Predictive Value of Metabolic Parameters in Pretreatment 18F-FDG PET/CT with Regard to Molecular Subtype, Immunohistochemistry, and Overall Survival in Primary Invasive Ductal Breast Cancer

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OBJECTIVE
The aim of the study was to evaluate the association between metabolic parameters obtained from pretreatment 18F-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) and molecular subtype, immunohistochemistry, and overall survival (OS) in female breast cancer (BC) patients with invasive ductal carcinoma (IDC).

METHODS
A total of 179 patients were included in the study, and their primary tumor histopathological features, molecular subtypes, axillary lymph node (ALN) involvement, distant metastasis, and OS were evaluated and compared 18F-FDG PET parameters. Among the PET parameters, maximum standardized uptake value (SUVmax), mean SUV, metabolic tumor volume (MTV), and total lesion glycolysis (TLG) of the tumor and ALNs were examined.

RESULTS
Based on molecular subtypes, the metabolic parameters of tumors were at their lowest in the Luminal A group and had the highest values within the triple-negative BC group. The triple-negative BC subtype was associated with a higher Ki67 proliferation index. Tumor SUVmax was higher in patients who were estrogen receptor negative, progesterone receptor (PR) negative, had nuclear grade III, and had distant metastasis (p=0.021, p=0.001, p<0.001, p=0.014, respectively). Patients with distant metastasis, ALN metastasis, and internal mammary lymph node involvement had higher tumor TLG and MTV (respectively, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001). Higher ALN SUVmax values were observed in patients with distant metastasis and those who were PR negative (p=0.016). The ALN TLG value was found to be higher in patients with distant metastases and ALN metastases compared to those without (p<0.001, p=0.049, p=0.025, respectively).

CONCLUSION
The study indicates that PET/CT is a highly dependable method for detecting ALN involvement. Furthermore, assessing metabolic tumor characteristics using 18F-FDG PET/CT before initiating primary IDC treatment might provide crucial diagnostic and prognostic insights that significantly contribute to managing the disease.

Keywords: 18F-fluorodeoxyglucose positron emission; invasive ductal breast cancer; metabolic tumor volume; overall survival; tomography/computed tomography; total lesion glycolysis.

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INTRODUCTION

Breast cancer (BC) is a heterogeneous tumor with a higher risk of recurrence or death. It is crucial to identify patients experiencing a risk of recurrence or progression, as there is currently no clinical method for an accurate assessment of the prognosis and survival of BC patients. The prognosis of BC depends on various immunohistochemical (IHC) factors.

Significant prognostic factors considered in BC include the size of the tumor, multifocality, lymph node spread, and distant metastases (DM). In addition, various histopathological and molecular features such as histopathological type, grade, hormone receptor status (estrogen receptor [ER] and progesterone receptor [PR]), human epidermal growth factor receptor (HER2) (cerb-B2) status, and the ki-67 proliferation index are other important factors.[1–4]

18-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography (18F-FDG PET/CT) has great prognostic significance in predicting malignant tumors, TNM staging, evaluation of therapeutic effects, the FDG parameter maximum standardized uptake value (SUVmax), metabolic tumor volume (MTV), and total lesion glycolysis (TLG) as a parameter of tumor metabolism and volume are important.[5–10] Among the SUV parameters, the most commonly used maximum SUVmax expresses the single voxel value representing the most intense 18F-FDG uptake in the tumor. It cannot accurately reflect glucose metabolism, especially in heterogeneous tumors such as BC.[5–10] Therefore, volumetric parameters such as TLG and MTV have been developed as semi-quantitative metrics for 18F-FDG accumulation. By comprehensively measuring the glucose metabolism parameters of tumor cells, it may be more valuable in reflecting the heterogeneity of tumors.[11–14]

Most of the studies have collectively evaluated all the different histological subtypes of BC, including invasive ductal breast carcinoma (IDC), invasive lobular breast carcinoma, mucinous carcinoma, and metaplastic carcinoma. There are a limited number of studies that have investigated the relationship between molecular subtypes, immunohistochemistry features, and overall survival (OS) determined from pretreatment biopsy specimens of the primary tumor with volumetric 18F-FDG PET/CT parameters at the initial staging of a homogenous group of patients diagnosed with invasive ductal breast cancer (IDBC).[14–16]

This study aims to evaluate metabolic parameters for the primary tumor of pretreatment 18F-FDG PET/CT in relation to molecular subtype, immunohistochemistry, and OS only in patients with IDC.

