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RESEARCH ARTICLE

Metabolic Tumor Burden Assessed by Dual Time Point [^{18}F]FDG PET/CT in Locally Advanced Breast Cancer: Relation with Tumor Biology

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Abstract

Purpose: The aim of the study was to investigate the influence of dual time point 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) positron emission tomography/x-ray computed tomography (PET/CT) on the standard uptake value (SUV) and volume-based metabolic variables of breast lesions and their relation with biological characteristics and molecular phenotypes.

Procedures: Retrospective analysis including 67 patients with locally advanced breast cancer (LABC). All patients underwent a dual time point [^{18}F]FDG PET/CT, 1 h (PET-1) and 3 h (PET-2) after [^{18}F]FDG administration. Tumors were segmented following a three-dimensional methodology. Semiquantitative metabolic variables (SUV_{max}, SUV_{mean}, and SUV_{peak}) and volume-based variables (metabolic tumor volume, MTV, and total lesion glycolysis, TLG) were obtained. Biologic prognostic parameters, such as the hormone receptors status, p53, HER2 expression, proliferation rate (Ki-67), and grading were obtained. Molecular phenotypes and risk-classification [low: luminal A, intermediate: luminal B HER2 (–) or luminal B HER2 (+), and high: HER2 pure or triple negative] were established. Relations between clinical and biological variables with the metabolic parameters were studied. The relevance of each metabolic variable in the prediction of phenotype risk was assessed using a multivariate analysis.

Results: SUV-based variables and TLG obtained in the PET-1 and PET-2 showed high and significant correlations between them. MTV and SUV variables (SUV_{max}, SUV_{mean}, and SUV_{peak}) where only marginally correlated. Significant differences were found between mean SUV variables and TLG obtained in PET-1 and PET-2. High and significant associations were found between metabolic variables obtained in PET-1 and their homonymous in PET-2. Based on that, only relations of PET-1 variables with biological tumor characteristics were explored. SUV variables showed associations with hormone receptors status ($p < 0.001$ and $p = 0.001$ for estrogen and progesterone receptor, respectively) and risk-classification according to phenotype (SUV_{max}, $p = 0.003$; SUV_{mean}, $p = 0.004$; SUV_{peak}, $p = 0.003$). As to volume-based variables, only TLG showed association with hormone receptors status (estrogen, $p < 0.001$; progesterone, $p = 0.031$), risk-classification ($p = 0.007$), and grade ($p = 0.036$). Hormone receptor negative tumors, high-grade tumors, and high-risk phenotypes showed higher TLG values. No association was found between the metabolic variables and Ki-67, HER2, or p53 expression.

Conclusion: Statistical differences were found between mean SUV-based variables and TLG obtained in the dual time point PET/CT. Most of PET-derived parameters showed high association

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with molecular factors of breast cancer. However, dual time point PET/CT did not offer any added value to the single PET acquisition with respect to the relations with biological variables, based on PET-1 SUV, and volume-based variables were predictors of those obtained in PET-2.

Key words: [^{18}F]FDG PET/CT, Breast cancer, Volume-based metabolic variables, Clinicopathological factors, Molecular phenotypes

Introduction

Breast tumor metabolism assessed by 2-deoxy-2-[^{18}F]fluoro-D-glucose PET/x-ray computed tomography ([^{18}F]FDG PET/CT) has shown multiple relations with histopathological and immunohistochemical factors [1–3]. The maximum standard uptake value (SUV_{max}) represents a single-pixel value, which reflects maximum intensity of [^{18}F]FDG activity in the tumor and ignores the extent of metabolic abnormality and changes in the distribution of a tracer within a lesion. This has lead to an increase of the interest on other different tumor burden variables accounting for all metabolically active regions within the tumor mass. Remarkable examples are the metabolic total volume (MTV) and total lesion glycolysis (TLG) that could serve as better predictors of clinical outcome than semiquantitative methods. However, there is a limited amount of information about the relationships between clinical and pathological factors of breast cancer and volume-based metabolic parameters. On the other hand, it is well known that the maximum glycolytic activity of tumor tissues occurs between 3 and 5 h after the administration of [^{18}F]FDG. This is why dual time point PET has been used in order to improve the characterization of breast lesions [4, 5].

The evolution of tumor activity metabolic variables, after [^{18}F]FDG administration, has been studied previously as well as their relations with tumor biology. However, to our knowledge, no previous study has addressed the changes in volume-based metabolic variables, or their relations with molecular tumor characteristics, in a dual time point acquisition [6–8].

Thus, the aim of the present study was to complement the previous knowledge in two ways. Firstly, to explore the relations between the metabolic parameters SUV_{max}, SUV_{mean}, SUV_{peak}, MTV, and TLG of the dual time point [^{18}F]FDG PET/CT and secondly, for conventional and delayed imaging, to analyze the correlations of all metabolic variables with biological characteristics and molecular phenotypes obtained from a sample of patients with locally advanced breast cancer (LABC).

