

Reproducibility of [¹¹C]Choline-Positron Emission Tomography and Effect of Trastuzumab

Laura M. Kenny¹, Kaiyumars B. Contractor¹, Rainer Hinz⁴, Justin Stebbing¹, Carlo Palmieri¹, Jie Jiang², Sami Shousha², Adil Al-Nahhas³, R. Charles Coombes¹, and Eric O. Aboagye¹

Abstract

Purpose: This study sought to evaluate the reproducibility of [¹¹C]choline-positron emission tomography and the effect of trastuzumab in breast cancer.

Experimental Design: Twenty-one patients with newly diagnosed and recurrent breast cancer stage II-IV had a baseline dynamic [¹¹C]choline-PET scan, 10 patients had a second [¹¹C]choline-PET scan to examine reproducibility, and 6 patients had a second scan within a month after trastuzumab. Analysis of [¹¹C]choline uptake was measured as the semiquantitative standardized uptake value at 30 and 60 minutes (SUV₃₀ and SUV₆₀), and quantitatively as the net irreversible retention of the radiotracer at steady-state (Ki) and plasma to tissue exchange at 60 minutes (IRF60min).

Results: Breast tumor lesions in all patients were visualized by [¹¹C]choline PET. The difference in tumor versus normal tissue uptake was significant for SUV₃₀, SUV₆₀, Ki, and IRF60 minutes (Wilcoxon $P < 0.0001$). At 60 minutes postinjection, $15.1 \pm 2.16\%$ of plasma radioactivity was due to unmetabolized [¹¹C]choline radioactivity. [¹¹C]Choline uptake was reproducible in breast tumor lesions ($r^2 = 0.9$ for SUV, 0.9 for Ki, and 0.8 for IRF60). Early responses to trastuzumab measured by [¹¹C]choline-PET were significant in three lesions occurring in two patients who responded clinically.

Conclusions: [¹¹C]Choline-PET uptake variables can be reproducibly assessed. Initial studies show that trastuzumab decreases [¹¹C]choline uptake. *Clin Cancer Res*; 16(16); 4236–45. ©2010 AACR.

Radiolabeled choline has been explored as a potential radiotracer for use in positron emission tomography (PET) oncology studies (1–4). Increased choline uptake reflects intracellular choline kinase and activity, which is higher in malignantly transformed cells than in normal human mammary epithelial cells (5, 6). Our group has recently explored the use of [¹¹C]choline in estrogen receptor (ER)-positive breast cancer and found a good correlation with tumor grade, a measure of tumor aggressiveness ($r = 0.7$; ref. 7). This study aimed to explore the utility of [¹¹C]choline in both ER-positive and ER-negative breast tumors, and to determine the test-retest variability of [¹¹C]choline pharmacokinetics in the absence of treatment in the same population.

Authors' Affiliations: ¹Department of Cancer Medicine, Imperial College London, Hammersmith Hospital; ²Department of Histopathology, Imperial College NHS Healthcare Trust, Charing Cross Hospital; ³Department of Nuclear Medicine, Imperial College NHS Healthcare Trust, Hammersmith Hospital, London, United Kingdom; and ⁴Wolfson Molecular Imaging Centre, University of Manchester, Manchester, United Kingdom

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Corresponding Author: Eric Aboagye, Imperial College London, Room 240, MRC Cyclotron Building, London W12 0NN, United Kingdom. Phone: 44-20-83833759; Fax: 44-20-83831969; E-mail: eric.aboagye@imperial.ac.uk

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Choline is an essential component of cell membranes and is thus necessary for cell division; it acts as a source of lipid second messengers through the Kennedy pathway (8). Following transport into the cell, the initial step results in the formation of phosphocholine from the phosphorylation of choline by the enzyme choline kinase (CK; in a reaction dependent on ATP and Mg²⁺); phosphocholine is then effectively trapped intracellularly (9). Human mammary epithelial cells oncogenically transformed by Her2 (5), Ras (10), Src (11), and Mos (11) exhibit increased levels of phosphocholine compared with untransformed cells due to increased constitutive activity of CK α . Alterations in membrane choline phospholipid metabolism are evident in breast cancer tumorigenesis (5). In mitogenic signaling, CK α is thought to play a critical role in the regulation of cell cycle progression through G₁→S transition and has been previously identified as a therapeutic target (12–15).

[¹¹C]Choline-PET has mainly been studied in prostate cancer diagnosis (2, 16–18), and to a lesser extent in other tumors such as glioma (19), lung cancer (20), and hepatocellular carcinoma (21). When compared with [¹⁸F]fluorodeoxyglucose (FDG), [¹¹C]choline shows higher sensitivity for the detection of lung tumors, prostate tumors, esophageal tumors in the mediastinum, and brain tumors measuring ≥ 5 mm (19). Other anatomic regions (liver, pancreas, small bowel) showed high physiologic uptake of choline, potentially limiting the role of [¹¹C]

