Original article

[¹⁸F]fluorothymidine-positron emission tomography in patients with locally advanced breast cancer under bevacizumab treatment: Usefulness of different quantitative methods of tumor proliferation

J.M. Marti-Climent a, *, I. Dominguez-Prado a, M.J. García-Veloso a, V. Boni b, I. Peñuelas a, I. Toledo c, J.A. Richter a

a Servicio de Medicina Nuclear, Clinica Universidad de Navarra, Pamplona, Spain
b Departamento de Oncología Médica, Clinica Universidad de Navarra, Pamplona, Spain
c Departamento de Medicina Preventiva y Salud Pública, Facultad de Medicina, Universidad de Navarra, Pamplona, Spain

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A B S T R A C T

Objectives: To investigate quantitative methods of tumor proliferation using 3-[¹⁸F]fluoro-3-deoxithymidine ([¹⁸F]FLT) PET in patients with breast cancer (BC), studied before and after one bevacizumab administration, and to correlate the [¹⁸F]FLT-PET uptake with the Ki67 index.

Material and methods: Thirty patients with newly diagnosed, untreated BC underwent a [¹⁸F]FLT-PET before and 14 days after bevacizumab treatment. A dynamic scan centered over the tumor began simultaneously with the injection of [¹⁸F]FLT (385 ± 56 MBq). Image derived input functions were obtained using regions of interest drawn on the left ventricle (LV) and descending aorta (DA). Metabolite corrected blood curves were used as input functions to obtain the kinetic Ki constant using the Patlak graphical analysis (time interval 10–60 min after injection). Maximum SUV values were derived for the intervals 40–60 min (SUV40) and 50–60 min (SUV50). PET parameters were correlated with the Ki67 index obtained staining tumor biopsies.

Results: [¹⁸F]FLT uptake parameters decreased significantly (p < 0.001) after treatment: SUV50 = 3.09 ± 1.21 vs 2.22 ± 0.96; SUV40 = 3.00 ± 1.18 vs 2.14 ± 0.95, KiLV[10–3] = 52[22–116] vs 38[13–80] and KiDA[10–3] = 49[15–129] vs 33[11–98]. Consistency interclass correlation coefficients within SUV and within Ki were high. Changes of SUV50 and Ki_DA between baseline PET and after one bevacizumab dose PET correlated with changes in Ki67 index (r-Pearson = 0.35 and 0.26, p = 0.06 and 0.16, respectively).

Conclusions: [¹⁸F]FLT-PET is useful to demonstrate proliferative changes after a dose of bevacizumab in patients with BC. Quantification of tumor proliferation by means of SUV and Ki has shown similar results, but SUV50 obtained better results. A correlation between [¹⁸F]FLT changes and Ki67 index was observed.

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[¹⁸F]fluorotimidina-PET en pacientes con carcinoma de mama localmente avanzado en tratamiento con bevacizumab: utilidad de diferentes métodos de cuantificación de la proliferación celular

R E S U M E N

Objetivos: Evaluar métodos cuantitativos de proliferación celular en PET con 3-[¹⁸F]fluoro-3-desoxitimidina ([¹⁸F]FLT), antes y después de una dosis de bevacizumab en pacientes con carcinoma de mama (CM), y correlacionar la captación de [¹⁸F]FLT con el índice Ki67.

Material y métodos: Se incluyeron 30 mujeres con CM no tratadas. Se realizó [¹⁸F]FLT-PET antes y 14 días después de una dosis de bevacizumab. La PET dinámica centrada en el tumor se inició simultáneamente con la infusión de [¹⁸F]FLT (385 ± 56 MBq). Se dibujaron regiones de interés en ventrículo izquierdo (VI) y aorta descendente (AD), obteniéndose funciones de entrada, que corregidas por metabolitos, se utilizaron para obtener la constante Ki de Patlak (intervalo: 10-60 min). Se calcularon valores máximos del SUV en los intervalos 40-60 min (SUV40) y 50-60 min (SUV50). Los parámetros PET se correlacionaron con el Ki67, obtenido en biopsias tumorales teñidas.


Palabras clave:
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* Corresponding author.
E-mail address: jmmartic@uanv.es (J.M. Marti-Climent).

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intracelular fueron elevados en los SUV y en los Ki. Los cambios de SUV50 y Ki, AD entre la PET basal y la PET tras bevacizumab se correlacionaron con los cambios en el Ki67 (r-Pearson = 0.35 y 0.26, p = 0.06 y 0.16, respectivamente).