Materials and Methods

Patients

Patients reported in this work were participants of an ongoing prospective multicenter study initiated in September 2009 with the following inclusion criteria: newly diagnosed breast cancer with clinical indication of neoadjuvant chemotherapy (NC) and absence of distant metastases confirmed by other methods previously to request the PET/CT for staging purpose. For the present study, patients with PET scans with a unique or predominant breast lesion uptake higher than background and with a clinical size of at least 2 cm greatest diameter in any projection on conventional imaging techniques (ultrasonography or mammography) were selected. The study included seven hospitals and was approved by the respective institutional review boards. Written informed consent was obtained from all patients.

Pre-Treatment Histopathological Analysis

The histopathological analysis of the primary tumor was performed on specimens obtained by core aspiration biopsy. The determination of tumor type and the histopathological grading were obtained. Immunohistochemistry was performed on paraffin-embedded material using primary antibodies for estrogen and progesterone receptors (ER/PR), epidermal growth factor receptor (HER2), and the proliferation index based on the Ki-67 antibody. ER, PR, and HER2 were scored as positive (+) or negative (−) as previously was referred [9].

Final positive lymph node status (positive or negative) was established by the clinician attending to histopathological confirmation, by fine needle aspiration biopsy (FNAB)/sentinel lymph node biopsy (SLNB). In cases of multiple pathologic lymph nodes, classification was based on ultrasonography and PET/CT. N stage was established, integrating histology and PET information and M stage by PET/CT according to the classification of the American Joint Committee on Cancer (AJCC) [10].

Molecular Classification of Subgroups

Patients were categorized into five molecular phenotypes [11] [luminal A, luminal B-HER2(−), luminal B-HER2(+), HER2(+) pure, and triple negative] on the basis of the different combinations of ER, PR, and HER2 status and Ki-67 labeling index. Furthermore, patients were classified in risk categories depending on tumor phenotype: high risk [basal like or HER2(+) pure], intermediate risk [luminal B-HER2(−) or luminal B-HER2(+)], and low risk [luminal A].

[^{18}F]FDG-PET/CT Imaging

Patients fasted for at least 6 h before the PET examination and had blood glucose levels less than 160 mg/dl at the time of injection. PET/CT was performed on the same dedicated whole-body PET/CT machine (Discovery DSTE-16 s, GE Medical Systems) following a standardized protocol in three-dimensional (3D) mode.

Fifty-seven patients underwent dual time point imaging with a mean interval of 127 min between the two phases (range between 112 and 138 min). 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 or early PET). The second examination imaged only the chest, with acquisition of one or two bed positions (PET-2 or delayed PET). Both acquisitions were performed following a standardized protocol [9].

Imaging Assessment and Lesion Segmentation

Two nuclear medicine physicians performed visual assessment, independently. For the present study, PET scans with a unique or dominant breast lesion uptake higher than background and with a size of at least 2 cm of greatest diameter were selected.

For the semiquantitative assessment, the PET images in DICOM format (Digital Imaging and Communication in Medicine) files were imported into the scientific software package Matlab (R2015b, The MathWorks, Inc., Natick, MA, USA) and pre-processed using a semi-automatic image segmentation procedure. All image visualizing and processing procedures were performed using in-house software in Matlab. The tumor was manually located and automatically segmented in three dimensions to acquire the metabolic and volumetric variables for each patient. The software allows enclosing the tumor within a box. In case of multiple breast lesions (multicenter or multifocal cancer), the largest one with the highest [^{18}F]FDG uptake was selected for the analysis.

The standard uptake values (SUV) were computed using the formula:

$$\mathrm{SUV} = \frac{S_V \times R_S \times W}{(R_{TD} \times D_F) \times e^{\left(-Ln(2)\times\frac{E_t}{H_F}\right)}}$$

The parameters S_V, R_S, W, R_{TD}, D_F, E_t, and H_f were stored value, rescale slope, patient weight, radiopharmaceutical injected dose, decay factor, elapsed time, and half-life, respectively.

Regions contained in the box with a SUV equal to or larger than 40 % the SUV_{max} were automatically included in the volume of interest (VOI).

Metabolic Variables Acquisition

In each VOI, semiquantitative parameters were obtained as maximum, mean, and peak standard uptake value (SUV_{max}, SUV_{mean}, and SUV_{peak}, respectively). The metabolic tumor volume (MTV) and the total lesion glycolysis (TLG) were also computed for each patient. The variables were defined as follows:

- SUV_{max} was defined as the maximum uptake in the VOI reflects the maximum tissue concentration of [^{18}F]FDG in the tumor.
- SUV_{mean} was taken as the average of the SUV values contained in the VOI.
- To compute SUV_{peak}, all possible averages SUV in cubes of 3 × 3 × 3 voxels included in the tumor were computed. The maximum value of those averages was assigned to SUV_{peak}.
- MTV was the volume of the VOI after the segmentation.
- TLG was taken as the product of SUV_{mean} by the MTV, providing an estimate of the total tumor glycolytic activity.