Translational Relevance

¹¹C]Choline-positron emission tomography (PET) is a marker of choline kinase activity, which is upregulated in tumorigenesis, and as such is a promising imaging technique in cancer. In breast cancer, we have recently shown that tumor ¹¹C]choline uptake by PET imaging correlates with grade in estrogen receptor (ER)-positive tumors. This current study evaluates repeatability of ¹¹C]choline-PET in both ER-positive and ER-negative tumors; we also assessed the effect of trastuzumab on choline uptake. We show that ¹¹C]choline tumor standardized uptake at 60 minutes is reproducible to within 9% (% coefficient of variation). We further provide initial evidence that ¹¹C]choline tumor uptake decreases after treatment with trastuzumab in patients who subsequently had a clinical response, a finding that warrants further evaluation of ¹¹C]choline as a response/resistance imaging biomarker.

choline-PET for tumor detection/monitoring in these sites (19). It is recognized that ¹¹C]choline uptake is not absolutely specific to cancer; other processes such as inflammation or infection may lead to increased uptake, although previous studies have shown that ¹¹C]choline is more sensitive in detecting lymph node metastases in the mediastinum from esophageal and lung tumors than FDG (1, 4, 22).

Before being routinely incorporated into clinical practice, a new radiotracer requires thorough validation to determine the difference between tumor and background uptake and the extent of the effect of metabolism on tumor uptake, and to quantify the intrinsic variability in the absence of treatment. The intrinsic variability of each pharmacokinetic parameter describing uptake and retention should be quantified to provide a robust objective criteria that can be used to determine responses similar to those for Response Evaluation Criteria in Solid Tumors and the more recent guidelines for FDG-PET (known as the Positron Emission Tomography Response Criteria in Solid Tumors; refs. 23, 24).

Choline has been proposed as a pathway marker of the extracellular receptor kinase/mitogen-activated protein kinase pathway (MAPK; ref. 10). Based on its regulation, we proposed that ¹¹C]choline pharmacokinetics would be modified by an agent such as trastuzumab. Breast tumor-derived cells expressing Her2 have high levels of phosphocholine (5). The human epidermal growth factor receptor (Her2/neu) is overexpressed in 20% to 30% of invasive breast cancers. Her2 promotes tumor cell proliferation through the RAS/MAPK pathway and inhibits apoptosis through phosphatidylinositol 3'-kinase-AKT-mammalian target of rapamycin (25). Trastuzumab (Herceptin, Genentech) is a humanized monoclonal antibody that targets the

extracellular domain of Her2, leading to reduced proliferation and survival of Her2 dependent tumors (26).

A reliable biomarker that can be used *in vivo* such as the one proposed here (¹¹C]choline-PET) would have an obvious advantage in being able to detect *de novo* resistance *in vivo* to agents such as trastuzumab so that alternative, more effective treatments can be offered to patients. Given that Her2 and other effectors of the Ras-MAPK pathway increase choline phosphorylation (5, 10, 27), we hypothesized that the retention of ¹¹C]choline will be attenuated through inhibition of Her2 and/or the MAPK pathway.

Materials and Methods

Patients

Our previous study assessed choline uptake only in ER-positive tumors (7). We included ER-negative tumors in this study to extend our knowledge of choline metabolism in breast cancer. Ethical approval for the study was granted by the Hammersmith Hospitals NHS Trust Research Ethics Committee. All patients gave fully informed consent to participate in the study, which was done under 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. Patient recruitment for the study was divided into two arms: (A) To determine the reproducibility of ¹¹C]choline-PET, patients had a baseline scan followed by a second scan within 1 week in the absence of treatment. (B) To assess response to trastuzumab, patients who were eligible for single-agent trastuzumab therapy had a baseline scan followed by a posttreatment scan within 1 month of initiating treatment (mean 9 days, range 2–28 days). Trastuzumab was given intravenously as a loading dose of 8 mg/kg followed by 6 mg/kg every 3 weeks (see Table 3). Patients in group B had a clinical response assessment scheduled for three or more cycles of trastuzumab.

Patients were recruited from the medical oncology clinics at Charing Cross Hospital, London. Inclusion criteria are as follows: ages ≥ 18 and ≤ 80 years, with histologic or cytologic evidence of breast cancer (American Joint Committee on Cancer stage II–IV) including at least one site of measurable disease ≥ 2 cm, a treatment-free interval of ≥ 3 weeks (for cytotoxic chemotherapy, hormonal therapy, or cytostatic agents), 4 weeks applied for radiotherapy to the imaging site, a life expectancy of ≥ 3 months, hemoglobin of ≥ 10 g/dL, granulocyte count of $\geq 1.5 \times 10^9/L$, platelet count of $\geq 100 \times 10^9/L$, adequate creatinine clearance (calculated ≥ 50 mL/min), adequate hepatic function (bilirubin ≤ 1.5 upper limit of normal, serum transaminases ≤ 2.5 upper limit of normal), Eastern Cooperative Oncology Group performance status 0–2 inclusive, and able to give fully informed consent and comply with the protocol. In addition, those patients in the drug treatment arm had to have breast tumors with Her2 receptor positivity of grade 3+ by immunohistochemistry, or positive by a fluorescent *in situ* hybridization test.

