late static PET scans, fused with their corresponding CT scans, are included in all cases. Lesions are highlighted with white arrows. Color scales have units of kBq/cc and Hounsfield Units for PET and CT, respectively. (A) Patient #1, exhibiting concordance between SUV and kinetic rate constants $K_1$ and $K_1/k_2$. 1st lymph node, marked with a red TAC and arrow (SUV = 10.7, $K_1 = 1.64$ min$^{-1}$, $K_1/k_2 = 4.91$, $k_3 = 0.21$ min$^{-1}$) exhibits both higher SUV as well as higher $K_1$ and $K_1/k_2$ than 2nd lymph node, marked with a blue TAC and arrow (SUV = 6.0, $K_1 = 0.59$ min$^{-1}$, $K_1/k_2 = 1.16$, $k_3 = 0.70$ min$^{-1}$). Input function TAC superimposed in black. (B) Patient #2, exhibiting discordance between SUV and kinetic rate constants: osseous metastasis marked with a red TAC and arrow (SUV = 5.0, $K_1 = 0.97$ min$^{-1}$, $K_1/k_2 = 4.17$, $k_3 = 0.18$ min$^{-1}$) has a similar SUV but different pharmacokinetics compared to the osseous metastasis marked with a blue TAC and arrow (SUV = 5.0, $K_1 = 0.41$ min$^{-1}$, $K_1/k_2 = 1.27$, $k_3 = 1.00$ min$^{-1}$). Input function TAC superimposed in black.
### Table 1. Summary of $^{11}$C-choline dynamic PET analysis. Mean ± standard deviation (range).

<table>
<thead>
<tr>
<th></th>
<th>SUV</th>
<th>$K_P$ (min$^{-1}$)</th>
<th>$K_C$ (min$^{-1}$)</th>
<th>$K_1$ (min$^{-1}$)</th>
<th>$K_1/k_2$</th>
<th>$k_3$ (min$^{-1}$)</th>
<th>$T^*$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All prostate cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesions (n=108)</td>
<td>3.60±2.16</td>
<td>0.26±0.17</td>
<td>0.28±0.22</td>
<td>0.79±0.98</td>
<td>2.92±3.52</td>
<td>0.36±0.30</td>
<td>9±7</td>
</tr>
<tr>
<td></td>
<td>(0.82-12.00)</td>
<td>(0.06-1.01)</td>
<td>(0.07-1.33)</td>
<td>(0.11-7.17)</td>
<td>(0.31-20.00)</td>
<td>(0.00-1.00)</td>
<td></td>
</tr>
<tr>
<td><strong>Local recurrences</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=24)</td>
<td>4.00±2.25</td>
<td>0.27±0.13</td>
<td>0.26±0.15</td>
<td>0.49±0.36</td>
<td>3.37±4.46</td>
<td>0.48±0.33</td>
<td>9±6</td>
</tr>
<tr>
<td></td>
<td>(1.75-8.96)</td>
<td>(0.10-0.59)</td>
<td>(0.11-0.68)</td>
<td>(0.16-1.58)</td>
<td>(0.46-20.00)</td>
<td>(0.10-1.00)</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph node</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metastases (n=65)</td>
<td>3.40±2.32</td>
<td>0.25±0.19</td>
<td>0.26±0.22</td>
<td>0.85±1.17</td>
<td>2.49±2.94</td>
<td>0.32±0.27</td>
<td>9±7</td>
</tr>
<tr>
<td></td>
<td>(0.82-12.00)</td>
<td>(0.06-1.01)</td>
<td>(0.07-1.33)</td>
<td>(0.10-7.76)</td>
<td>(0.06-12.39)</td>
<td>(0.04-1.00)</td>
<td></td>
</tr>
<tr>
<td><strong>Osseous metastases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=19)</td>
<td>3.86±1.24</td>
<td>0.31±0.14</td>
<td>0.35±0.20</td>
<td>0.99±0.92</td>
<td>3.93±4.11</td>
<td>0.39±0.31</td>
<td>9±7</td>
</tr>
<tr>
<td></td>
<td>(1.71-5.60)</td>
<td>(0.11-0.68)</td>
<td>(0.11-0.88)</td>
<td>(0.16-3.37)</td>
<td>(0.71-13.10)</td>
<td>(0.05-1.00)</td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory lymph</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nodes (n=60)</td>
<td>1.63±0.70</td>
<td>0.11±0.04</td>
<td>0.11±0.04</td>
<td>0.34±0.17</td>
<td>1.10±0.58</td>
<td>0.21±0.18</td>
<td>10±5</td>
</tr>
<tr>
<td></td>
<td>(0.80-4.92)</td>
<td>(0.06-0.26)</td>
<td>(0.06-0.27)</td>
<td>(0.10-0.95)</td>
<td>(0.39-3.15)</td>
<td>(0.06-1.00)</td>
<td></td>
</tr>
<tr>
<td><strong>Gluteus maximus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>muscle (n=73)</td>
<td>1.02±0.40</td>
<td>0.07±0.03</td>
<td>0.13±0.08</td>
<td>0.13±0.08</td>
<td>1.34±1.00</td>
<td>0.20±0.11</td>
<td>20±16</td>
</tr>
<tr>
<td></td>
<td>(0.43-2.02)</td>
<td>(0.03-0.16)</td>
<td>(0.04-0.31)</td>
<td>(0.04-0.32)</td>
<td>(0.32-5.71)</td>
<td>(0.04-0.31)</td>
<td></td>
</tr>
<tr>
<td><strong>Adipose tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=73)</td>
<td>0.26±0.07</td>
<td>0.02±0.01</td>
<td>0.06±0.04</td>
<td>0.07±0.04</td>
<td>0.72±0.27</td>
<td>0.03±0.03</td>
<td>65±69</td>
</tr>
<tr>
<td></td>
<td>(0.13-0.46)</td>
<td>(0.01-0.10)</td>
<td>(0.02-0.22)</td>
<td>(0.02-0.29)</td>
<td>(0.28-1.82)</td>
<td>(0.00-0.18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$K^p$</td>
<td>$K^c$</td>
<td>$K_1$</td>
<td>$K_{1/2}$</td>
<td>$k_3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-----------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate cancer lesions</td>
<td>0.94</td>
<td>0.91</td>
<td>0.70</td>
<td>0.44</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=108)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory lymph nodes</td>
<td>0.93</td>
<td>0.91</td>
<td>0.54</td>
<td>0.27</td>
<td>0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluteus maximus muscle</td>
<td>0.86</td>
<td>0.77</td>
<td>0.78</td>
<td>0.36</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=73)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.64</td>
<td>0.53</td>
<td>0.53</td>
<td>0.18*</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=73)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*significance not reached