The percentage difference in the SUV_{max} or retention index (RI) between PET-1 and PET-2 was calculated according to the formula: [(SUV-2) − (SUV-1)]/(SUV-1).

Statistical Analysis

Normality of the variables was analyzed using the Kolmogorov-Smirnov test. Based on the results of this analysis, Spearman's test was used to study the correlations between all semiquantitative variables SUV_{max}, SUV_{mean}, SUV_{peak}, MTV, TLG, and age.

First, we computed the correlations between the PET-1 and PET-2 metabolic tumor variables. Due to its non-parametric nature, Spearman's correlation coefficient was considered. Values below 0.1 were taken as indicators of no correlation between the variables while correlation values over 0.7 (and p value < 0.05) were taken as indicators of strong correlation. Based on significant and high associations were found between any metabolic variable obtained in PET-1 with the same variable obtained in PET-2, only PET-1 variables were used for the following analysis.

The association of semiquantitative variables with clinicopathological prognostic factors and biological subtypes (qualitative variables) and the RI with the risk categories were evaluated using the Kruskal-Wallis test. This test measures the difference of medians between groups of qualitative variables and identifies the significant groups. The Mann-Whitney test was used for categorical variables. According to the age at the diagnosis, patients were divided into two groups, ≤45 years or > 45 years. Furthermore, in order to convert Ki-67 to a categorical variable, two groups

were defined (high grade with a Ki-67 ≥ 14 % and low grade Ki-67 < 14 %).

We also computed the area under the curve (AUC) of all metabolic quantitative variables using the receiver operator characteristics (ROC) analysis. The point on the curve furthest from the line of no discrimination was considered the optimum threshold and the cutoff value.

Finally, a multivariable logistic regression analysis with the most significant quantitative variables was used to obtain the odds ratio for each metabolic variable. These variables were binarized using the cutoff value threshold obtained from their respective ROC curves. Also, the dependence variable, that is the phenotype risk, was divided in two groups: high risk [basal like and HER2(+) pure] and low/intermediate risk [luminal B-HER2(−), luminal B-HER2(+), and luminal A]. Wald's test was used for this analysis.

A significance level (p value) of $p < 0.05$ was used in all statistical tests. All p values obtained from multiple comparisons were corrected using Bonferroni's method.

Results

Sixty-seven patients were included for the present study, 57 of them with a dual time point PET/CT acquisition. Disease characteristics of the studied population including among others, histopathological analysis, molecular classification, and PET information are summarized in Tables 1 and 2.

Significant differences were found between mean SUV_{max}, SUV_{mean}, SUV_{peak}, and TLG obtained in PET-1 and PET-2 ($p < 0.001$ for SUV-based variables and $p = 0.008$ for TLG). No statistical significant differences were found for the MTV ($p = 0.291$).

Significant relations were observed between SUV_{max} and SUV_{peak} with other metabolic variables in PET-1 and PET-2 with the only exception of MTV. Detailed results are shown in Tables 3 and 4.

SUV_{mean} showed significant relations with the TLG both in PET-1 ($r = 0.730$; $p < 0.0001$) and PET-2 ($r = 0.786$; $p < 0.0001$).

Integrated metabolic variables, as MTV and TLG, showed significant association in PET-1 ($r = 0.825$; $p < 0.0001$) and PET-2 ($r = 0.848$; $p < 0.0001$).

MTV showed a very weak, yet significant, association with SUV_{mean} in PET-1 ($r = 0.259$; $p = 0.033$) and PET-2 ($r = 0.370$; $p = 0.004$).

Most metabolic variables obtained in PET-1 and PET-2 showed high and significant associations. The only exceptions were found between the MTV and the SUV variables, both in the PET-1 and PET-2. Table 5 shows all the results.

The mean ± SD RI was 14.07 ± 16.35. Although high-risk tumors had higher RI (mean ± SD of 17.68 ± 18.18) with respect to intermediate and low-risk tumors (mean ± SD of 12.32 ± 15.17 and 12.89 ± 18.84, respectively), no significant differences between the RI with the risk categories were found ($p = 0.619$).

Table 1. Patient's characteristics

Characteristics	Population	Percentage
Histology		
IDC	63	95.5
ILC	3	4.5
ER		
Positive	45	68.2
Negative	21	31.8
PR		
Positive	36	54.5
Negative	30	45.5
HER2		
Positive	19	28.8
Negative	47	71.2
p53		
Positive	18	48.6
Negative	19	51.4
Ki-67		
High (≥ 14 %)	53	89.8
Low (< 14 %)	6	10.2
Tumor grade		
I	3	4.8
II	30	47.6
III	30	47.6
Clinical T stage		
T2	35	53.0
T3	14	21.2
T4	17	25.8
Phenotype		
Luminal A	5	7.6
Luminal B HER2−	26	38.8
Luminal B HER2+	16	23.9
HER2	4	6.0
Triple negative	16	23.9
Risk phenotype		
Low	5	7.5
Intermediate	42	62.6
High	20	29.9
Metabolic focci		
Unifocal	50	74.6
Multifocal	10	14.9
Multicentric	7	10.5
Metabolic necrosis		
Yes	10	14.9
No	57	85.1
Metabolic stage		
I	1	1.6
II	21	33.3
III	30	47.6
IV	11	17.5
Lymph node histopathologic involvement		
Yes	49	83.1
No	10	16.9
N metabolic stage		
N0	15	22.7
N1	36	54.5
N2	5	7.6
N3	10	15.2

