Original Article

18F-FDG semi-quantitative parameters and biological prognostic factors in locally advanced breast cancer


Aim: To analyse the correlation between 18F-FDG uptake assessed by PET/CT in locally advanced breast tumours and histopathological and immunohistochemical prognostic factors.

Material and methods: Thirty-six women with breast cancer were prospectively evaluated. PET/CT was requested in the initial staging previous to adjuvant chemotherapy (multicentric study). All the patients underwent an 18F-FDG PET/CT with a dual-time-point acquisition. Both examinations were evaluated qualitatively and semiquantitatively with calculation of SUVmax values in PET-1 (SUV-1) and in PET-2 (SUV-2) and the percentage variation of the standard uptake values (retention index) between PET-1 and PET-2.

Clinical and metabolic stages were assessed according to TNM classification. The biological prognostic parameters, such as the steroid receptor status, p53 and c-erbB-2 expression, proliferation rate (Ki-67), and grading were determined from tissue of the primary tumour. Metabolic and biological parameters were correlated.

Results: A positive relationship was found between semiquantitative metabolic parameters and biological parameters. SUV-1 and SUV-2 values did not show significant statistical correlation (p<.05) except for the clinical tumour size.

About the biological parameters, retention index showed the best results with positive and significant relation (p<.05) with estrogen and progesterone receptor status and Ki-67. Isolated SUV values did not show significant relation to these parameters.

Conclusion: Retention index showed the best relation with biological parameters compared to isolated SUVmax values. These data suggest that SUV change over time is a prognostic marker.

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Parámetros semicuantitativos de 18F-FDG y factores pronósticos biológicos en el cáncer de mama localmente avanzado

Objetivo: Analizar la correlación entre la captación de 18F-FDG valorada por la PET-TC en cáncer de mama localmente avanzado y factores pronósticos histopatológicos e inmunohistoquímicos.

Material y métodos: Se valoraron prospectivamente 36 mujeres con cáncer de mama. La PET-TC fue requerida en la estadificación previamente al tratamiento quimioterápico (estudio multicéntrico). A todas se les realizó una PET-TC con 18F-FDG en 2 fases. Ambas fueron valoradas cualitativa y semicuantitativamente con cálculo del SUVmax en la PET-1 (SUV-1) y en la PET-2 (SUV-2) así como el índice de retención.

Los estadígrafos clínicos y metabólicos fueron evaluados siguiendo la clasificación TNM. Se determinaron los parámetros biológicos pronósticos del tumor primario, como el estado de los receptores esteroideos, la expresión del p53 y c-erbB-2, el índice de proliferación (Ki-67) y el grado histológico. Los parámetros biológicos e histológicos fueron correlacionados.

Resultados: Se encontró una relación positiva entre los parámetros metabólicos semicuantitativos y los biológicos. Los valores de SUV-1 y SUV-2 no mostraron una correlación estadísticamente significativa excepto para el tamaño tumoral.
Introduction

Preoperative prediction of patient prognosis is becoming increasingly important because more and more women with breast cancer are given neoadjuvant chemotherapy with the aim of downstaging their disease and increasing the feasibility of breast-conserving surgery.

$^{18}$F-FDG (FDG) uptake may provide important clinical and biological information that can be of prognostic significance. In addition, it has been proposed that dual-time-point FDG-PET may improve the sensitivity and accuracy of FDG-PET in assessing patients with primary breast cancer.\(^1\)

The biological characteristics of the tumours are considered important prognostic factors in patients with breast cancer and may influence glucose metabolism as detected by PET.\(^2\)

Patients with locally advanced breast tumours have a worse prognosis due to the lymph node involvement and a higher incidence of distant metastases. Thus, following the recommendation of NCCN Task Force Report, FDG PET/CT is indicated in locally advanced tumours and is not recommended in stages I and II because of the low prevalence of metastatic disease and the high rate of false positives.\(^3\)

The aim of this prospective analysis was to determine the possible correlation between FDG uptake and well-established prognostic markers in women with locally advanced breast cancer.

Material and methods

This prospective and multicentric study (Investigation project, grant: 2009/40) was approved by the review boards of the seven enrolled hospitals. Written consent was obtained from all patients.