MATERIALS AND METHODS

This retrospective study included 179 female patients with IDC of the breast who had received 18F-FDG PET/CT imaging for staging between January 2015 and May 2018. All patients were initially diagnosed by a fine needle and/or core needle tru-cut biopsy. The exclusion criteria were as follows: (1) Those without breast or axillary lymph node (ALN) biopsy results; (2) those without immunohistochemistry results; (3) diagnosis of secondary malignancy patients; (4) bilateral BC; (5) non-IDC histopathology; (6) pregnancy; (7) male BC; (8) insufficient clinical data; and (9) those without follow-up.

The patient and tumor characteristics are summarized in Table 1. Histopathological features of the primary tumor (histological grade, ER/PR/HER2 status, Ki-67 proliferation index) were obtained from the biopsy reports. ER and PR were considered positive when immunoreactive cell nuclei were <1%.[17] The IHC test uses a chemical dye to stain the HER2 proteins. The IHC gives a score of 0–3+ that measures the amount of HER2 proteins on the surface of cells in a BC tissue sample. If the score is 0–1+, it is considered HER2 negative. If the score is 2+, it is considered borderline. A score of 3+ is considered HER2 positive. If the IHC test results are borderline, it is likely that a fluorescence in situ hybridization (FISH) test will be done on a sample of the cancer tissue to determine if the cancer is HER2 positive. It gives a positive or negative score on the FISH test. The Ki-67 proliferation rate was counted in hotspot areas, and the percentage of nuclear Ki-67-positive tumor cells was reported.

Categorization of Molecular Subtypes

According to the recommendations of the 12th International Breast Conference, the patients were categorized into five subtypes, as follows:[1] Luminal A (LumA): ER-positive and/or PR positive, HER2 negative, and Ki-67 >14%[2] Luminal B-HER2 negative (LumB−): ERPositive and/or PR-positive, HER2 negative, and Ki-67 of at least 14%.[3] Luminal B-HER2 positive (LumB+): ER-positive and/or PR-positive, HER2 positive, and any Ki-67 index[4] HER2 positive (HER2+): ER negative, PR negative, HER2 positive[5] Triple-negative Breast Cancer (TNBC): ER negative, PR negative, HER2 negative.[1]
18F-FDG PET/CT Image Technique
All patients underwent PET/CT after fasting for at least 6 h and then had their blood glucose levels checked. A serum glucose level was measured below 200 ng/dL and intravenous injection of 8–12 mCi (296–444 MBq) (approximately 8.1 MBq of FDG per kilogram of body weight) 18F-FDG was administered. Whole-body PET/CT imaging was performed on a biograph (Siemens Biograph 6, Chicago, IL, USA) using a full-ring high-resolution (HI-REZ) LSO PET and a six-slice CT scanner (Siemens Biograph 6, Chicago, IL, USA). Firstly, a non-enhanced CT scan was performed with the following parameters: 40–60 mAs, 140 kV, and 5-mm section thickness. Positron emission tomography scanning with 3 min per bed position was then acquired on the identical transverse field of view in the caudocranial direction. PET image datasets were reconstructed iteratively using the ordered subset expectation maximization algorithm with CT-based attenuation correction.

18F-FDG PET/CT Image Analysis
Qualitative and quantitative (or semi-quantitative) image analysis was carried out by two experienced nuclear medicine specialists with significant experience in reading 18F-FDG PET/CT scans. All image analysis was performed using General Electric Advantage Workstation (AW workstation Volume Viewer 3) software (GE Healthcare, Waukesha, WI, USA). The

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<th>Table 1  Characteristics of patients</th>
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Min: Minimum; Max: Maximum; SD: Standard deviation; TNBC: Triple-negative breast cancer; HER2: Human epidermal growth factor receptor; SUV\textsubscript{max}: Maximum standardized uptake value. Follow-up (months), BC-related exitus (n)
maximum intensity projection and attenuation-corrected PET/CT fusion images were evaluated in three planes (transaxial, coronal, and sagittal). Maximum SUV, mean SUV, MTV, and TLG of the tumor and ALN were recorded. Maximum SUV was based on body weight and was calculated using the following formula: \( \text{injected dose [MBq]} ÷ \text{body weight [g]} \). The tumor contours were semi-automatically delineated by using a threshold of 42% of the SUV \( \text{max} \) within the lesion to calculate MTV. TLG was calculated by multiplying the SUV mean by the MTV. A volumetric region of interest was drawn to fully include the primary tumor and/or ALNs. The volumetric region of interest border was adjusted semi-automated if the volume extended beyond the borders of the primary lesion on checking the sagittal and coronal images.