The missing data are not available
IDC invasive ductal carcinoma, *ILC* invasive lobular carcinoma, *ER* estrogen receptor, *PR* progesterone receptor

Any metabolic variable obtained in PET-1 showed high and significant association with the same variable obtained in PET-2. Based on the associations between the PET-1 and PET-2 metabolic variables, only PET-1 variables were used to study their correlations with biological characteristics and molecular phenotypes. Figs. 1 and 2 show examples of

Table 2. Metabolic tumor variables obtained in PET-1 and PET-2

	PET-1	PET-2
SUV_{max}		
Mean	9.00	10.71
Median	7.01	8.09
Standard deviation	5.52	7.55
Maximum	24.12	30.81
Minimum	1.80	2.55
SUV_{peak}		
Mean	7.04	8.28
Median	5.47	6.11
Standard deviation	4.43	6.04
Maximum	19.42	25.46
Minimum	1.49	1.92
SUV_{mean}		
Mean	5.54	6.53
Median	4.22	4.70
Standard deviation	3.45	4.56
Maximum	15.81	18.47
Minimum	1.10	1.60
MTV		
Mean	16.21	14.52
Median	9.88	9.39
Standard deviation	19.12	18.25
Maximum	109.73	102.78
Minimum	2.54	2.35
TLG		
Mean	109.02	121.10
Median	37.41	44.15
Standard deviation	202.20	237.57
Maximum	1351.20	6.11
Minimum	3.79	1500.29

SUV standard uptake value, *MTV* metabolic tumor volume, *TLG* total lesion glycolysis

low- and high-risk phenotype lesions with their metabolic results.

SUV variables were found to be associated with hormone receptor status (ER: $p < 0.001$ in all cases and PR: $p = 0.001$ in all cases) and risk-classification attending phenotype (SUV_{max}, $p = 0.003$; SUV_{mean}, $p = 0.004$; SUV_{peak}, $p = 0.003$).

With respect to volume-based variables, only TLG showed association with hormone receptors status (ER: $p < 0.001$ and PR: 0.031), risk-classification ($p = 0.007$), and grade ($p = 0.036$). Hormone receptors negative tumors, high-grade tumors, and high-risk phenotypes showed higher SUV and volume values compared to the others (Table 6).

No association was found between SUV or volume-based variables with the Ki-67, HER2, p53 expression, or nuclear grade. Only the TLG was associated with the molecular phenotypes ($p = 0.007$). Neither relations were found with the clinical T stage, except for the SUV_{peak} ($p = 0.04$), metabolic stage, N metabolic stage, metabolic foci pattern, metabolic necrosis, lymph node histopathologic involvement, and patient age (cutoff of 45 years).

Table 4. Relations between SUV_{peak} in PET-1 and PET-2 with other metabolic variables

$n = 67$	SUV_{mean} PET-1	MTV PET-1	TLG PET-1
SUV_{peak} PET-1	0.994	0.301	0.720
	< 0.0001	0.013	< 0.0001
$n = 57$	SUV_{mean} PET-2	MTV PET-2	TLG PET-2
SUV_{peak} PET-2	0.995	0.399	0.801
	< 0.0001	0.002	< 0.0001

Results are presented as Rho Spearman's and p values
SUV standard uptake value, *MTV* metabolic tumor volume, *TLG* total lesion glycolysis

No significant relations were found between Ki-67, as continuous variable, and SUV or volume-based variables. However, when we considered Ki-67 as a categorical variable (high grade with a Ki-67 ≥ 14 % and low grade with a Ki-67 < 14 %), significant relations were found for the volume-based variables (MTV, $p = 0.006$; TLG, $p = 0.020$); however, SUV variables did not show significant relations (SUV_{max}, $p = 0.652$; SUV_{mean}, $p = 0.707$; SUV_{peak}, $p = 0.598$).

ROC curves were computed for all the metabolic variables, and the accuracy of the test was obtained depending on whether PET variables separated the patients with and without high-risk phenotype. Typically, the 95 % confidence interval was between 0.65 and 0.90. The AUC was 0.737, 0.757, 0.757, 0.752, and 0.647 for TLG, SUV_{max}, SUV_{peak}, SUV_{mean}, and MTV, respectively. The cutoff values with the best sensitivity and specificity for the prediction of high-risk phenotype were $SUV_{max} = 6.95$, $SUV_{mean} = 4.42$, $SUV_{peak} = 5.48$, MTV = 10.02, and TLG = 67.10.

In the multivariable logistic regression analysis for the prediction of high-risk phenotype, the only significant variable was the SUV_{max} ($p < 0.001$) with an odds ratio value of 10 (Table 7).