Patients

From October 2009 to December 2010, all patients with the following inclusion criteria were studied: large (stage IIB) and/or locally advanced breast cancer (IIA–IIIC) with indication of neoadjuvant chemotherapy. Exclusion criteria were clinical stage IV, previous surgery to the breast or axilla or administration of a previous treatment.

All patients underwent digital mammography, breast ultrasonography and FDG PET/CT before receiving neoadjuvant chemotherapy.

Tumour histology and biological parameters were evaluated by the core needle biopsy before neoadjuvant chemotherapy.

Patients fasted for at least 4 h before the FDG administration and had blood glucose levels less than 160 mg/dl at the time of injection.

Image acquisition and analysis

FDG PET/CT was performed in a reference hospital, following a standardized protocol, in three-dimensional (3D) mode, 3 min/bed position with a dedicated whole-body PET/CT machine, placed in our reference centre for the study. Transmission scans were performed for all patients to provide attenuation correction with CT. The PET section thickness was 3.8 mm. Iterative reconstruction and scatter correction of image were done.

All the patients underwent PET/CT with a dual-time-point acquisition. The first examination was performed as whole-body images from head to thigh 60 min after intravenous administration of approximately 370 MBq of $^{18}$F-FDG (PET-1). The second examination imaged the chest only, with acquisition of one or two bed positions 3 h after FDG administration (PET-2).

Both examinations were evaluated qualitatively and semiquantitatively with calculation of SUVmax values in PET-1 (SUV-1) and in PET-2 (SUV-2). The percentage variation of the standard uptake values (retention index, RI) between PET-1 and PET-2 was obtained attending to the formula: $RI = \frac{SUV-2 - SUV-1}{SUV-1} \times 100$.

PET/CT images were interpreted by two nuclear medicine specialists blinded to the patients record. If the interpretation differed, consensus was reached with the help of a third physician.

The SUVmax of the breast cancer was measured by manually marking a cubical volume of interest (VOI) around the tumour.

Clinical and metabolic sizes of the primary tumour were established attending the biggest diameter assessed by morphological techniques (digital mammography and breast ultrasonography) and the maximum diameter of the lesion in the axial delayed PET imaging respectively.

The metabolic N status was visually evaluated in axilla and in internal mammary, supra and infraclaviclar regions. A lymph node with a detectable metabolism, higher than background activity in adjacent fat that increasing in the delayed PET/CT was considered positive.

Clinical and metabolic stages were determined according to the American Joint Committee on Cancer (AJCC) 7th edition by clinical examination, mammogram and ultrasonography (clinical stage) and FDG PET/CT (metabolic stage) in all the patients.\(^4\) An example is shown in Fig. 1.

The presence or absence of metastatic disease was evaluated in FDG PET/CT.

Prognostic factors

In breast tumours there are several prognostic factors in different phases of validation. In Table 1 are referred prognostic factors attending to their importance and clinical evidence.

<table>
<thead>
<tr>
<th>Category</th>
<th>Prognostic factors</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Axillary involvement, grade and histological type of tumour, mitotic rate, ER/PR expression.</td>
</tr>
<tr>
<td>2</td>
<td>C-erb-B2, proliferation markers (MIB-1), vascular invasion and p53.</td>
</tr>
<tr>
<td>3</td>
<td>Microvascular density, ploidia, GERF, TGFrα, bcl-2, p52 and D-catepsine.</td>
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</table>
In this study we selected category 1 and some category 2 prognostic factors to establish their relation with metabolical parameters.

**Histopathological analysis**

The histopathological analysis was performed on the specimens obtained by gross-needle aspiration biopsy and surgical procedures.

The determination of the tumour type (four main groups: infiltrating ductal carcinoma, infiltrating lobular carcinoma, lobular carcinoma “in situ” and ducal carcinoma “in situ” with occasional subtypes from the first one) and the histopathological grading (1, well differentiated; 2, moderately differentiated; 3, poorly differentiated) were performed on formalin fixed, paraffin embedded tumour tissue, cut at 5 µm sections and stained with haematoxylin and eosin.

Immunohistochemistry was performed on paraffin embedded material using primary antibodies for oestrogen and progesterone receptors, p53 and c-erbB2 and the proliferation index was accounted with the Ki-67 antibody.

