Original Article

Usefulness of positron emission mammography in the evaluation of response to neoadjuvant chemotherapy in patients with breast cancer

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Abstract: Our study examines the association between two Positron Emission Mammography (PEM) semi-quantitative parameters: PUVmax (maximum uptake value) and LTB (lesion to background) baseline and the end of Neoadjuvant chemotherapy (NAC) with pathologic response in each of the following breast cancer subtype: Triple negative breast cancer (TPN), HER2-positive, and ER-positive/HER2-negative cancers. One-hundred and eight patients, 71 with invasive ductal carcinoma and 37 with infiltrating lobular carcinoma were evaluate with ¹⁸F-FDG-PEM scans before and after of NAC. We assessed the impact of 2 PEM semi-quantitative parameters for molecular subtype correlated with pathologic response according Miller-Payne grade (MPG). After NAC, an overall reduction of 2 PEM semi-quantitative parameters was found. Neither breast cancer subtypes nor Ki67 modified chemotherapy responses. Compared to PUVmax, an overall increase of LTB was found in baseline condition, independent of the expressed immunophenotype. Post-treatment values of PUVmax revealed a significant reduction compared to baseline values (4.8 ± 0.26 vs. 1.9 ± 0.18; P < 0.001) and LTB exhibited a significant decay after the first course of NAC (15.8 ± 1.36 vs. 5.5 ± 0.49; P < 0.001). Using the Kruskal-Wallis H test which showed no correlation between the different molecular subtypes and the MPG and PUVmax and LTB (P = 0.52). Two PEM semi-quantitative parameters demonstrated a statically significant correlation and equivalence across the different breast cancer subtypes correlated with pathologic response according to MPG. PEM did not allow for prediction of NAC response in terms of breast cancer biomarkers, it is not discarded that this technology might be helpful for individual treatment stratification in breast cancer.

Keywords: Positron emission mammography, breast cancer, neoadjuvant chemotherapy, ¹⁸F-FDG-PEM

Introduction

Locally advanced breast cancer is a subset of breast cancer characterized by the most advanced breast tumors in the absence of distant metastasis [1]. According to the American Joint Committee on Cancer, clinical scenarios, including in the locally advanced disease are stages III-B-III, advanced nodal disease and inflammatory carcinomas [1, 2]. Neoadjuvant chemotherapy (NAC) has an important role in patients with locally advanced cancers, treating distant micrometastases, downstaging tumors, improving operability, and sometimes allowing breast-conserving surgery to take place [3, 4]. In response to NAC, tumor cells express metabolic responses. The measurement of glucose metabolic rates after NAC is recognized as an important diagnostic tool due to an early decrease in glucose uptake is related to a better disease-free and overall survival [4, 5]. Positron emission tomography (PET/CT) has shown to be useful in different stages of breast cancer (BC) such as staging, evaluate recurrence, and treatment response assessment, where the sensitivity is poor in lesions less than 10 mm; in these scenario current clinical evidence suggest that ¹⁸F-FDG high resolution positron emission mammography (high resolution PEM) has higher sensitivity that PET/CT for very small lesions (< 10 mm) and more accu-
rate for detected unsuspected additional lesions [6, 7]. The advantages of such dedicated system include improved geometric sensitivity, higher spatial resolution, shorter imaging time, and reduced attenuation compared with whole-body positron emission tomography systems [8, 9].

To measure glucose metabolism, high resolution PEM use two metabolic parameters: the maximum PEM uptake value or PUVmax and the lesion-to-background ratio or LTB [9, 10]. PUVmax is an uncorrected value that suffers from the effects of the background signal. Meanwhile, the attenuated-corrected ratio (LTB) incorporates the background signal, but the method for measuring the background is unspecified and varies from report to report. Thus, the clinical significance of this improvement has not yet been fully established [9].

On the other hand, it is known that breast carcinomas consist of different subtypes depending on hormone receptors, such as ER+ tumors, HER2 overexpression, and the cell proliferation marker Ki67. All of these biomarkers play a critical role in the glucose metabolism, correlating with the survival outcomes of patients [11-15].