Discussion

The SUV_{max} is a sensitive indicator of metabolic activity and tissue proliferation in breast cancer. However, this is

Table 3. Relations between SUV_{max} in PET-1 and PET-2 with the rest of metabolic variables

$n = 67$	SUV_{peak} PET-1	SUV_{mean} PET-1	MTV PET-1	TLG PET-1
SUV_{max} PET-1	0.994	0.994	0.258	0.692
	< 0.0001	< 0.0001	0.035	< 0.0001
$n = 57$	SUV_{peak} PET-2	SUV_{mean} PET-2	MTV PET-2	TLG PET-2
SUV_{max} PET-2	0.997	0.995	0.379	0.787
	< 0.0001	< 0.0001	0.003	< 0.0001

Results are presented as Rho Spearman's and p values
SUV standard uptake value, *MTV* metabolic tumor volume, *TLG* total lesion glycolysis

Table 5. Relations between metabolic variables obtained in PET-1 and PET-2

n = 57	PET-2				
PET-1	SUV_{max}	SUV_{peak}	SUV_{mean}	MTV	TLG
SUV_{max}	0.982	0.984	0.981	0.354	0.768
	< 0.0001	< 0.0001	< 0.0001	0.006	< 0.0001
SUV_{peak}	0.982	0.986	0.982	0.390	0.795
	< 0.0001	< 0.0001	< 0.0001	0.003	< 0.0001
SUV_{mean}	0.980	0.983	0.985	0.348	0.766
	< 0.0001	< 0.0001	< 0.0001	0.008	< 0.0001
MTV	0.271	0.287	0.251	0.955	0.747
	0.041	0.030	0.059	< 0.0001	< 0.0001
TLG	0.677	0.690	0.664	0.760	0.864
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Results are presented as Rho Spearman's and p values
SUV standard uptake value, *MTV* metabolic tumor volume, *TLG* total lesion glycolysis

influenced by multiple factors, among others, tumor size [12, 13]. For this reason, we excluded tumors smaller than 2 cm of long axial axis as other authors did previously [14]. Kaida et al. [15] were less restrictive and excluded patients with tumors smaller than 10 mm based on the full-width at half-maximum of PET and those with negative [^{18}F]FDG uptake for breast cancer.

With respect to the dual time point acquisition in [^{18}F]FDG PET/CT, a different metabolic behavior with time has been reported depending on the tumor biology. A decrease of SUV_{max} over time of 50 % was found in luminal A tumors while a 25 % decrease was observed in triple negative tumors. However, no statistical differences of the retention index were found between the different groups [1]. In the present analysis, although high-risk tumors had a mean RI higher compared with intermediate- and low-risk categories, no significant differences were found.

In the present work, the relation between metabolical variables obtained in PET-1 and PET-2 was studied, finding a high and significant association between SUV variables with TLG. On the other hand, significant differences were found between mean SUV-based variables and TLG obtained in PET-1 and PET-2. Thus, tumor metabolism was higher in PET-2 compared with PET-1. However, no differences were found for the MTV, probably explained by the use of the 40 % of the SUV_{max} in the segmentation process, instead of a fixed value.

We have previously reported a good linear relation between SUV_{max} in PET-1 and SUV_{max} in PET-2 (R^2 = 0.948) allowing predicting SUV_{max} in PET-2 as a function of SUV_{max} in PET-1 [16]. In the present study, significant associations were found both between SUV and volume-based variables obtained in the same PET acquisition (Tables 3 and 4) and the cross-relation between PET-1 and PET-2 variables (Table 5). To our knowledge, no previous reported work has addressed this relation. Thus, dual time point PET does not seem to add any useful