Oestrogen and progesterone receptors were scored as positive or negative using a cut-off of 10% nuclear immunostaining. P53 nuclear staining was evaluated as positive or negative. C-erbB2 was evaluated as positive when the membrane immunostaining was complete in more than 30% of tumoral cells (3+) and when they were less than 30% (2+) but the analysis with in situ hybridization with fluorescence (FISH) showed her2 gene amplification. The Ki-67 proliferation index was considered high when it was over 30% of nuclear immunostaining.

Final N status (positive or negative) was established histopathologically guided by ecography or sentinel node biopsy.

**Statistical analysis**

All statistical tests were two-sided with a significance level of p < 0.05. SPSS 18.0.1 for Windows was used for all analyses.

All semiquantitative data were expressed in terms of mean ± SD. The Spearman’s rank-order correlation coefficient was used to measure the association between SUVmax and the numerical prognostic variable, and the association between SUVmax and categorical measures was assessed by Mann–Whitney U and Kruskal–Wallis tests (clinical markers and molecular biomarkers).

### Results

The study included 36 women with locally advanced breast cancer (mean age 45 ± 12.5 years).

The mean clinical size of the primary breast lesion attending to morphologic techniques was 46.7 ± 25.1 mm. The mean metabolical size was 37.5 ± 24.1 mm.

Clinical and metabolical stages assessed according to the AJCC are shown in Table 2. 25% of patients were diagnosed of advanced disease attending to the metabolical assessment.

Mean tumour SUV-1 and SUV-2 was of 8.05 ± 6.53 and 9.05 ± 7.70 respectively. The mean RI was of 13.4% ± 17.7.

Tables 3 and 4 show the correlation between the SUVmax in the breast tumours and histopathological and immunohistochemical prognostic factors.

A significant positive and moderate relation was observed between SUV-1 and clinical size although the correlation was weak for the other metabolical parameters (SUV-2 and RI). With regard to the metabolical size the relation was weak and not significant.

Attending the proliferation rate, high proliferative tumours with an overexpression of Ki-67 showed high glucose metabolism with a significant and moderate relation for the RI. This relation was not significant for isolated semiquantitative parameters, SUV-1 and SUV-2 (Table 3).
Attending to our results, RI was the double 9,11–13.

Some previous studies found significantly In several other reports, no significant In our study a the relation although,  although breast cancer recurrence is a potential indication for FDG PET/CT, the management of locally advanced breast tumours supports the current indication of this technique, principally for diagnostic purposes and the assessment of response to chemotherapy.

Pathologic grade is a major predictive factor in breast carcinoma and is used to describe the differentiation of tumour tissue reflecting the degree of malignancy. The relation between SUV and histological grade is explained because tumours with high glucose metabolism proliferate actively, and tumours with high proliferation activity usually show high histological grade. In our study a positive relation, although not significant, between high tumour grade and SUVmax value was found. This finding is in agreement with previous reports. 7–11

Ki-67 is a nuclear antigen expressed in the G1, G2 and S phases of the cell cycle but not in the G0 phase. There is an increased FDG uptake during both the S phase and the G1/G2 phases. About the proliferation markers, some authors who studied proliferation marker Ki-67 found a positive correlation between the percent of Ki-67-labelled cells and SUV uptake. 7–11 In our results the relation was moderate and significant for the RI.

There have been contradictory reports on steroid hormone receptor status and 18F-FDG uptake. Some studies showed no correlation between hormone receptor status and SUV values. 9,11–13 However, many recent series showed higher SUV in ER negative tumours. 7,14–16 Attending to our results, RI was the double in estrogen receptor negative tumours or progesterone receptor negative ones compared to tumours with hormone receptor expression.