Some histopathological classifications are useful to evaluate tumoral response to NAC. We chose the Miller-Payne grading (MPG) system to assess the pathological response. The MPG system are based on tumor cellularity in the excision/mastectomy specimen compared with the pretreatment core biopsy as follows: grade 1, no reduction in overall cellularity; grade 2, minor (< 30%) loss of cellularity; grade 3, estimated 30%-90% reduction in tumor cells; grade 4, > 90% loss of tumor cells; and grade 5, no invasive carcinoma (IC) [14, 15].

Nevertheless, there is a need to define standardized metabolic criteria of early response to the therapy considering the heterogeneity of subtypes, and to supervise the effectiveness of 18F-FDG-high resolution PEM in monitoring the benefits of chemotherapy.
The main objective of the present study was to examine the association between two PEM examination-parameters: PUVmax (tissue concentration [mCi/g] × weight [g]/injected FDG dose [mCi]); and LTB (lesion to background ratio [the ratio of the lesion’s PUVmax to the average background value]) measured at the baseline PEM examination and the end of first course of NAC, and pathologic response in each of the following breast cancer subtype: Triple negative breast cancer (TPN), HER2-positive, and ER-positive/HER2-negative cancers.

Methods and materials

Patients

This is a retrospective study was approved by the institutional review board with waivers of written informed consent. From August 2012 and May 2015, 108 consecutive patients with clinical stage II or III breast cancer underwent two \(^{18}\)F-FDG-high resolution PEM studies, before starting NAC chemotherapy and the end of the first course of NAC. The NAC regimens, the clinical stages and the molecular breast cancer subtypes and the rest of characteristics of study population are summarized in Table 1. The exclusion criteria were confirmed distant metastasis and previous NAC or hormone therapy. All patients underwent two PEM studies, before the treatment and the end of the first course of NAC.

High-resolution PEM and quantification of the \(^{18}\)F-FDG uptake

High-resolution PEM imaging was performed in a commercially available high-resolution PEM unit (PEM Flex, Naviscan PET Systems, Inc., Rockville, MD). Technical specifications have

Figure 1. NAC responses according to the breast cancer subtype. \(^{18}\)F-FDG uptake values reflected as PUVmax and LTB at baseline (A) and interim (B) PEM evaluation for response assessment. Overall, LTB was increased in comparison with PUVmax. Meanwhile, all breast cancer subtypes pooled together exhibited a reduction in both uptake parameters in response to NAC (C and D). Similar responses to NAC were observed in LUM (E), HER2 (F) and TPN tumors (G). Error bars indicate the standard errors of the mean (SEM). *P < 0.05, **P < 0.01 and ***P < 0.01. Abbreviations: NAC: neoadjuvant chemotherapy, LUM: molecular subtypes of breast cancer, including luminal A and luminal B, HER2: human epidermal growth factor receptor 2, TPN: triple negative breast cancer.
been previously described by Yamamoto et al. [9]. For the quantification of \(^{18}\)F-FDG uptake, a region of interest (ROI) was drawn around the lesion in question, and the maximum absorption was recorded as PUVmax following the formula: tissue concentration (mCi/g) × weight (g)/injected FDG dose (mCi). The ratio of the lesion’s PUVmax to the average background value (LTB), was obtained brief, 2-cm circular ROI was drawn on the slice of nipple, and the ROI was drawn in a homogeneous area of 6 normal breast tissue. The position of this ROI was equal in both studies.

Patients were required to fast for four h before the administration of 185 MBq of \(^{18}\)F-FDG and their serum glucose levels was below 150 mg/dl. The high-resolution PEM scan was performed 45 min after FDG injection. The acquisition time was 5 min, and the view mammograms were cephalocaudal (CC) and mediolateral oblique (MLO). High-resolution PEM produces a 12-slice tomographic image display; the slice thickness is equal to the compressed breast thickness divided by 12. Two nuclear medicine physician independently measured semi-quantitative uptake without clinical data using a workstation (MIM viewer PEM 1.0, MIM Software Inc., Cleveland, OH, USA).

### Histopathological classification

Breast cancer immunophenotypes were classified as follows: luminal (ER+), HER2 overexpressing (ER-/PR-/HER2+), and triple negative (ER-/PR-/HER2-). Previous studies have subclassified luminal tumors as luminal A or luminal B; however, because these luminal subtypes are clinically similar and not well distinguished by only ER, PR, and HER2 status, all luminal tumors were pooled for further analysis.