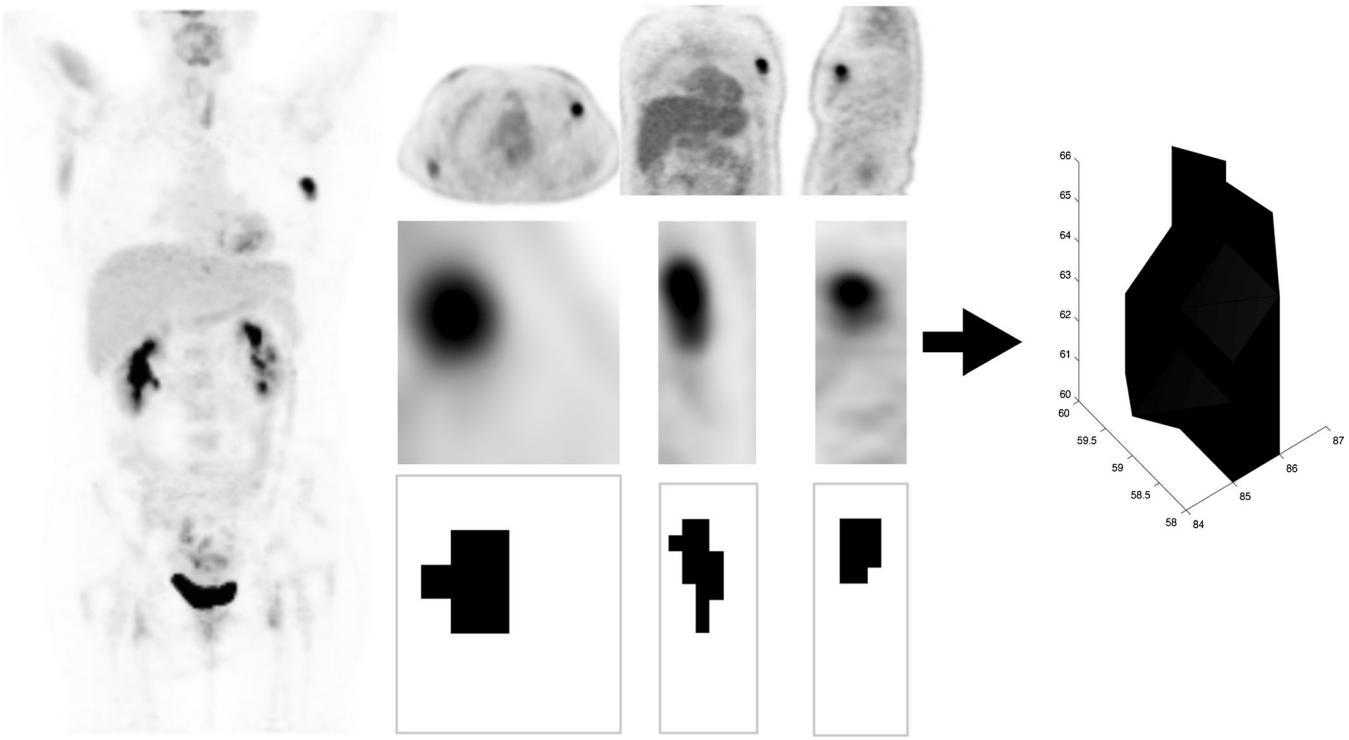

Fig. 1. Lesion segmentation and metabolic variables of a luminal A tumor (low-risk phenotype category). Maximum intensity projection PET image and axial, coronal, and sagital projections showing the location, metabolic morphology, and 3D segmented image of the breast tumor. The obtained metabolic variables were SUV_{max} = 9.57, SUV_{peak} = 7.03, SUV_{mean} = 5.80, MTV = 3.13 cm^3, and TLG = 18.16.