**Conclusiones:** La [*F]*FLT-PET refleja los cambios en la proliferación celular tras una dosis de bevacizumab en pacientes con CM. La cuantificación de la proliferación por medio del SUV y la Ki arrojó resultados similares, si bien fueron mejores con el SUV50. Los cambios en [*F]*FLT se correlacionaron con los cambios en el índice Ki67.

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**Introduction**

Breast cancer is the most frequent neoplasia in women and the most frequent cause of death in women between 35 and 55 years of age. Five-year overall survival is estimated to be 65%, with large disparities between stages. At the time of the diagnosis success to 20% of breast cancer patients present a locally advanced breast cancer (LABC) without distant metastases. The current standard of care for LABC is preoperative systemic therapy (PST). In general, 60–90% of patients achieve clinical response to PST. Complete pathologic remissions (pCR) are, however, noted in only 3–30% of patients in most breast cancer trials. These patients have improved survival compared with patients achieving a less than pCR. An ideal setting for preoperative chemotherapy would be to give the most sensitive and effective chemotherapy drug regimen to the patient to increase the fraction of pCR achieved. In the last years, special attention was directed on the identification of molecular profiles and predictive biomarkers associated with pCR. PST offers the ability to discern treatment effect in vivo and ultimately may allow the identification of useful biomarkers for individualization of treatment based on clinical, pathologic, image-guided, or molecular assessment of the tumor during initial treatment. The monoclonal antibody to the vascular endothelial growth factor (VEGF) bevacizumab (Avastin, Genetech) is routinely used in different tumor types, including metastatic breast cancer, based on results from the E2100 phase III study conducted in 722 patients. This study showed that bevacizumab substantially improves the progression-free survival, but not overall survival, of metastatic breast cancer when compared with single-agent paclitaxel. Currently, its role in the preoperative setting together with the identification of predictive biomarkers has become an important field of research. Wedam et al. showed that bevacizumab alone followed by bevacizumab with doxorubicin/docetaxel in patients with inflammatory breast cancer resulted in a 70% clinical response.

Currently, no validated biomarkers exist for appropriate selection of patients with cancer for antiangiogenic therapy; however, a number of systemic, circulating, tissue and imaging biomarkers are emerging. PET is a non-invasive imaging modality that offers the possibility to visualize in vivo different metabolic cellular processes and tumor biology. Recently, García García–Esquinazas et al. have demonstrated that SUVmax percentage changes (ΔSUVmax) in PET–CT could accurately predict pCR to neoadjuvant chemotherapy (NAC) of breast cancer patients. Forty-three women with stage II–III breast cancer were included in their study. FDG PET–CT was performed at baseline and after NAC, and ΔSUVmax were compared with pathologic findings at surgery. They obtained an accuracy of 90.7% to predict tumor response. (*[F]*F) kinetic studies have been investigated during treatment of recurrent high-grade brain tumors with bevacizumab and irinotecan. High correlation was found between [*F]*FLT uptake in tumor (SUV) and influx rate constant (Ki), indicating that [*F]*FLT PET is a potential useful imaging biomarker for therapy monitoring with prognostic value. Thus, imaging tumor proliferation with [*F]*FL PET could offer a new approach to assessing tumor growth kinetics. The ability of [*F]*FL PET to provide an early measure of treatment response has been demonstrated in several preclinical and clinical studies with a variety of tumor types. The PET data have also been correlated with biological factors. García Vicente et al. correlated biological parameters and semiquantitative metabolic parameters in 36 women with locally advanced breast cancer.

This study was included in a multicenter, prospective, phase II clinical trial, designed to identify biomarkers of response to bevacizumab therapy in the preoperative setting in patients with untreated LABC. A dose of bevacizumab alone (15 mg/kg) was administered in cycle 1, and bevacizumab in combination with adriamidine and docetaxel were administered for cycle 2 through cycle 5. After PST all patients underwent surgery. We sought to determine whether tumor sampling techniques can be replaced with non-invasive imaging methods to assess tumor changes following neoadjuvant bevacizumab treatment. The status of biomarkers and tumor imaging profiles before treatment, together with changes after treatment, may allow prediction of sensitivity or resistance to anti-VEGF agents and provide an opportunity to understand their mechanism of action.

The objectives of the present report were to evaluate different quantitative methods to monitor tumor proliferative changes using [*F]*FLT-PET in patients with LABC, studied before and after a single infusion of bevacizumab, and to study if changes observed in [*F]*FLT before and after the antiangiogenesis therapy correlates with those observed in the immunohistochemical biomarker of proliferation Ki67.