### Statistical analysis

Quantitative values were shown as mean ± the standard error of the mean. The Wilcoxon signed-rank test was used to compare each uptake parameter at baseline vs. those values obtained in the interim evaluation and the Mann-Whitney test was used for comparisons between PUVmax and LTB. Differences in the proportion of the responses were analyzed by Fisher’s exact test. The association between PUVmax and LTB was evaluated with a Pearson correlation test. Additionally, we performed a two-sample equivalence test by using the responses to treatment expressed as percentages that were calculated as follows:

\[
\Delta \text{PUVmax} (%) = 100 \times \frac{(\text{PUVmax}_F - \text{PUVmax}_I)}{\text{PUVmax}_I}
\]

\[
\Delta \text{LTB} (%) = 100 \times \frac{(\text{LTB}_F - \text{LTB}_I)}{\text{LTB}_I}
\]

Correlations with pathologic response (Wilcoxon rank sum test) pathological response and correlation/equivalence between PUVmax and LTB. We analyzed the MPG and PUVmax and LTB measurements for molecular subtype using the Kruskal-Wallis H test.

All hypothesis testing was performed with \(\alpha = 0.05\), and statistically significant differences are designated with an asterisk. SPSS 17.0 (SPSS Inc, Chicago, IL, USA) was used for data analysis.

### Results

Median tumor size was 52 mm (22-110). 65% of patients (71 out of 108 patients) had invasive ductal carcinoma, whereas 35% (37 out of 108 patients) had infiltrating lobular carcinoma.

### Evaluation of response to NAC, according to the immunophenotype

Two hundred and sixteen \(^{18}\)F-FDG PEM scans were performed in all patients. The first was carried out at the beginning of treatment (baseline); and the other one, was performed after

### Table 2. Percentage of metabolic response

<table>
<thead>
<tr>
<th>Treatment response (%)</th>
<th>n</th>
<th>PUVmax (% ± SE)</th>
<th>LTB (% ± SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUM</td>
<td>84</td>
<td>50.6 ± 6.39</td>
<td>48.5 ± 5.50</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>HER2</td>
<td>13</td>
<td>65.9 ± 6.50</td>
<td>59.9 ± 7.12</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>TPN</td>
<td>11</td>
<td>65.3 ± 7.50</td>
<td>66.8 ± 15.55</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Ki67 &lt; 14%</td>
<td>40</td>
<td>44.5 ± 5.55</td>
<td>37.9 ± 7.37</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Ki67 ≥ 14%</td>
<td>68</td>
<td>47.4 ± 5.50</td>
<td>47.1 ± 5.57</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Abbreviations: LUM, molecular subtypes of breast cancer including luminal A and luminal B; HER2, human epidermal growth factor receptor 2; TPN, triple negative breast cancer; SE = standard error; PUVmax, maximum uptake value; LTB, lesion to background.
the first cycle of NAC (interim) with a median interval of 13 days (range 3-28 d). Compared to PUVmax, an overall increase of LTB was found in baseline condition, independent of the expressed immunophenotype. This phenomenon was also observed after NAC (Figure 1A; 4.8 ± 0.26 vs. 1.9 ± 0.18; ***P < 0.001). Likewise, LTB exhibited a significant decay after the first course of NAC (Figure 1D; 15.8 ± 1.36 vs. 5.5 ± 0.49; ***P < 0.001).

Finally, changes in both uptake parameters were analyzed for each immunophenotype, and it was found a reduction in PUVmax and LTB values for LUM (Figure 1E; PUVmax: 4.9 ± 0.33 vs. 1.9 ± 0.19; LTB: 16.0 ± 1.95 vs. 5.8 ± 0.63, P < 0.001), HER2 (Figure 1F; PUVmax: 5.2 ± 0.75 vs. 2.3 ± 1.0; LTB: 12.5 ± 1.55 vs. 6.2 ± 2.37, P < 0.05), and TPN (Figure 1G; PUVmax: 5.3 ± 0.51 vs. 2.0 ± 0.92; LTB: 14.7 ± 3.14 vs. 4.65 ± 1.9; P < 0.01). Comparing to LUM, TPN and HER2-positive patients exhibited a tendency to increase metabolic response based on the change in both semiquantitative parameters, although the differences were not statically significant (Table 2).