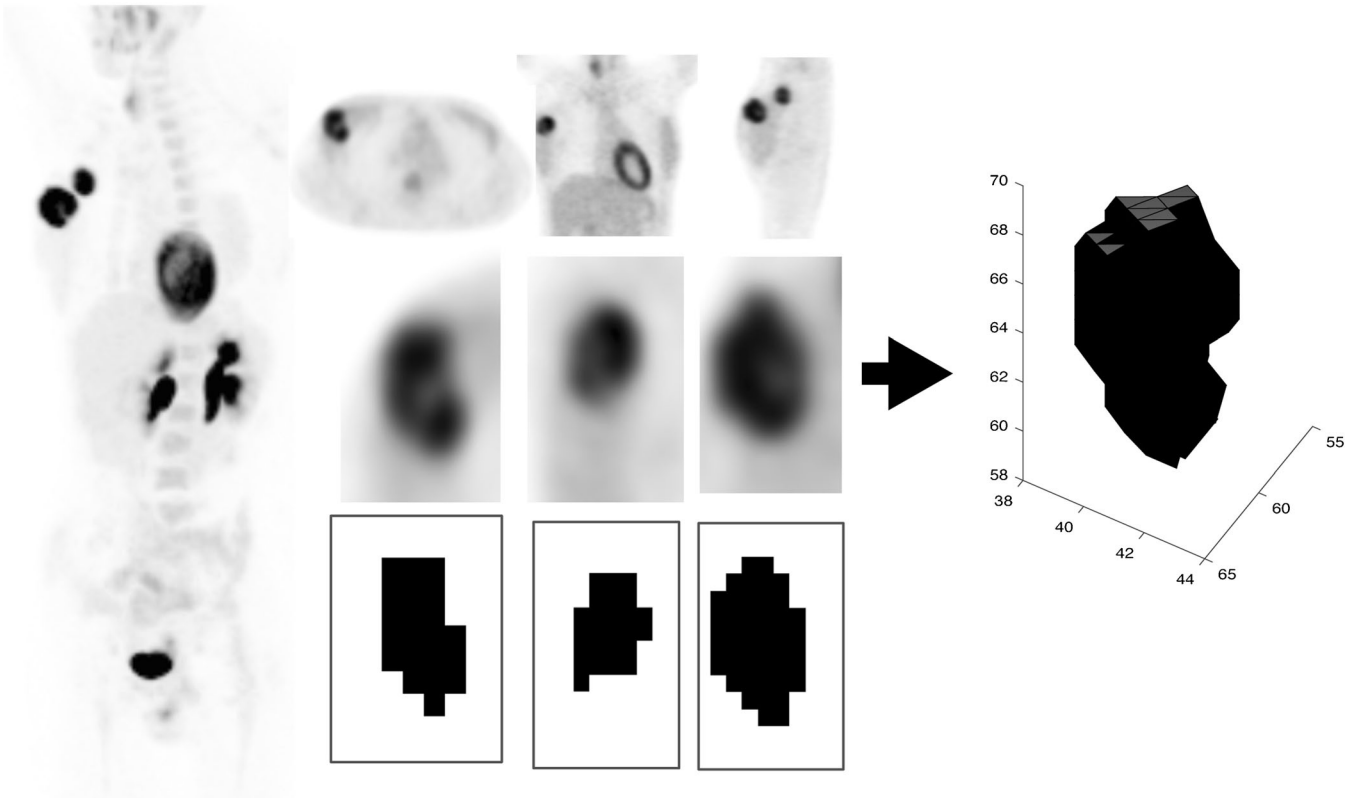

Fig. 2. Lesion segmentation and metabolic variables of a basal tumor (high-risk phenotype category). Maximum intensity projection PET image and axial, coronal, and sagital projections showing the location, metabolic morphology, and 3D segmented image of the breast tumor. The obtained metabolic variables were SUV_{max} = 11.66, SUV_{peak} = 9.00, SUV_{mean} = 7.15, MTV = 17.90 cm^3, and TLG = 127.91.

information with respect to the standard single acquisition since all the PET-2 metabolic variables studied could be predicted from the obtained in PET-1.

MTV is able to represent the total volume and activity of the metabolically active cancer cells and may be less sensitive to statistical noise. Even more, TLG combined from SUV and MTV represents both the degree of [^{18}F]FDG uptake and the size of the metabolically active tumor. However, the selection of a validated optimal threshold to delineate the tumor and determine the volume-based variables seems to be a challenge taking into account the different clinical context as lesions size, tumor biology, and background breast activity that can be influenced by patient age and hormonal status.

The methods used for lesion segmentation are classified in fixed or adaptive. Some authors have used a threshold of maximum activity for each lesion, i.e., a fraction of SUV_{max} [17]. Others used fixed absolute values [15, 18]. In the present study, we decided to choose the 40 % of the maximum uptake for each analyzed breast lesion. Based on we did not use a fixed threshold for tumor segmentation, partial volume correction was not performed.

With respect to the metabolic variables and clinical and metabolic stages, previous works have found significant relations between volume-based metabolic variables and the presence of metabolic lymph node involvement and distant metastases [14]. Kaida et al. [15] described associations between volumetric parameters and SUV_{max} with pathological T stage and pathological N status. We did not find any relation of metabolic variables with clinical or metabolic stages, except for an association between SUV_{peak} and T3 and T4.

The value of some PET-derived parameters as biomarkers has been previously described [19]. In breast cancer, correlation between the [^{18}F]FDG uptake and the expression of proliferation-associated antigen Ki-67 was found [20]. In the present work, a significant association was found between Ki-67 groups and volume-based metabolic variables although no relations were observed for the SUV-based variables.

Few works have explored the relations between tumor biological characteristics and volume-based variables such as MTV and TLG. These works found that TLG and/or MTV were significantly associated with high nuclear grade but no significant relations were found with hormone receptor or HER2 expression [14, 15]. In our study, TLG was found to be correlated to estrogen and progesterone receptor status and showed statistical differences between GI, GII, and GIII (with larger values in more dedifferentiated tumors) and MTV did not show any

Table 6. Dispersion measures and the Mann-Whitney test for SUV and volume-based variables with some risk biological variables