Table 1. Patient characteristics

Patient	Tumor type	AJCC clinical stage*	Histologic grade	Estrogen receptor staining
1	Lobular	II	2	ER++
2	Inflammatory	IV	3	Negative
3	Ductal	II	3	Negative
4	Ductal	II	3	Negative
5	Ductal	IV	2	ER+
6	Ductal	IV	2	ER++
7	Mucinous	II	2	ER+++
8	Ductal	III	2	ER+++
9	Ductal	IV	2	ER++
10	Ductal	IV	3	Negative
11	Ductal	III	3	Negative
12	Ductal	II	2	ER+++
13	Ductal	II	3	Negative
14	Ductal	II	3	ER+
15	Ductal	II	3	Negative
16	Ductal	III	3	Negative
17	Ductal	III	2	Negative
18	Ductal	III	3	Negative
19	Ductal	III	3	ER+++
20	Ductal	IV	3	Negative
21	Ductal	IV	3	ER++

Radiotracer synthesis and scanning

[¹¹C]Choline was synthesized through the method previously described by Pascali et al. (28). The target dose for radioactivity was 370 MBq for each scan (equivalent to 1.036 mSv). A short transmission scan lasting 2 minutes for subject positioning, and a second longer transmission scan for attenuation correction were done before radiotracer injection. All patients were scanned on a CTI/Siemens HR+ ECAT962 tomograph (Siemens). The field of view was centered on the target tumor lesion (s) that had been identified previously by clinical/radiological examination.

[¹¹C]Choline was administered through bolus intravenous injection over 10 to 30 seconds followed by a 6 mL normal saline flush. Patients were scanned for 65 minutes in two-dimensional mode. Data were reconstructed by filtered back projection and binned into time frames as follows: 1 × 30 (background), 5 × 10, 2 × 20, 2 × 30, 5 × 60, 3 × 120, 2 × 180, 3 × 300, and 3 × 600 seconds.

Blood sampling and [¹¹C]choline metabolite analysis

The concentration of [¹¹C]choline in arterial plasma was measured using continuous arterial blood sampling over time during the scan to quantify the arterial plasma input function. Blood was sampled through a 22G cannula inserted in the radial artery at a rate of 300 mL/h for the first 10 minutes after radiotracer injection. Total blood radioactivity was measured using an online radioactivity counter. This was compared with that in discrete samples taken at baseline and at 2.5, 3.5, 5, 7.5, 10, 15, 20, 30, 45, 50,

and 60 minutes. The relative contributions of the [¹¹C]choline parent fraction (and the metabolite, [¹¹C]betaine) were determined using reverse-phase high-performance liquid chromatography as previously described (7).

Image and data analysis

Image analysis of tumor and normal tissues was done using Analyze software. The perimeter of each suitable lesion was manually defined by visual inspection of 20 to 60 minutes summed image derived from the dynamic scan. The time course of radioactivity from each region of interest was extracted and displayed as time versus radioactivity curves, decay corrected and normalized to body surface area.

The standardized uptake value at 30 and 60 minutes (SUV₃₀ and SUV₆₀) was calculated by normalizing tumor [¹¹C]choline activity data at 30 and 60 minutes postinjection time points to body surface area and injected dose. SUV = measured tissue activity/([injected activity/body surface area]); AUC was the integral of the SUV over the imaging period. Modified Patlak analysis, which takes account of tissue and plasma metabolites of [¹¹C]choline, was used to derive Ki, a measure of irreversible retention within a region of interest. For flux constant measurements, the presumption of the graphical method used is that no metabolite is retained in the tumor (7, 29–32). Spectral analysis was used to calculate the tumor impulse response function (plasma to tissue exchange) at 60 minutes (IRF60) using the metabolite-corrected plasma input function and the tumor time-activity curve (33). K1 was

calculated using a two-tissue compartmental model as previously described (29).

Statistical analysis

The difference between tumor and normal tissue uptake was evaluated on a lesion-by-lesion basis using the Wilcoxon matched pairs test. Analysis of reproducibility of PET pharmacokinetic parameters was done on a lesion-by-lesion basis. For reproducibility measurements, individual parameter values for the two pretreatment scans were plotted against each other; high reproducibility was represented by all data distributed along a line of unity. Pearson's correlation coefficient and Wilcoxon signed rank test were used to assess reproducibility.

We also assessed the normality of the data as a probability plot of the ordered relative differences versus the normal probability (cumulative frequency of ranks) for each lesion. Relative differences in parameter estimates were defined as follows: $(\text{scan A} - \text{scan B}) / (\text{mean value for scans A and B})$. Intraclass correlation coefficient (ICC), a measure of reliability of the two measurements, was assessed; if the measurements were identical, the

ICC will be 1. A two-way random mixed model with absolute agreement was used. The variance of the relative difference for the different parameters was tested using the *F* test, which compares the SDs of two normally distributed data sets.