**Correlation and equivalence between PUVmax and LTB according to the immunophenotype**

If a background signal interferes with the PUVmax value, the linear relationship between PUVmax and LTB can exhibit a biased relationship. To discard this possibility, a Pearson correlation analysis was used to test the linearity in the relation between both uptake parameters. Additionally, it was applied an equivalence test to determine whether the means for PUVmax and LTB are close enough to be considered equivalent.

The Pearson correlation indicates a direct association between both parameters in all of the conditions tested (P < 0.05). This is denoted in Figure 2A-D. Moreover, the confidence intervals at the 95% supported the equivalence between PUVmax and LTB (all cases 95% CI = -7.65, 7.38; LUM 95% CI = -2.21, 3.41; HER2 95% CI = -7.65, 0; TPN 95% CI = 0, 10.0; Figure 2).
Evaluation of response to NAC, according to the expression of Ki67, and correlation/equivalence between PUVmax and LTB

In parallel with the immunophenotype recordings, it was assessed the treatment response, according to the expression of Ki67. **Figure 3** shows that PUVmax and LTB at baseline, were significantly higher in patients with Ki67 ≥ 14% than in patients with Ki67 < 14% (PUVmax: 5.9 ± 1.21 vs. 3.7 ± 0.45; LTB: 18.0 ± 1.7 vs. 12.1 ± 1.7; P < 0.01) (**Figure 4A** and **4B**). Graph results also summarizes the reduction of PUVmax and LTB in both groups (Ki67 < 14% and Ki67 ≥ 14%) after NAC (PUVmax: Ki67 < 14%: 3.7 ± 0.45 vs. 1.9 ± 0.5, P < 0.05; Ki67 ≥ 14%: 5.9 ± 1.21 vs. 2.0 ± 0.77, P < 0.001; LTB: Ki67 < 14%: 12.1 ± 1.7 vs. 4.9 ± 0.5, P < 0.05; Ki67 ≥ 14%: 18.0 ± 1.7 vs. 5.3 ± 0.91, P < 0.001).

Compared with patients with Ki67 < 14%, tumors with Ki67 ≥ 14% also show a tendency to exhibit an increased metabolic response (**Table 2**).

Lastly, the measurements of the correlation between PUVmax and LTB revealed that there were significant associations between PUVmax and LTB in patients with high and low expression of Ki67 (**Figure 4C** and **4D**). In agreement with these results, equivalence test shows that metabolic responses were indistinguishable between PUVmax and LTB, corroborating the equivalence among both parameters (**Figure 4E**); Immunohistochemical stains for Ki-67 in patients with high and low expression of Ki67 (**Figure 4F** and **4G**). Using the Kruskal-Wallis H test which showed no correlation between the different molecular subtypes and the MPG and PUVmax and LTB (P = 0.52), but if a correlation was found between the response rate by MPG and both semiquantitative parameters (**Table 3**).

**Discussion**

Multiple research groups recommend the use of metabolic imaging as a diagnostic and predictive tool, imaging modality of choice is whole body 18F-FDG PET/CT [16, 17]. However, the fundamental limitation of this technique remains its difficulty in accurately characterizing the metabolic activity of lesions smaller than 2.5 cm due to partial volume effects and smoothing. Information obtained from semiquantitative analysis of PEM appears to be beneficial for quantifying the metabolic activity of lesions smaller than those seen with whole...
PEM in patients with breast cancer

Figure 4. NAC responses according to the expression of Ki67, and correlation and equivalence between PUVmax and LTB. Graph results show increased PUVmax and LTB values at baseline in patients with high expression of Ki67. Meanwhile, a significant reduction of both uptake parameters at the interim was recorded in patients with high and low expression of Ki67 (A and B). Analysis of correlation indicates a strong association between PUVmax and LTB in both groups (C and D), while equivalence between both uptake parameters also was corroborated (E). Immunohistochemical stains for Ki67 in Luminal A (< 14%) (F) and Luminal B (> 14%) (G) based on Eye-10 method (× 100).

Body PET/CT [19]. The technological advancement of PEM will allow progress not only in making the diagnosis of the disease at an early stage, but also in determining early tumor responses to treatment, which is now a key component for optimizing therapies [7]. Here, we provide a detailed description of the changes in tumors metabolic responses to NAC by using PEM. In view of the fact that variations in glucose metabolism are related to specific tumor markers we also stratify those metabolic responses according to the subtypes of breast cancer and to the expression of Ki67 [19, 20].