			Metabolic variables				
			SUV_{max}	SUV_{mean}	SUV_{peak}	MTV	TLG
Biological variables	Risk phenotype:						
	Statistical descriptors:						
	Low	Mean	6.32	3.86	4.77	17.23	47.12
		Median	4.57	2.59	3.50	6.55	18.17
		SD	3.52	2.28	2.52	27.37	65.89
	Intermediate	Mean	8.06	4.96	6.26	12.72	77.30
		Median	5.75	3.49	4.58	9.68	33.58
		SD	5.72	3.58	4.56	10.86	127.64
	High	Mean	11.65	7.18	9.24	23.28	191.47
		Median	11.57	7.24	9.57	15.99	99.62
		SD	4.81	2.97	3.90	28.23	312.46
	Mann-Whitney test (p values):		0.003	0.004	0.003	0.107	0.007
	Low vs intermediate		0.581	0.535	0.605	0.285	0.227
	Low vs high		0.021	0.021	0.021	0.135	0.014
	Intermediate vs high		0.002	0.002	0.002	0.079	0.008
	Estrogen receptor:						
	Statistical descriptors:						
	Positive	Mean	7.61	4.69	5.78	12.69	63.91
		Median	5.71	3.42	4.57	8.51	28.18
		SD	5.14	3.28	4.03	12.52	97.16
	Negative	Mean	12.19	7.48	9.70	24.27	210.44
		Median	11.66	7.33	9.63	17.21	108.35
		SD	5.31	3.19	4.34	21.89	316.72
	Mann-Whitney test (p values):						
	Positive vs negative		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Progesterone receptor:						
	Statistical descriptors:						
	Positive	Mean	7.36	4.55	5.69	13.88	69.07
		Median	5.53	3.39	4.43	9.88	33.58
		SD	5.18	3.33	4.07	13.68	106.89
	Negative	Mean	11.10	6.82	8.77	19.36	160.29
		Median	10.98	6.95	8.43	12.22	82.36
		SD	5.44	3.30	4.42	24.45	274.99
	Mann-Whitney test (p values):						
	Positive vs negative		0.001	0.001	0.001	0.350	0.031
	Tumor grade						
	Statistical descriptors:						
	Grade I	Mean	5.52	3.35	4.05	6.91	18.33
		Median	3.74	2.32	2.68	7.73	18.17
		SD	3.51	2.14	2.59	3.45	0.54
	Grade II	Mean	8.47	5.25	6.60	12.26	77.81
		Median	6.08	3.58	4.60	9.68	30.98
		SD	5.75	3.65	4.60	10.77	116.99
	Grade III	Mean	10.47	6.40	8.21	20.39	156.56
		Median	8.57	5.04	6.52	16.43	79.46
		SD	5.49	3.39	4.43	24.27	274.95
	Mann-Whitney test (p values):		0.085	0.089	0.077	0.116	0.036
	GI vs GII		0.210	0.234	0.188	0.347	0.381
	GI vs GIII		0.133	0.103	0.103	0.188	0.024
	GII vs GIII		0.079	0.092	0.084	0.079	0.051

significant relation, except for the ER expression. No significant associations were found between MTV and nuclear grade. TLG was correlated with molecular tumor characteristics probably due to its combined nature (volumetric and metabolic). Based on that, our study seems to support TLG as a better indicator of clinicopathological factors of breast cancer than SUV_{max} or MTV [15]. In our case, TLG had the additional advantage, with respect to SUV variables, of showing association with tumor grade.

Table 7. Odd ratios of metabolic tumor variables obtained in PET-1 and confidence intervals

	OR	CI 95 % [max–min]
SUV_{max}	10.0	32.26–3.05
SUV_{peak}	7.5	21.28–2.64
SUV_{mean}	7.5	21.28–2.64
MTV	4.0	9.17–1.75 s
TLG	6.6	16.95–2.58

OD odd ratio, *CI* confidence interval

With regard to molecular phenotypes, Kajary et al. [14] observed the largest differences in TLG between the luminal A and the triple negative group, with overlapping values for the other groups. The lowest median value of MTV was also found in the luminal A group, and the highest in HER2(+) cancers. Luminal B-HER2(−) cancers have in average lower SUV_{max}, SUV, and TLG as compared to luminal B-HER2(+) cancers. The opposite was found for MTV. Luminal B-HER2(+) have lower SUV average, MTV, and TLG compared with HER2(+) cancers, but the medians of SUV_{max} were similar. Kaida et al. [15] found higher SUV and volume-based metabolic variables in triple negative tumors compared to the others. In our dataset, the more aggressive phenotypes, such as triple negative and HER2 (+) pure, had higher SUV and TLG than the intermediate- and low-risk tumors. In the present work, higher values of SUV-based variables and TLG were found in the high-risk phenotype group with respect to the others. Using a multivariable logistic regression analysis, we found that the most significant variable was the SUV_{max}. Thus, in our sample, patients with elevated SUV_{max} had a 90 % risk of belonging to the high-risk phenotype category.

About the limitations, considering that MTV and TLG of [^{18}F]FDG PET/CT are suggested to be better indicators of whole tumor burden than SUV_{max}, we analyzed only the representative lesion responsible of the T staging. The fact that some patients had multifocal lesions could affect our results. However, multifocal and multicentric lesions represented only the 25 % of our sample.

Conclusion

Statistical differences were found between mean SUV-based variables and TLG obtained in the dual time point PET/CT. PET-derived parameters, especially the ones most directly related to tumor glycolysis, such as the SUV variables and TLG, could act as predictors of tumor biology based on their association with histopathological factors of breast cancer, although in the multivariable logistic regression analysis for the prediction of high-risk phenotype, the most significant variable was the SUV_{max}.

Dual time point PET/CT did not offer any added value to the single PET acquisition with respect to the relations with biological variables, based on PET-1 SUV and volume-based variables were predictors of those obtained in PET-2.

Compliance with Ethical Standards. The study included seven hospitals and was approved by the respective institutional review boards. Written informed consent was obtained from all patients.

Conflict of Interest

The authors declare that they have no conflict of interest.

Disclaimer

All the authors have participated in the writing and revision of this article and take public responsibility for its content.
The present publication is approved by all authors and by the responsible authorities where the work was carried out.
All the authors confirm the fact that the article is not under consideration for publication elsewhere.

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