The 95% reference ranges for spontaneous fluctuations in the parameters was calculated as $1.96 \times \text{SD}$ of the relative difference in the two pretreatment scans. Data from reproducibility analyses were then used to determine if treatment with trastuzumab resulted in a significant difference in [¹¹C]choline pharmacokinetics. [¹¹C]Choline response to treatment was the relative difference between posttreatment and the pretreatment measurement; that is, $(\text{scan}_{\text{pre}} - \text{scan}_{\text{post}}) / (\text{scan}_{\text{pre}})$. Where two pretreatment scans were available, their average was used. The Mann-Whitney test was used to assess the significance of the change in [¹¹C]choline uptake between (a) clinical responders (complete or partial response, stable disease) and (b) progressive disease. A *P* value of ≤ 0.05 was considered significant. Statistical analyses were done using SPSS for Windows version 12 (SPSS) and GraphPad Prism version 3.0 (Graph Pad Software).

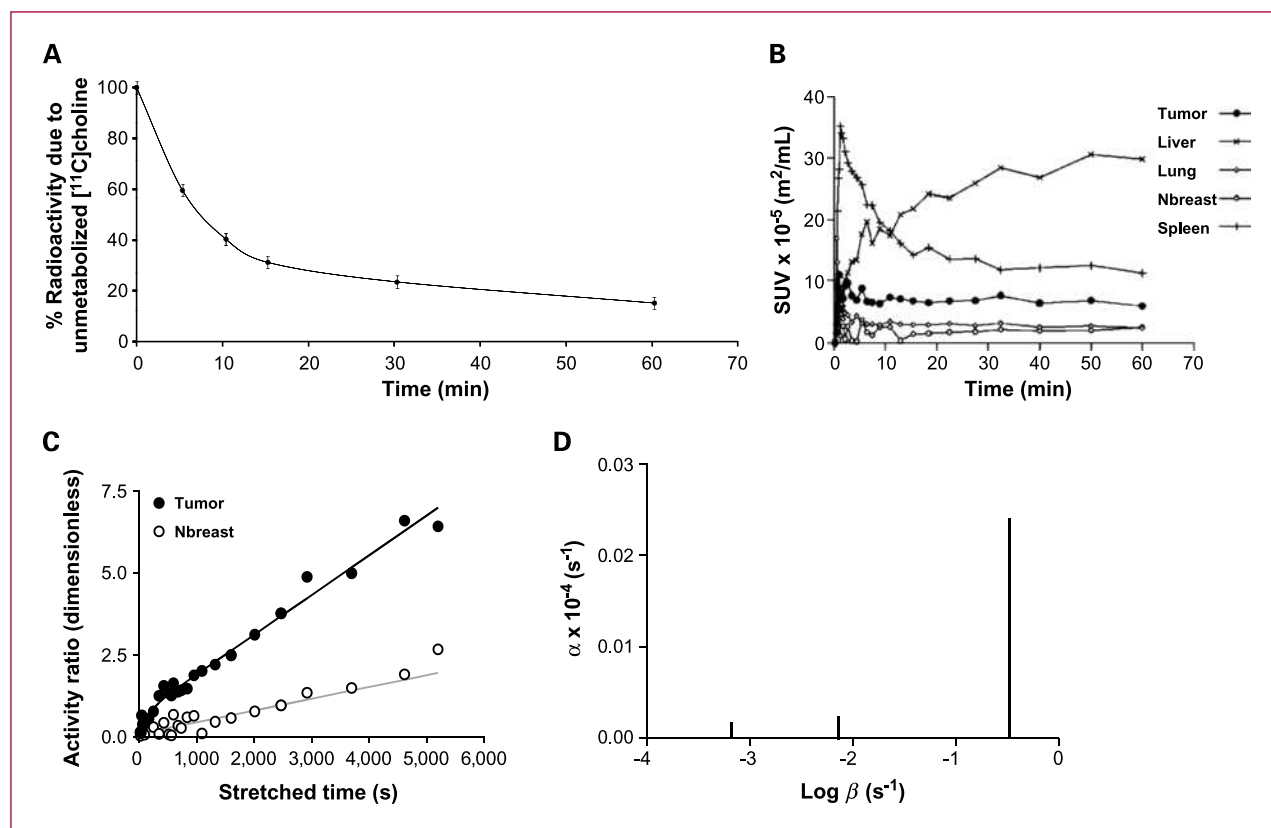


Fig. 1. A, summary of [¹¹C]choline metabolism in plasma. The plot represents the proportion of unmetabolized [¹¹C]choline in arterial plasma expressed as percentage of total plasma radioactivity measured in 20 patients. At 60 min, the mean activity was $15.2 \pm 2.2\%$, with the remainder due to a single metabolite, [¹¹C]betaine. B, time versus radioactivity curve for tumor and normal tissues in patient 4. C, comparison of a modified Patlak model in tumor and normal tissue. D, range of distinct kinetic components within the tumor lesion determined by spectral analysis—log β values to the right of the graph represent reversible components including blood volume (rightmost) and trapped components (leftmost).

Results

Patient characteristics

In total, 21 patients between 39 and 71 years old entered the study, all of whom had a baseline [¹¹C]choline scan; 10 patients had a second scan for repeatability analysis, and 6 patients had scans pre- and post-trastuzumab. The groups were well balanced with regard to stage (eight stage II, six stage III, and seven stage IV). Eleven of the 21 patients had tumor samples that were ER positive, whereas the remainder were ER negative. The scans were well tolerated. The mean radioactivity administered per scan was 347.43 ± 6.29 MBq (specific activity $361-1,316$ MBq/ μ mol). The characteristics of the study patients are shown in Table 1.