FDG uptake was higher in infiltrating ductal than in lobular carcinomas like other authors reported previously. 12,17,18 although, to our knowledge, non-biological explanation has been identified. Oncoprotein c-erbB-2 overexpression is a well-known factor of tumour aggressiveness and poor prognosis. Ueda et al. found a significant relationship between 18F-FDG uptake and c-erbB-2 oncogene expression. 12 In several other reports, no significant influence of c-erbB-2 overexpression on SUV was found as in our work although tumours with c-erbB-2 overexpression showed higher RI compared to tumours with no overexpression (mean of 17% vs 13%). This finding might suggest that c-erbB-2 has no major influence on glycolytic pathways. 7,8,11–16

Overexpression of the tumour suppressor gene p53 is a common feature of malignant breast tumours. It has been suggested that overexpression of p53 reflects tumour aggressiveness and decreased survival. 20 Some previous studies found significantly higher SUV values in patients with non-functional p53, 5,11,15 and others found no correlation between 18F-FDG uptake and p53 status 21 but used an immunohistochemical (IHC) method to determine p53 status. We do not find significant correlation although

### Table 3
Correlation of clinical and metabolical sizes and proliferation index (Ki-67) with semiquantitative metabolic parameters.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Pearson</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Clinical size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUV-1</td>
<td>0.46</td>
<td>0.008</td>
</tr>
<tr>
<td>SUV-2</td>
<td>0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>RI</td>
<td>0.19</td>
<td>0.33</td>
</tr>
<tr>
<td>Metabolical size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUV-1</td>
<td>0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>SUV-2</td>
<td>0.09</td>
<td>0.66</td>
</tr>
<tr>
<td>RI</td>
<td>0.17</td>
<td>0.40</td>
</tr>
<tr>
<td>Ki-67 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUV-1</td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td>SUV-2</td>
<td>0.37</td>
<td>0.06</td>
</tr>
<tr>
<td>RI</td>
<td>0.52</td>
<td>0.007</td>
</tr>
</tbody>
</table>

The mean of SUVmax value was lower, but not significant, in invasive lobular than in invasive ductal carcinoma.

Considering tumour grade, mean SUVmax was higher in grade 3 than in grades 2 and 1 (p > 0.05).

Positivity for estrogen receptors or progesterone receptors was associated with lower mean SUVmax values but the differences were higher and significant for the RI values (Figs. 2 and 3).

On the other hand, overexpression of c-erbB-2 showed no significant impact on SUVmax values although the differences were higher for RI than for SUVmax values (Fig. 4).

Tumours with positive p53 also had higher SUVmax values (p > 0.05) compared to negative p53 tumours.

### Discussion

Although breast cancer recurrence is a potential indication for FDG PET/CT, the management of locally advanced breast tumours supports the current indication of this technique, principally for diagnostic purposes and the assessment of response to chemotherapy.

Pathologic grade is a major predictive factor in breast carcinoma and is used to describe the differentiation of tumour tissue reflecting the degree of malignancy. The relation between SUV and histological grade is explained because tumours with high glucose metabolism proliferate actively, and tumours with high proliferation activity usually show high histological grade. In our study a positive relation, although not significant, between high tumour grade and SUVmax value was found. This finding is in agreement with previous reports. 7–11

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### Table 4
Comparison of maximum standardized uptake values and retention index (RI) with pathological and biological variables.

<table>
<thead>
<tr>
<th>Biological characteristics</th>
<th>n</th>
<th>Mean SUV-1 ± SD</th>
<th>Mean SUV-2 ± SD</th>
<th>RI ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological grade (p value)</td>
<td>11</td>
<td>8.50 ± 5.04</td>
<td>10.61 ± 8.04</td>
<td>21.42 ± 21.81</td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>7.43 ± 5.96</td>
<td>9.04 ± 8.04</td>
<td>11.41 ± 14.40</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>8.50 ± 5.04</td>
<td>10.61 ± 8.04</td>
<td>21.42 ± 21.81</td>
</tr>
<tr>
<td>Progesterone receptor (p value)</td>
<td>12</td>
<td>7.26 ± 6.59</td>
<td>8.74 ± 8.74</td>
<td>8.95 ± 10.53</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>7.12 ± 4.16</td>
<td>8.48 ± 5.81</td>
<td>18.75 ± 20.35</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>8.20 ± 4.95</td>
<td>10.14 ± 7.58</td>
<td>13.11 ± 15.21</td>
</tr>
<tr>
<td>C-erb-B2 (p value)</td>
<td>14</td>
<td>8.63 ± 6.93</td>
<td>10.85 ± 9.76</td>
<td>17.28 ± 20.63</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>7.12 ± 4.16</td>
<td>8.48 ± 5.81</td>
<td>13.11 ± 15.21</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>8.20 ± 4.95</td>
<td>10.14 ± 7.58</td>
<td>13.11 ± 15.21</td>
</tr>
<tr>
<td>p 53 (p value)</td>
<td>11</td>
<td>9.44 ± 5.45</td>
<td>11.84 ± 8.78</td>
<td>18.09 ± 20.76</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>5.79 ± 4.20</td>
<td>6.99 ± 6.15</td>
<td>11.82 ± 13.02</td>
</tr>
</tbody>
</table>
p53 positive tumours showed higher semiquantitative values of SUV-1 and SUV-2 with a greater difference for the RI.