This retrospective study demonstrated that LUM, HER2 and TPN had similar PUVmax and LTB values in baseline PEM scan. Also, a substantial reduction of PUVmax and LTB after NAC occurred in pooled data of all subtypes of breast cancer. Moreover, metabolic responses for each subtype were as considerable as those recorded in the pooled data.

Regarding percentages of chemotherapy responses, we found no substantial changes neither across subtypes nor between PUVmax and LTB. In all cases, response rates to NAC were greater than 40%. Based on previous demonstrations, it is feasible that this percentage may predict a good rate of pathological complete response [21, 22].

On the other hand, correspondences between PUVmax and LTB suggests that background activity does not affect metabolic measurements. In favor of this observation, we also recorded high positive correlations between both uptake parameters. Moreover, the equivalence test indicates that the metabolic responses measured with LTB were within the margin of the PUVmax. Since equivalence implies that PUVmax and LTB are similar enough that there is no practical consequence to assuming that they are equal, this corrobo-
Table 3. Miller-Payne grade with molecular subtypes and correlation with PEM values

<table>
<thead>
<tr>
<th>MPG</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2</th>
<th>TPN</th>
<th>x²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13 37.14</td>
<td>16 32.65</td>
<td>7 54.84</td>
<td>6 54.54</td>
<td>2.83</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>12 34.28</td>
<td>22 44.89</td>
<td>3 23.07</td>
<td>4 36.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8 22.85</td>
<td>4 8.16</td>
<td>2 15.38</td>
<td>1 9.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 5.71</td>
<td>2 4.08</td>
<td>1 7.69</td>
<td>0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35 100</td>
<td>49 100</td>
<td>13 100</td>
<td>11 100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MPG, Miller-Payne grade; HER2, human epidermal growth factor receptor 2; TPN, triple negative breast cancer.

Many breast cancer subtypes have good chemotherapy response, however, seems to be reflected in the percentage of response that were improved in TPN and HER2-positive related with LUM, although without being statistically significant. We suggest that the lack of significance could be the consequence of a low proportion of HER2 and TPN cases. Therefore, we cannot rule out the possibilities that these may be chance findings or may reflect no changes in the metabolic activity of tumors, requiring a much larger sample size to improve this observation.

However, if correspondences in the interim responses to NAC between subtypes will confirmed; then, our results might be in accordance with the hypothesis that cell killing during chemotherapy follows a first-order kinetic model, where a large absolute change in the number of viable tumor cells must occur early in the course of therapy to achieve a complete or subtotal tumor response. In fact, it is previously described that, even in nonresponding tumors, the number of tumor cells may decrease by up to 90%, resulting in a corresponding decrease of $^{18}$F-FDG uptake [28].

Remarkably, patients with high levels of Ki67 showed high pre-treatment values of both metabolic parameters in comparison with patients with low levels of Ki67. This finding confirms that the overexpression of cell proliferation markers induces exacerbated metabolic responses [29, 30]. Nevertheless, posttreatment PUVmax or LTB were similar between patients with low and high levels of Ki67. Also, the response rates were comparable between groups and, once more, differences between PUVmax and LTB were not registered.

Even though our results are not conclusive, we might infer that Ki67 has not a prognostic role...
in NAC responses. However, results expressed as percentage newly leads us to observe a tendency for patients with Ki67 ≥ 14%, who eventually had increased responses, supposing a more adept response to chemotherapy in the neoadjuvant setting [31]. Still, with no conclusive data, the main question remains whether Ki-67 provides prognostic information for interim-PEM reporting [32].

Our study has some limitations. First, the number of patients studied was relatively small and, second, the median follow-up duration was quite short. Therefore, the subgroup analysis does not have much power, and the lack of statistical significances should be interpreted with caution. Despite these limitations, our data suggest that PUmax and LTB have a similar potential to reflect early chemotherapy responses in breast cancer, showing the value of the high resolution efficiency.

Conclusions

LTB demonstrated a statically significant correlation and equivalence with PUmax across the different breast cancer subtypes correlated with pathologic response according to MPG, suggesting that PUmax has a low-affectation of the background signal. On the other hand, there is a need for further development of follow-up studies that can accurately distinguish biomarkers influencing breast cancer prognosis between subjects.

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Disclosure of conflict of interest

None.

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