Comparison of tumor and normal tissue uptake of [¹¹C]choline

All 21 patients were included in this part of the analysis, which sought to evaluate the differences in pharmacokinetic parameters describing [¹¹C]choline uptake and retention between breast tumors/metastases and normal tissues on a lesion-by-lesion basis (total number of tumor lesions = 25). Tracer uptake was visible in all tumor lesions; no new lesions were identified by [¹¹C]choline-PET, which had not been previously identified by other clinical/radiological methods. Data for the various parameters in breast tumor/metastases and corresponding normal tissues are shown in Supplementary Table S1 and are depicted graphically in Supplementary Fig. S1A to D. Comparisons of SUV and Ki in ER-positive and ER-negative tumors are shown in Supplementary Fig. S1E and F. No arterial samples were available from patient 20; therefore, only semiquantitative analysis (SUV) was done in this particular patient.

Metabolism of [¹¹C]choline

Arterial plasma samples were available for metabolite analysis in 20 patients and showed that the fraction of radioactivity in plasma due to [¹¹C]choline decreased

rapidly postinjection such that at 60 minutes, the mean (\pm SE) radioactivity fraction in arterial plasma due to [¹¹C]choline was $15.15 \pm 2.17\%$ (range 7.98–41.99%; see Fig. 1A). The ¹¹C-radioactivity profile in plasma showed [¹¹C]choline and one other component more polar than [¹¹C]choline, [¹¹C]betaine; evidence that this is the main metabolite of choline in humans has been published elsewhere (32, 34). In five patients who had arterial sampling pre- and post-trastuzumab, there was no significant difference in metabolism of choline (Wilcoxon $P = 0.44$), indicating that the drug had not affected radiotracer metabolism.

Tumor [¹¹C]choline pharmacokinetics

The uptake of [¹¹C]choline was significantly higher in the tumor compared with the corresponding normal tissue (i.e., normal breast tissue or lung tissue) for SUV₃₀, SUV₆₀, IRF60, and Ki (Wilcoxon $P < 0.0001$). Tumor uptake was lowest in a skin metastasis from a ductal tumor (Ki = 4.08×10^{-4} mL plasma/s/mL tissue, SUV₆₀ 2.86×10^{-5} m²/mL) and highest in a lung metastasis (Ki = 18.21×10^{-4} mL plasma/s/mL tissue, SUV₆₀ 12.89×10^{-5} m²/mL). Examples of typical time versus radioactivity curves of [¹¹C]choline uptake in tumor and corresponding normal tissues are shown in Fig. 1B and Supplementary Fig. S2. There was high uptake in the liver and spleen thought to be due to choline metabolism and blood pooling, respectively.

There was a good fit of the Patlak and spectral models to the raw tumor data (see Fig. 1C). Tumor Ki calculated using plasma corrected for choline metabolism but non-metabolite-corrected tissue data as an input function was 17.9% higher than Ki calculated using modified Patlak analysis; the linear part of the Patlak plots suggests that a net irreversible retention component of the tracer is present within our 60-minute data, which was also evident in the spectrum of kinetic components (Fig. 1D).

Reproducibility of [¹¹C]choline PET

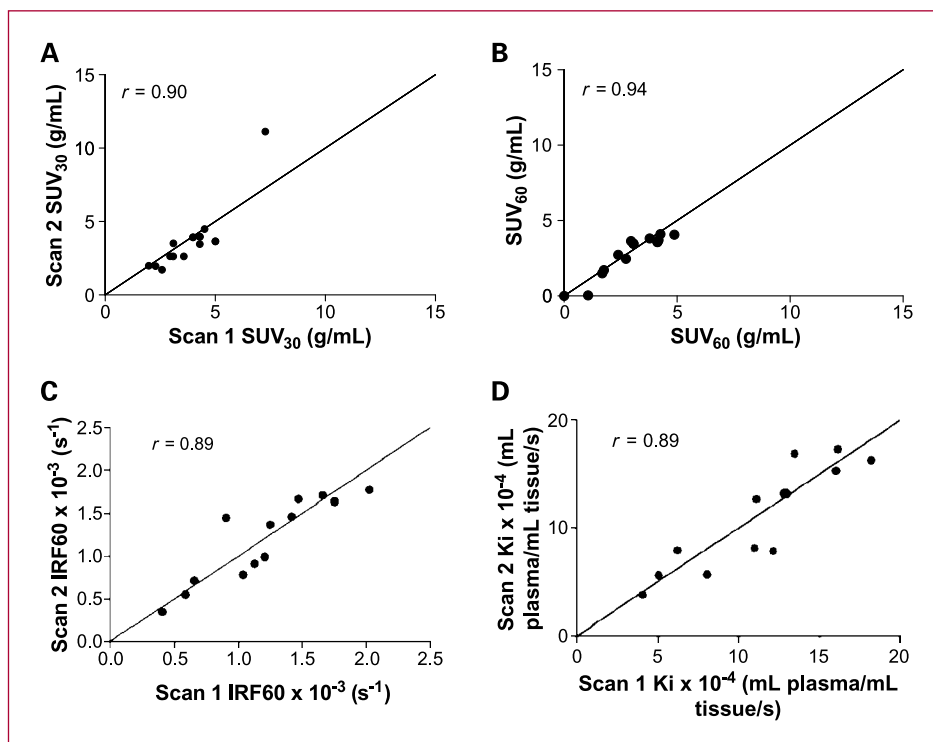
Ten patients were able to complete two [¹¹C]choline scans to determine reproducibility of radiotracer uptake