The tumors were classified and staged according to the World Health Organization classification and the TNM staging system.[18]

Tumor 18F-FDG PET parameters were compared with the patient’s clinical, immunohistochemistry, and molecular characteristics. All patients received at least one therapy protocol. All patients had at least 5 years of follow-up. Clinical follow-up was performed until the date of death or the last follow-up date of June 2022 with maximum intervals of 6 months (median follow-up: 30 months, range: 12–90 months).

### Statistical Analysis

IBM SPSS Statistics for Windows, version 26 (IBM Corp. Armonk, N.Y. USA) was used for statistical analysis. In the evaluation of the study data, descriptive statistical methods (mean, standard deviation, median, minimum, and maximum) were applied. Quantitative evaluations were made using the Shapiro–Wilk test, Kolmogorov–Smirnov, and graphical evaluations. Student’s t-test was used for two-group comparisons of normally distributed quantitative data, and the Mann–Whitney U-test was used for two-group comparisons of non-normally distributed data. One-way ANOVA Test was used for comparisons of normally distributed groups of three or more; Kruskal–Wallis test was used for comparisons of non-normally distributed data of groups of three or more; Bonferroni Dunn test was used for pairwise comparisons. Pearson Chi-Square test and Fisher-Freeman-Halton Exact test were used to compare qualitative data. Spearman’s correlation analysis was used to evaluate the relationships between variables. McNemar, Kappa concordance test, and diagnostic screening tests (sensitivity, specificity, PPV, and NPV) were used to evaluate the agreement between ALN biopsy and ALN SUV \( \text{max} \) results. OS rates were calculated by Kaplan–Meier analysis. Univariable and multivariable cox regression models were used to investigate the prognostic factors. Findings that were statistically significant in univariable analysis were included in the multivariable backward model. Significance was evaluated at the \( p<0.05 \) level. The primary endpoints were to evaluate the association between metabolic parameters obtained from pretreatment 18F-FDG PET/CT and molecular subtype, immunohistochemistry, and TNM stage. The secondary endpoint was to determine OS in female BC patients with IDC.

### RESULTS

Tumors were detected in various breast quadrants among the patients, with 36 cases located in the inner quadrant, 107 cases in the outer quadrant, and 36 cases in the retroareolar area. In 18 patients (9.94%), the internal mammary lymph nodes (IMLN) were found to be involved, and out of these, four patients did not have any ALN metastases. A statistically significant difference was found in the rates of IMLN involvement according to breast quadrant \( (p=0.007; p<0.01) \), with a higher incidence of IMLN involvement in the inner quadrant group compared to the outer quadrant group.

In 98 patients, multifocality of the tumor was detected. Based on the existence of multifocality, there was a statistically significant difference in the rates of ALN positivity \( (p=0.007; p<0.01) \), with a higher incidence of IMLN involvement in the inner quadrant group compared to the outer quadrant group.

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There was no statistically significant difference in patients’ axillary TLG, tumor SUVmax, and tumor TLG measurements based on the presence of multifocality (p>0.05).

Out of a total of 179 cases, 137 (76.5%) had positive ALN metastasis on biopsy, while 135 (75.4%) were found to be positive on PET/CT. Of the 42 cases with negative ALN biopsy results, 35 were negative on PET/CT and 6 were positive. Of the 137 cases with positive ALN biopsy results, 129 were positive on PET/CT and eight were negative. Therefore, the sensitivity of PET/CT was 94.16%, the specificity was 85.71%, the positive predictive value was 95.56%, the negative predictive value was 81.82%, and the accuracy was 92.18%.

The rate of distant metastasis was statistically higher in the presence of positive ALN compared to negative ALN (36.5% vs. 16.7%, p=0.016). ALNs SUVmax, TLG, and MTV, tumor SUVmax, TLG, and MTV values showed statistically significant differences according to the stage (p=0.001, <0.001, 0.014, <0.001, <0.001, respectively). These values were higher in the advanced stages. MTV values were higher in patients with distant metastasis, ALN metastasis, and involvement of IMLN (p<0.001, p<0.001, p<0.001, respectively).

Tumor TLG value was found to be higher in ER negative and PR negative patients compared to positive ones (respectively, p=0.002, p=0.001). Tumor MTV value was found to be higher in ER negative and PR negative patients compared to positive ones (p=0.039, p=0.007, respectively).