The high FDG uptake in tumours with non-functional p53 are explained because mutation of p53 impair the repressive effect of p53 on GLUT1 and GLUT4 gene promoters. Furthermore, in non-functional p53 tumours there is a loss of expression of TIGAR (TP53-induced glycolysis and apoptosis regulator) that can also explain the high FDG uptake.
Early breast tumours, with less biologically aggressive features, are less glycolitic than more advanced ones. Our mean SUV values were higher than previously reported values in tumours in an early stage. Crippa et al.\(^2\) found a mean SUV of 5.6 for invasive ductal carcinoma. On the other hand our mean SUV values were in concordance with the reported by Basu et al.\(^2\) in patients with axillary lymph nodes and distant metastasis (mean SUV-1: 7.7 ± 6.2, SUV-2: 8.9 ± 7.1) with a very similar RI (15.7 ± 10.8%). These same authors found similar results in lesions bigger than 2 cm and in grade 3 tumours in a sample of triple negative breast cancer. We do not assess the triple negative status due to the small amount of patients (7 patients). Thus the association between glucose metabolism in more advanced stages of the disease and some biological tumoral characteristics reflects a higher degree of relatively aggressiveness.\(^2\)

In breast cancer, the prevalence of metastatic disease rates between 3 and 9% including patients in all the stages.\(^1\) In more advanced neoplasms, as inflammatory type, the prevalence increases to 20–38%. Several studies have shown an important role of FDG PET/(CT) in the locoregional and distant workup of patients with inflammatory or locally advanced (stage III) breast cancer.\(^2\) Therefore PET/CT can not only evaluate the proliferation potency of a tumour but also determine systemic and regional lymph node metastasis through a single examination. In our work PET/CT detected advanced disease in the 25% of the patients changing significantly the clinical management and prognosis.

Some breast cancers have low metabolic activity, with low contrast between the tumour and the background activity.\(^1\) So we decided to perform a delayed image, 3 h after FDG administration, to get a better metabolical definition of the lesion in order to obtain its axial measure, although with limitations due to the spatial imaging resolution. We did not use the hanging breast technique reported by Vidal-Sicart et al.\(^2\) that can achieve higher lesion visualization as well as higher semiquantitative values in comparison with standard procedure.

The present study, like others published before, demonstrates that an association exists between FDG uptake, express as SUV, and specially RI with other well-known important prognostic variables in breast cancer. The relation between tumoral glucose retention with other biological factors has not been fully assessed and can address the importance of RI as a prognostic factor that must be studied in future works.\(^10,2\)

The limited number of patients in some groups could be a limitation in our study and at the moment we are working on increasing the sample of patients and the follow-up to find more significant results and study the association of semiquantitative parameters and the disease evolution.

Principally category 1 pathological and biological factors are also known to confer poorer prognosis and probably a higher risk of occult distant metastases. On top of that, some studies suggested that the intensity of FDG uptake in the primary tumour could have prognostic value.\(^30,3\) If these results are demonstrated in other works, FDG PET could be proposed as a method for patient stratification before chemotherapy.

Conclusion

The higher incidence of metastases in locally advanced breast tumours (25% in our series) compared to patients with early breast tumours justifies the current indication for FDG PET/CT. Significant correlation was found between glucose metabolism expressed as PI and several histological and biological factors as hormone receptor status and Ki-67 overexpression.

These data suggest that SUV change over time is a prognosis marker in locally advanced breast tumours.

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Conflict of interests

The authors have no conflict of interests to declare.

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References