Table 2. Reproducibility of [¹¹C]choline-PET in breast tumor lesions

	COV	Pearson correlation coefficient <i>r</i>	<i>r</i> ²	Wilcoxon <i>P</i>	SD of mean relative difference (%)	<i>F</i> test	ICC
SUV ₃₀	15.4	0.9	0.8	0.1	20.3	1.0	0.9
SUV ₆₀	8.4	0.9	0.9	0.5	12.4	0.7	0.9
SUV _{MAX60}	16.9	0.8	0.7	0.9	24.8	0.8	0.8
AUC	9.4	0.9	0.9	0.7	14.8	0.6	0.9
Ki	14.7	0.9	0.8	0.8	21.2	0.8	0.9
IRF60	13.4	0.9	0.8	0.5	19.5	1.0	0.9
K1	15.3	0.8	0.6	1.0	22.4	0.5	0.8

Abbreviations: COV, coefficient of variation; SUV₃₀ and SUV₆₀, average SUV value at 30 and 60 minutes, respectively; SUV_{MAX60}, maximum SUV value at 60 minutes; AUC, AUC at 60 minutes; Ki, Patlak Ki corrected for radioactive metabolites of [¹¹C]choline present in plasma and tissue; IRF60, IRF value at 60 minutes; K1, delivery of [¹¹C]choline determined by compartmental analysis.

Fig. 2. Repeatability for the PET pharmacokinetic parameters: A, SUV₃₀; B, SUV₆₀; C, Ki; D, IRF60, with the corresponding Pearson correlation coefficient value (*r*).



and retention. In total, 13 lesions were assessed on an individual basis. The mean number of days (\pm SD) between the first and second scans was 3.75 ± 1.75 days, during which none of the patients received any treatment. Most (70%) of the patients had relapsed disease; the remainder were newly diagnosed. [¹¹C]Choline uptake was found to be reproducible for all the parameters under investigation. The Pearson correlation coefficient (*r*) for Ki (modified Patlak) was 0.89, $P < 0.0001$, and for SUV was $r = 0.945$, $P < 0.0001$ (see Table 2). Results of the correlation analysis are also depicted graphically in Fig. 2A to D for the different imaging parameters. The pharmacokinetic parameters studied were all reproducible, with ICC values approaching 1 in nearly all cases. The semi-quantitative parameters seemed only minimally (nonsig-

nificant) superior compared with the modeled parameters Ki and IRF60.

Relationship of [¹¹C]choline-PET parameters with ER status

There was a significant difference in [¹¹C]choline uptake measured by Ki between ER-positive and ER-negative tumors (Mann-Whitney = 0.032) with significant overlap. The difference was not significant for SUV₆₀ or SUV₃₀ (Mann-Whitney $P = 0.11$ and 0.14 , respectively).

Measurement of response to trastuzumab with [¹¹C]choline

Six patients with eight lesions were imaged before and after treatment with trastuzumab. Posttreatment scans

Fig. 3. [¹¹C]choline-PET images in a patient who responded to trastuzumab. The posttreatment scan was conducted 3 d after initiating single-agent trastuzumab (8 mg/kg).