There was no statistical difference between HER2 and nuclear grade with tumor SUVmax and tumor TLG value (respectively, p=0.089, p=0.067, p=0.493, respectively). There was no statistical difference between HER2 and tumor SUVmax values (p=0.80).
DM were detected in 57 patients. Tumor SUV_{max} value was found to be higher in patients with distant metastasis (lymph node, bone, liver, lung, pleura, and brain), ER negative, PR negative, and nuclear Grade III patients compared to their counterparts. (p=0.021, p=0.001, p<0.001, and p=0.014, respectively). Tumor TLG and MTV values were found to be higher in patients with distant metastasis, ALN metastasis, and IMLN involvement (respectively, p<0.001, p<0.001, and p<0.001). Axillary TLG value was found to be higher in patients with DM and ALN metastases compared to those without (p<0.001, p=0.049, and p=0.025, respectively). Axillary SUV_{max} values were found to be higher in patients with DM and those with negative PR compared to their counterparts (p<0.001, p=0.023, respectively).

According to the TNM classification, 34 patients were Stage I (19%), 69 Stage II (38.5%), 19 Stage III (10.6%), and 57 Stage IV (31.8%).

Forty-five (25.1%) patients died. Out of 45 patients, 36 were Stage IV, 5 were Stage III, and IV were Stage II. There were two patients with luminal A, 14 patients with luminal B HER-, nine patients with luminal B HER+, four patients of HER2+, and 16 patients of TNBC. The median survival of all patients was 87 months. The 3-year survival rate was 83.8%, the 5-year survival rate was 70.7%, and the 7-year survival rate was 64.8% (Fig. 1). In univariate analysis, ALN TLG, molecular subtype, Ki67 value, tumor size, IMLN involvement, and presence of DM were found to be statistically significant (p=0.049, p=0.046, p<0.001, p=0.003, and p<0.001). In multivariate analysis, molecular subtype, tumor size, and presence of distant metastasis were found to be independent prognostic factors that affected OS (p=0.020, p=0.037, and p<0.001) (Table 2).

**DISCUSSION**

As BC is a heterogeneous disease, volumetric parameters such as MTV and TLG other than SUV_{max} are necessary to reflect the tumor burden. However, the results from previous studies that report the prognostic role of volumetric parameters in BC show inconsistency. [3,4]

Few studies have investigated the relationship between the baseline metabolic volume of primary IDBC measured by 18F-FDG PET/CT scans, molecular subtype, immunohistochemistry, and OS. The previous studies have generally assessed all histopathological subtypes together and have reported highly variable results. [3,4,13]

The majority of studies have shown that 18F-FDG uptake, measured by SUV_{max}, TLG, MTV, and other parameters, is associated with different molecular subtypes of BC. [3,12,13,18] This study evaluated the correlation of pretreatment 18F-FDG PET/CT with metabolic parameters for the primary tumor, molecular subtypes, immunohistochemistry, and OS only in female patients with IDC. In our study, we found that tumor SUV_{max}, tumor MTV, and tumor TLG values were the lowest in the Luminal A group and the highest in the TNBC group. In addition, we found that tumor TLG and MTV values were higher in ER-negative and PR-negative patients compared to positive ones, which is consistent with findings from some studies. [12,13] Ege et al. [14] found that ER and PR negativity were only associated with an increased SUV_{max} value. Groheux et al. [3] found significant associations with SUV_{max} and TLG. Furthermore, Lemarignier et al. [19] determined that increased volumetric parameters were associated with PR negativity. Conversely, Kaida et al. [6] determined that PR negativity was not associated with any of the volumetric parameters and Osborne et al. [20] found no significant association between SUV_{max} and PR status.

Our study, along with several other studies, [1,3,10,21] found no statistically significant relationship between HER-2 status and tumor SUV_{max}. In contrast, Ueda et al. [22] and Kajáry et al. [12] found a significant relationship between tumor SUV_{max} and HER2. In addition, our study revealed a significantly higher uptake of 18F-FDG in triple-negative BCs when compared to non-triple-negative tumors, which is consistent with the findings of previous studies. [3,9,12,21] Furthermore, we observed a significant correlation for all metabolic parameters studied.

The previous studies have pointed out that Ki67 is positively correlated with 18F-FDG uptake despite its insignificance. [9,12,13] Ege et al. [14] and Qu et al. [13] suggest that the Ki-67 index is an important parameter, mostly for the HER2+ subtype. In our study, we found a correlation between TNBC and the Ki-67 index. ALN metastasis is one of the most important prognostic factors in BC. There are still conflicting results regarding the relationship between volume-based PET/CT parameters of ALN metastasis and the primary mass. Groheux et al. [3] did not find an association between SUV_{max} or other metabolic parameters and ALN metastasis. According to Yoo et al. [23] studies found that the TLG of primary breast tumor had predictive value for ALN metastasis in IDC. Our findings
revealed significant correlations between primary tumor and ALN 18F-FDG PET/CT parameters in terms of not only SUV\textsubscript{max} but also TLG and MTV similar to some previous research.\cite{6,12,14}

García Vicente et al.\cite{10} found that molecular subtypes based on IHC classification are associated with axillary nodal status. In our study, we found that axillary SUV\textsubscript{max} values were higher in patients with negative PR status, while there was no statistical difference between axillary SUV\textsubscript{max} and ER status, cerb-B2, and nuclear grade. In addition, there was no statistical difference between axillary TLG and ER status, PR status, cerb-B2, and nuclear grade in our study.