**Material and methods**

**Patient selection**

Thirty women with newly diagnosed, biopsy-proven, untreated LABC were enrolled (mean age: 50 ± 10 years [35–69 years]). Patients were required to be at least 18 years old, be cancer-free for the previous 5 years, not be pregnant or breastfeeding, and able to give informed consent and to lie still for the duration of the study. This study, as a part of a clinical trial, was conducted under protocols approved by the institutional review board/Independent Ethics Committee, Human Subjects Division and Radiation Safety Committee at each study center and patients signed informed consent at the hospital before enrollment.

**[*F]*FLT synthesis**

[*F]*FLT was automatically synthesized and prepared for human use in our institution. Syntheses and quality control strictly followed the Standard Operating Procedures (SOPs) and were subject to inspection. The labeling yield, radiochemical purity, and specific radioactivity of [*F]*FLT were checked and recorded after each production.

All reagents were obtained with the highest purity available and were used without further purification. The precursor 3-N-Boc-5′-O-dimethoxysteryl-3′-O-nosyl-thymidine was purchased from ABX Advanced Biomedical Compounds (Germany).
The synthesis process was based on other previously published methods\textsuperscript{18} and developed by our team using Eckert & Ziegler “Modular Lab System”. Briefly, [\textsuperscript{18}F]fluoride was obtained by irradiation of \textsuperscript{H\textsubscript{2}}\textsuperscript{18}O, concentrated by azotropic distillation and 25 mg of the Boc precursor in 1 mL of acetonitrile were added to the dried \textsuperscript{[18}F]fluoride residue. The nucleophilic substitution reaction was carried out at 110 °C, the reaction mixture hydrodized with 1 N HCl, neutralized and injected into the semi-preparative HPLC system (C18 column). Using \textsuperscript{H\textsubscript{2}}\textsuperscript{O}/EtOH (92/8) as mobile phase, [\textsuperscript{18}F]FLT eluted at around min 19. The product fraction was filtered into a sterile multidose vial through two 0.22 μm sterile filters. The final yield of the synthesis was usually 15% (uncorrected) with a radiochemical purity >97%.

PET scan and reconstruction

Each patient was scheduled to have two PET evaluations. Baseline [\textsuperscript{18}F]FLT scans were scheduled preceding the commencement of treatment. Patients continued in the study irrespective of whether [\textsuperscript{18}F]FLT tumor uptake was identified on the baseline scan to explore whether lesions initially undetectable by [\textsuperscript{18}F]FLT might become visible during treatment. On-treatment [\textsuperscript{18}F]FLT PET scans were performed 14 days after a single bevacizumab dose (15 mg/kg), in order to capture information regarding initial DNA damage to cells and early response to treatment.

The PET scans were carried out in an ECAT EXACT HR+ scanner (CTI-Siemens, Knoxville, USA). The patient was placed with the tumor centered in the field of view (15.2 cm).\textsuperscript{19} A 5 min transmission study with three \textsuperscript{68}Ge sources was conducted in order to perform the attenuation correction. Simultaneously with a bolus injection of 385 ± 56 MBq (10.4 ± 1.5 mCi) [\textsuperscript{18}F]FLT, a dynamic acquisition started in 2D mode with a total duration of 60 min, with a sequence 6 × 5 s, 6 × 10 s, 3 × 20 s, 5 × 30 s, 5 × 60 s, 8 × 150 s, 6 × 300 s.

The reconstruction was performed as 128 × 128 matrices using ordered-subset expectation maximization (OSEM) with two iterations and 8 subsets followed by a postsmoothing of the reconstructed image using a 5 mm FWHM Gaussian filter. Corrections were applied to account for scattered photons, random events and photon attenuation. Each frame of the dynamic study had 63 axial slices.

Other procedures

Within 24 h after the completion of the PET studies, before and after treatment with bevacizumab, an ultrasound-guided biopsy of the tumor was performed. The Ki67 index of cell proliferation in these biopsies was determined.

[\textsuperscript{18}F]FLT quantification

For the quantification of [\textsuperscript{18}F]FLT PET studies, Patlak graphical analysis\textsuperscript{20} was carried out with an input function obtained from the image (image derived input function, IDIF).\textsuperscript{21,22} Two input functions were obtained using spherical volumes of interest (VOI) (15 mm diameter) drawn on the left ventricle (LV) and on the descending aorta (DA) in the early images 25–50 s after administration of [\textsuperscript{18}F]FLT as shown in Fig. 1a. These volumes were applied to the dynamic study to obtain, from the mean value functions, the input curves for the kinetic model (Fig. 1b).