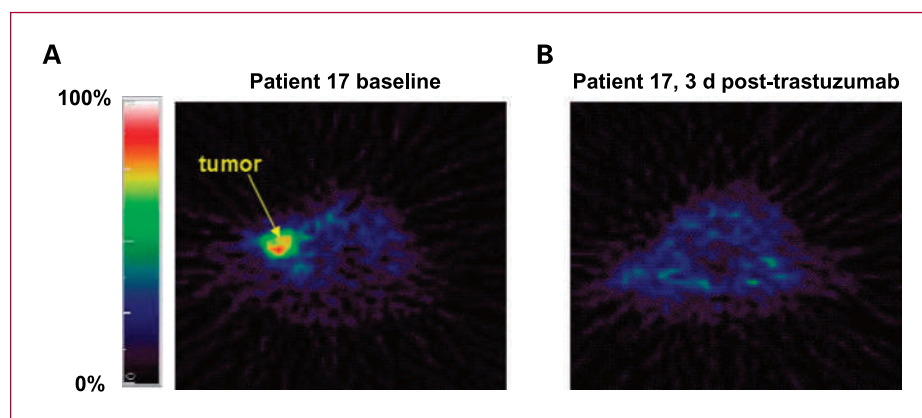


Table 3. Percentage change in [¹¹C]choline parameters post-trastuzumab

Patient	Lesion	Time (d) post-trastuzumab	SUV ₆₀	AUC	Ki	IRF60	K1	Clinical response*
14	Axilla	25,2 [†]	-47.6	-31.4	-47.6	-42.5	-37.4	CR
17	Cervical node	13	-63.9	-46.6	-31.6	-59.1	-70.0	PR
17	Axilla	13	-62.2	-55.9	-29.5	-58.3	-62.8	PR
18	Breast	28	-17.3	-12.4	12.0	-6.5	-24.9	PD
19	Breast	2	13.9	13.1	0.1	2.2	-33.2	SD
20	Lung met1	3	-47.8	-22.8	n/a	n/a	n/a	n/a*
20	Lung met	3	-39.9	-39.5	n/a	n/a	n/a	n/a*
21	Lymph node	4	18.2	17.8	48.6	43.4	+20.3	PD

NOTE: Statistically significant changes for each parameter are highlighted in gray.

Abbreviations: n/a, not applicable; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease.

*Patient 20 died from brain metastases before clinical response of the imaged area in the lungs was due to be done.

[†]Patient 14 received two cycles of trastuzumab before the second PET scan.

were done a median of 3.5 days (range 2–28 days) after single-agent trastuzumab. Typical changes in [¹¹C]choline-PET images in a patient with a partial clinical response (patient 17) are shown in Fig. 3. The percentage changes in parameter values from baseline and the corresponding clinical responses (complete response, partial response, stable disease, and progressive disease) are shown in Table 3. As we proposed to evaluate the most suitable pharmacokinetic parameter using dynamic imaging, the field of view was centered on one region of interest; whole body scanning was not done in this study. Hence, the utility of [¹¹C]choline response measurements in metastases outside the field of view (e.g., brain lesions as in patient 20) was not evaluable, making comparison between PET response and overall clinical response difficult.

Only one patient had a complete response clinically; this occurred in a palpable axillary node, which became impalpable. One other patient had a partial response in two lesions—all of these regions had significant decreases in [¹¹C]choline uptake. One lesion with progressive disease (patient 21) showed an increase in [¹¹C]choline uptake, which was more significant when measured using the modeled parameters Ki and IRF60 than the semiquantitative parameters SUV₆₀ and AUC. The small number of lesions in this study prohibits any definitive statistical analysis to assess if the [¹¹C]choline-PET parameter changes post-trastuzumab are different between responders and nonresponders.

The 95% confidence limits of the reproducibility data were used to determine if parameter changes for individual lesions post-trastuzumab were truly statistically significant when compared with baseline measurements. As seen in Table 3, there was no absolute agreement for the significant responses among pharmacokinetic parameters for each lesion. In summary, for SUV₆₀, there were five lesions that showed a significant decrease; these corresponded with three clinical responses in two patients. Comparison with

clinical response was not possible in the two other lesions due to progression outside the imaging field; the results for AUC were similar. Many of the changes in the kinetic parameters were nonsignificant post-trastuzumab. The modeled parameter Ki detected CR and PD, but not PR, whereas IRF60 only detected PR.

Discussion

We have shown in this study that serial measurements of choline metabolism by [¹¹C]choline-PET can be done reliably and used for monitoring response to drug therapy in patients with breast cancer. [¹¹C]Choline-PET is gaining prominence as an alternative radiotracer to [¹⁸F]FDG-PET, particularly in prostate cancer staging and measurement of residual disease postsurgery (2, 18). We recently showed, for the first time, that [¹¹C]choline-PET also has utility in ER-positive breast cancer. The present study extends that knowledge to ER-negative breast cancer with a more detailed analysis of radiotracer kinetics, and importantly verifies the reliability of performing serial [¹¹C]choline-PET studies.

Comparison of tumor to normal tissue localization of [¹¹C]choline-derived radioactivity by PET indicated high tumor to normal tissue contrast for both ER-positive and ER-negative breast cancer. In general, the technique shows promise for imaging supradiaphragmatic disease; the high physiologic uptake in the liver and spleen will present a challenge for imaging in those regions. To define an optimal time for assessment of breast tumors by [¹¹C]choline-PET, we examined both metabolism of the radiotracer in arterial blood and the time course of tumor radiotracer uptake. In agreement with the pilot studies of Roivainen and coworkers, [¹¹C]choline was metabolized such that only 15.1% of radioactivity was present at 60 minutes postinjection; the only radioactive metabolite observed was the oxidation product of choline oxidase, [¹¹C]betaine.

Objectively, these data would indicate that time points ≤ 30 minutes may be more appropriate to avoid significant contribution of [¹¹C]betaine to the tumor signal as has been observed in animal tumor models (27). Notably, however, the ability of [¹¹C]choline-PET to discriminate breast tumors from normal tissues was found to be similar whether data were assessed at 30 minutes (SUV₃₀) or 60 minutes (SUV₆₀). This latter observation is possibly due to very rapid and efficient phosphorylation and trapping of [¹¹C]choline in tumors, supported by observation of high net irreversible uptake (Ki) and the presence of irreversible components in the spectrum of kinetic components (Fig. 1D). The time versus radioactivity curves remain at steady state after ~ 10 minutes, making it unlikely that [¹¹C]betaine contributes significantly to the intracellular tumor signal, for example, through donation of the radiolabeled methyl group to produce labeled methionine from homocysteine (35). Thus, although present at significant levels in blood, [¹¹C]betaine does not seem to alter the outcome of the data acquisition at 30 or 60 minutes.