We detected false negative ALN particularly in the presence of micrometastatic lymph nodes, similar to the study conducted by Greco et al.\cite{24} Overall, ALN metastasis was positive by biopsy in 137 (76.5\%) of 179 cases, with 129 of 137 patients being positive and eight negative in PET/CT. Consequently, the negative predictive value of PET/CT was 81.82\%, and the accuracy was 92.18\%.

Inner tumor location and positive ALN status were associated with IMN adenopathy. Adam et al.\cite{25} also noted that internal mammary nodal metastases may have more prognostic significance than axillary metastases due to the higher metastatic or invasive potential of internal mammary metastases relative to the metastatic potential of axillary nodal disease. Our study found that in 18 patients (9.94\%), the IMLN was found to be involved, and out of these, four patients did not have any ALN metastases. There was a statistically significant difference in the rates of IMLN involvement according to breast quadrant, with a higher incidence of IMLN involvement in the inner quadrant group compared to the outer quadrant group (p=0.007; p<0.01). In addition, metastasis to the IMLNs in BC patients is associated with increased rates of distant metastasis and lower rates of OS.\cite{26}

In our study, we found that axillary and tumor volumetric parameters values showed statistically significant differences according to stage, with higher values in advanced stages. This finding is consistent with the study of Kaida et al.\cite{6}. In addition, Ueda et al.\cite{22} reported that advanced-stage BC patients have high SUV\textsubscript{max} values.

Tumor size is one of the parameters of TNM staging. In our study, all volumetric parameters correlated with the tumor size similar to previous studies.\cite{12–14} In univariate and multivariate analyses, tumor size was found to be an independent prognostic factor that affected OS in our study.

In our study, multivariate analysis revealed that molecular subtype, tumor size, and the presence of DM were independent prognostic factors that significantly affected OS. This result is in agreement with the findings of Jo et al.\cite{16} who identified high MTV as the only independent prognostic predictor in their multivariate survival analysis. Furthermore, in their study, Jo et al.\cite{16} found that large tumor size, high Ki-67 expression, high AJCC prognostic stage, high SUV\textsubscript{max}, high MTV, and high TLG were all significant predictors of poor relapse-free survival in their univariate survival analysis. An et al.\cite{27} reported that MTV was significantly associated with ALNM and survival in multivariate cox regression analysis. Sen and Turna\cite{28} reported that baseline high SUV\textsubscript{max} was associated with poor prognostic features. Patients with a high SUV\textsubscript{max} at baseline had larger tumors, more ER negativity, a higher tumor grade, and a higher occurrence of TNBC and HER2 enriched type. In addition, Koo et al.\cite{9} reported the highest FDG uptake in patients with poor prognostic features, such as high grade, hormone receptor negativity, triple negativity, and metaplastic tumors. Besides, multivariate analyses significantly associated SUV\textsubscript{max} with invasive tumor size, higher histologic grade, positive ALN status, and tumor subtype.

The greatest strength of this study is that there was a homogeneous cohort of BC patients that included only female patients with IDC and had a minimum follow-up period of 5 years. However, this study has several limitations, such as its retrospective design, the IHC testing of tumors in various pathology laboratories, and the inclusion of a cohort of patients with tumors <2 cm in diameter.

In addition, it is necessary to mention the NCCN guideline and the indications for the use of 18F-FDG PET/CT in BC. The NCCN guideline does not recommend 18F-FDG PET/CT in early BC (Stage I, II, or operable III). 18F-FDG PET/CT is most helpful in situations where standard staging studies are equivocal or suspicious, especially in the setting of locally advanced or metastatic disease.\cite{29}

**CONCLUSION**

This study demonstrated that PET/CT can show high sensitivity and accuracy in determining ALN involvement in staging. Moreover, assessing metabolic tumor parameters on pretreatment 18F-FDG PET/CT in primary IDBC can predict diagnostic and prognostic value and can be a useful tool to contribute to disease management.
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