The input functions were corrected for the presence of metabolites. For this purpose we used the metabolite fractions according to the Schiepers formula\textsuperscript{23}:

\[
\text{Metabolite fraction} = 0.42[1 - \exp(-0.029t)]
\]

Fig. 1. Patlak analysis: (A) example of volumes of interest in the left ventricle and descending aorta structures for image derived input function determination (B).

Patlak analysis was performed using the PMOD 3.0 software (PMOD Technologies Ltd., Adliswil, Switzerland) in the time interval between 10 and 60 min after injection, determining the influx constant (Ki). Two constants were obtained, considering the input function from the left ventricle (Ki,LV) and the curve from the descending aorta (Ki,DA).

The dynamic study was used to obtain PET images equivalent to a static PET study at the end of the dynamic sequence, corresponding to the time intervals 40–60 min and 50–60 min, which corresponds to studies of 20 and 10 min starting 40–50 min after administration of the radiopharmaceutical. These images allowed quantifying the standardized uptake value (SUV) SUV40 and SUV50 respectively. SUV values were derived from the maximum uptake in the tumor.

Changes in tumor proliferation before and after treatment were calculated according to the formula:

\[
\% \text{ change} = \left( \frac{[\textsuperscript{18}F]FLT \text{ post-treatment} - [\textsuperscript{18}F]FLT \text{ pre-treatment}}{[\textsuperscript{18}F]FLT \text{ pre-treatment}} \right) \times 100
\]

Statistical analysis

We analyzed the descriptive parameters, the correlation and the concordance between the quantitative variables PET (SUV and Ki constant), and PET parameters were also correlated with cell proliferation index Ki67. Statistical analysis was performed using SPSS software (version 15.0; IBM).

Results

The statistical parameters of SUV values and Ki influx constant of both scans are summarized in Table 1, and the corresponding box plots are shown in Fig. 2. The four parameters used to quantify cell proliferation showed a statistically significant reduction after treatment compared to baseline values. Mean decrease of SUV50, SUV40, Ki,LV and Ki,DA were 26%, 27%, 27% and 31% respectively, while the constant Ki67 felt 14 units. Statistically significant differences (p < 0.05) between the influx constants Ki,LV and Ki,DA
in the post-bevacizumab treatment PET scan were also observed. An example of the bevacizumab treatment effect in a patient is illustrated in Fig. 3, where a decrease of the four PET quantitative parameters studied is presented.

Changes in the SUV50 and Ki_DA PET parameters between basal state and after bevacizumab treatment showed a moderate correlation with changes in the Ki67 index (Pearson r = 0.35 and 0.26, respectively). SUV50 changes were more accurate (p = 0.06) than changes in Ki_DA (p = 0.16). Changes in SUV40 and Ki_LV showed a weaker correlation with the Ki67 reduction than SUV50 and Ki_DA (Fig. 4). The percentage of patients that had lower [\(^{18}\)F]FLT uptake after the bevacizumab treatment was 97 and 93% according to SUV50 and SUV40 measurements respectively.

The agreement within the two SUV measures and within the two influx constants were studied evaluating the interclass correlation coefficient (Table 2). The accordance degree was almost perfect (ICC > 0.81) in the 4 comparisons, showing the interchangeability of quantitative data between SUV40 and SUV50 and between Ki_LV and Ki_DA.

The correlation between the SUV values in tumors with the corresponding Ki influx constant, obtained at baseline and after treatment, is illustrated in Fig. 5. A stronger and significant correlation was found for Ki obtained with the input function from the left ventricle than the one obtained from the descending aorta (Table 3).

**Discussion**

\[^{18}\text{F}\]FLT is phosphorylated by thymidine kinase-1 (TK1) and trapped inside the cell. The application of \[^{18}\text{F}\]FLT phosphorylation as a biomarker of cell proliferation is based in the assumption that cellular \[^{18}\text{F}\]FLT trapping is a representation of TK1 activity, which reflects the DNA synthesis. \[^{18}\text{F}\]FLT-PET has been shown

**Table 1**

Descriptive statistics, mean ± standard deviation or median [range], for \[^{18}\text{F}\]FLT quantitative parameters of basal and post treatment scans.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>Post-treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV50</td>
<td>3.09 ± 1.21</td>
<td>2.22 ± 0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SUV40</td>
<td>3.00 ± 1.18</td>
<td>2.14 ± 0.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ki_LV (10(^{-3}) min(^{-1}))</td>
<td>52[22–116]</td>
<td>38[13–80]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ki_DA (10(^{-3}) min(^{-1}))</td>
<td>49[15–129]</td>
<td>33[11–98]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 2**

Interclass correlation coefficient within PET quantitative parameters.