The retention of choline within tumor cells, in PET studies, is largely due to the activity of CK, which is overexpressed in breast cancer (9). Estrogen receptor status had no effect on [¹¹C]choline tumor uptake (SUV₆₀ $P = 0.11$) but had an effect with respect to retention (Ki $P = 0.03$), in contrast to previous work by Ramirez de Molina et al. (9) where higher CK α expression was seen in ER-negative tumors. However, this occurred with a large overlap and would have to be confirmed in larger studies. A recent magnetic resonance spectroscopy study by Chen et al. (36) suggested that ER activity had negligible effect on membrane choline phospholipid metabolism. The mechanism for increased choline uptake and retention in ER-negative and ER-positive tumors in this study is thought to be due to elevated CK expression (5, 6), although upregulation of the choline transmembrane transporter may also be important at early time points (37). There was no significant difference between Her2-negative and Her2-positive patients with respect to [¹¹C]choline uptake (data not shown).

For all imaging methods, knowledge of the precision of repeated quantitative end points is a prerequisite for serial measurements, and by extension, reliability for detecting response to drug therapy. In this first test-retest study of [¹¹C]choline-PET in any cancer, we show that serial studies can be done with high reproducibility. ICC values for SUV₃₀, SUV₆₀, SUV_{60MAX}, AUC, Ki, and IRF₆₀ were ~ 0.9 or greater (Table 2); as expected, macropharmacokinetic parameters (K1, K3) were more variable. We showed that [¹¹C]choline SUV₆₀ was reproducible to within 9% (% coefficient of variation). [¹¹C]Choline-PET was less reproducible than 3'-deoxy-3'-[¹⁸F]Fluorothymidine (FLT) and FDG (38, 39). This could be attributed to reduced counts due to the use of ¹¹C compared with ¹⁸F, or to more rapid metabolism of [¹¹C]choline; unfortunately, there are limited data on the reproducibility of ¹¹C-labeled PET radiotracers in human tumors for comparison. These studies again

justify the use of SUV₃₀ and SUV₆₀ in the assessment of choline metabolism by PET. The [¹¹C]choline-PET objective response was defined in this study as the reduction in imaging parameters below the 95% confidence limits in the test-retest study. Thus, with the current protocol (without correction for respiratory motion), a decrease of 39.8 for SUV₃₀, 24.2% for SUV₆₀, 41.6% for Ki, 59.3% for IRF₆₀, and 27% for AUC will be classified statistically as response. As with other oncology radiotracers, the precision of the modeled pharmacokinetic parameters (Ki, IRF₆₀) were not necessarily superior to semiquantitative parameters (SUV). Whether the accuracy of the two approaches differs in the assessment of drug therapy remains to be seen.

The final part of this study sought to determine whether [¹¹C]choline could be used to measure response to treatment, in this case treatment of Her2-positive patients with trastuzumab. We selected this patient group because Her2 overexpression increases the levels of phosphocholine in immortalized mammary epithelial cells (5). Using the objective response criteria defined above, significant reductions in [¹¹C]choline parameters were observed in some patients within 1 month of initiating trastuzumab therapy (Table 3). That response to trastuzumab was detectable; the technique allows detection of changes outside the 95% reference range of spontaneous fluctuations. The small number of patients included in this part of the study (partly attributed to the switch of clinical treatment protocols within the hospital from single-agent trastuzumab to combination regimen during the trial period) precludes definitive inferences to be made regarding the optimal parameter for assessing response, and whether both increases (seen for Ki in a patient with progressive disease) and decreases (seen in responding patients) are definitive for categorizing clinical response. To date, no previous PET studies using [¹¹C]choline have evaluated tumor response to a therapeutic agent. Most studies have analyzed choline metabolism in tumors using magnetic resonance spectroscopy. Meisamy et al. (40) studied a group of breast cancer patients undergoing neoadjuvant treatment with doxorubicin-based chemotherapy with quantitative ¹H-magnetic resonance spectroscopy. A positive correlation was found between percentage change in total choline (% Δ tcho) and change in longest diameter. Interestingly, % Δ tcho at 24 hours posttreatment was able to distinguish between responders and nonresponders. Another recent study by Baek et al. (41) evaluated the relationship between ¹H-magnetic resonance spectroscopy measurements of total choline compounds (phosphocholine, glycerophosphocholine, and choline) and pathologic complete response in patients receiving neoadjuvant treatment for breast cancer, and found that, in responders, the change in choline metabolism was greater than change in tumor size. However, the reduction in total choline between responders and nonresponders only became significant at 69 days. Whether the PET technique allows earlier assessment of response will become apparent in future studies.

In conclusion, [¹¹C]choline-PET can be used in serial measurements of breast tumors with high precision; there seems to be a decline in choline uptake in response to trastuzumab in some patients; and further study is warranted for the use of choline as a response marker.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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