<table>
<thead>
<tr>
<th>PET</th>
<th>Compared parameters</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal SUV50</td>
<td>SUV50</td>
<td>0.992</td>
</tr>
<tr>
<td>Post-treatment SUV50</td>
<td>SUV40</td>
<td>0.992</td>
</tr>
<tr>
<td>Basal Ki_LV</td>
<td>Ki_DA</td>
<td>0.933</td>
</tr>
<tr>
<td>Post-treatment Ki_LV</td>
<td>Ki_DA</td>
<td>0.874</td>
</tr>
</tbody>
</table>

Fig. 2. Box-plot of the quantitative parameters SUV and influx rate constant Ki (min\(^{-1}\)) before and after an infusion of bevacizumab.

Fig. 3. \[^{18}\text{F}\]FLT PET scan in the breast tumor before (A) and after (B) a single infusion of bevacizumab. A decrease of the PET parameters was observed: SUV40 from 4.57 to 3.33, SUV50 from 4.35 to 3.52, Ki_LV from 0.109 to 0.078 and Ki_DA from 0.128 to 0.098.
quantitative methods in patients with LABC, studied before and after a single infusion of bevacizumab, within a multicenter, prospective, phase II clinical trial.

Van der Weerd et al. suggested the use of the ascending aorta as the structure of choice for defining IDIF and a large region of interest (ROI with 15 mm diameter, approximately) to minimize the effects of statistical noise. They also found that ascending aorta and DA had lower interobserver variation when applied to myocardial metabolic rate of glucose determination, compared with the use of left ventricle. De Langen et al. used IDIF with ROIs in the ascending aorta and aortic arch, but in our study the VOI were not always possible to be correctly drawn because the scanner field of view was centered in the breast tumor. Our results showed that Ki changes after an infusion of bevacizumab evaluated using IDIF obtained from the DA had a slightly higher correlation with the Ki67 index change than with those Ki changes obtained using the LV.

Ki and SUV obtained from the maximum values showed similar correlation with the proliferation index Ki67, although SUV obtained from a static acquisition required a shorter protocol and data analysis easier to be performed than needed for the influx constant evaluation. The latter was simplified using a modeled metabolic fraction that allowed the use of image derived input functions, without the need of blood sampling and further analysis.

Correlation between SUV values and the influx constant, pre and post the bevacizumab administration.

to be useful in predicting breast cancer responses to therapy and it has been investigated during treatment of recurrent high-grade brain tumors with bevacizumab and irinotecan. In this study we have investigated the usefulness of different \[^{18}\text{F}]\text{FLT}-\text{PET}

**Table 3**

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV50</td>
<td>Ki_LV</td>
<td>0.877</td>
</tr>
<tr>
<td>SUV50</td>
<td>Ki_DA</td>
<td>0.694</td>
</tr>
<tr>
<td>SUV40</td>
<td>Ki_LV</td>
<td>0.868</td>
</tr>
<tr>
<td>SUV40</td>
<td>Ki_DA</td>
<td>0.684</td>
</tr>
</tbody>
</table>

Fig. 4. Correlation between Ki67 change and PET change before and after a bevacizumab infusion (Spearman Rho).

Fig. 5. Correlation between SUV values and the Patlak influx constant, pre and post the bevacizumab administration.
In conclusion, [18F]FLT-PET is a non-invasive imaging technique that allowed the evaluation of tumor proliferation, by assessing the activity of the enzyme thymidine kinase. The [18F]FLT-PET quantification has demonstrated proliferative changes after a single dose of bevacizumab in patients with LABC. In our population, quantification of tumor proliferation through the SUV and the Patlak influx constant, with input function derived image, have shown similar results in the evaluation of bevacizumab therapy in patients with LABC previously untreated. Nevertheless, it is suggested to extend these quantitative methods to a longer series of patients, to corroborate our results.

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**Conflicts of interest**

The authors have no conflicts of interest to declare.

**Acknowledgements**

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