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The Value of ^{18}F-FDG PET/CT Imaging Combined With Pretherapeutic Ki67 for Early Prediction of Pathologic Response After Neoadjuvant Chemotherapy in Locally Advanced Breast Cancer

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Abstract: To evaluate the value of ^{18}F-fluorodeoxyglucose-positron emission tomography/computed tomography (^{18}F-FDG PET/CT) and pretherapeutic Ki67 in predicting pathologic response in locally advanced breast cancer (LABC) after neoadjuvant chemotherapy (NAC).

As a training set, total 301 LABC patients treated with NAC were retrospectively analyzed to evaluate the potential predictive value of pretherapeutic Ki67 for pathologic complete response (pCR) after NAC. Another 60 LABC patients were prospectively included as a validation set to evaluate the value of Ki67 combined PET/CT as pCR predictors. Ki67 was assessed in pretherapy core needle biopsy specimens and PET/CT scans were performed at baseline (before initiating NAC), after the 2nd, and 4th cycle of NAC. Maximum standardized uptake value (SUVmax) and its changes relative to baseline (ΔSUVmax%) were used as parameters of PEC/CT.

In the training set, Ki67 was a predictor of pCR to NAC, with area under the curve (AUC) of 0.624 ($P = 0.003$) in receiver-operating characteristic (ROC) analysis. In the validation set, Ki67 alone did not show significant value in predicting pCR in the validation set. ΔSUVmax% after then 2nd or 4th course are predictors of pCR to NAC with the AUC of 0.774 ($P = 0.002$) and 0.791 ($P = 0.002$), respectively. When combined with ΔSUVmax% after the 2nd and 4th course NAC, Ki67 increased the value of ΔSUVmax% in predicting pCR with the AUC of 0.824 ($P = 0.001$). Baseline SUVmax and after 2nd, 4th course NAC had no predictive value for pCR, but SUVmax after the 2nd and 4th course showed remarkable predictive value for nonpathologic response (Grade 1 in Miller-Payne Grading System) with the AUC of 0.898 ($P = 0.0001$) and 0.801 ($P = 0.003$).

Both PET/CT and Ki67 can predict pCR to NAC in LABC patients in the early phases of treatment. PET/CT combined Ki67 is a better pCR predictor for response to NAC. This helps the physician to predict the probability of pCR, and facilitates the optimization of individual treatment plan in case of ineffective and/or excessive chemotherapy.

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Abbreviations: LABC = locally advanced breast cancer, NAC = neoadjuvant chemotherapy, NPV = negative predictive value, pCR = pathologic complete response, PET/CT = positron emission tomography/computed tomography, PPV = positive predictive value, ROC = receiver-operating characteristic, SUV = standardized uptake value.

INTRODUCTION

Neoadjuvant chemotherapy (NAC) has been regarded as the standard treatment for patients with locally advanced breast cancer (LABC) because NAC can significantly downstage the primary tumor and enhance the probability of successful breast-conserving surgery.[1,2] Effective response to NAC usually implicates a favorable outcome especially in patients who have achieved pathologic complete response (pCR).[3] However, pCR is achieved only in the minority (approximately 20%) of breast cancer patients, and the response rate to NAC varies among different intrinsic subtypes of breast cancer.[4–6] Therefore, early prediction of therapeutic effectiveness to optimize individual patients' treatment in case of ineffective and excessive chemotherapy is essential.

Ki67 is a cell proliferation marker, which is exclusively detected during active phases of cell cycle (G1, S, G2, and mitosis).[7] The percentage of Ki67-positive tumor cells has been confirmed to be a prognostic factor with regard to progression-free survival and overall survival.[8,9] However, data on Ki67 as a predictive marker are scarce. Several researchers have assessed the predictive significance of pretherapeutic Ki67 by core-needle biopsy.[10–14] Two studies reported no correlation between pretherapeutic Ki67 and response to NAC.[10,11] However, some researchers indicated that the expression of pretherapeutic Ki67 was also a pCR predictor to NAC.[12–14] Patients with high Ki67 level were more likely to respond to NAC, even in estrogen receptor (ER)/progesterone receptor (PgR) positive patients who were considered to be less chemosensitive.[15] So, the role of Ki67 as a predictive marker is still controversial. In addition, Ki67 is a biomarker with large heterogeneity in tumor tissue. Ki67 alone as a predictive biomarker for predicting pCR to NAC is not convincing enough.

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^{18}F-fluorodeoxyglucose (^{18}F-FDG) is widely used in positron emission tomography/computed tomography (PET/CT) as a radiopharmaceutical. Tumors with high FDG uptake in PET/CT usually have strong glucose metabolism and proliferation activity. ^{18}F-FDG PET/CT used for early response assessment in the oncology field[16–18] has been reported to be a useful tool to differentiate responders from nonresponders early in breast cancer according to the changes of FDG uptake.[19,20] Recently, FDG-PET has also been shown to predict pathologic response to NAC with the parameter of relative changes in FDG standardized uptake value (SUV) after 1 or 2 cycles and the best time point for prediction was at the 2nd cycle of NAC.[21]

Both Ki67 and FDG are proliferation markers with the potential to predictive response to NAC. Whether combination of 2 biomarkers will enhance the predictive power for pCR to NAC is unclear. Nobody has explored the approach. Therefore, in our study, we firstly explore the predictive value of pretherapeutic Ki67 for pCR through retrospectively analysis of 301 LABC patients treated with NAC as a training set. Then, we prospectively collected 60 patients as a validation set to confirm the predictive significance of Ki67 (a proliferation marker) and PET/CT (a metabolism detective tool) and to explore the predictive value of combining both of them.

PATIENTS AND METHODS

Patients

As a training set, 301 patients with LABC (stage II and III) who were treated with NAC followed surgery in our institution (Fudan University Shanghai Cancer Center) from with evaluable pretherapeutic Ki67 score and pathologic response to NAC were retrospectively analyzed. Patients with inflammatory breast cancer, metastasis, previous breast cancer, or other malignant tumor history or with severe comorbidity were excluded. In order to confirm the predictive value of Ki67 and ^{18}F-FDG PET/CT and to explore the predictive possibility of combination both of them, women ages between 18 and 70 years with newly diagnosed, noninflammatory, LABC, who could undergo PET-CT examination and at least 4 cycles of NAC, were consecutively and prospectively enrolled into our study as the validation data set. The clinical stage was assessed by physical examination and imaging procedures (breast ultrasound and/or magnetic resonance imaging). Histological type and expression of ER, PgR, HER2, and Ki67 in primary tumor tissues were assessed by core needle biopsy. PET/CT were performed at baseline (before initiating NAC), and after 2nd, 4th cycle of NAC. Patients with serious diseases unable to tolerate chemotherapy, previous chemotherapy or radiotherapy history, or with unexpected metastasis at baseline PET/CT were excluded. The study was approved by the Ethical Committee and Institutional Review Board of the Fudan University Shanghai Cancer Center. All patients in validation data set provided informed consent.

NAC Regimens

Patients in training set received NAC based on paclitaxel plus carboplatin (PC). Trastuzumab was used in some patients according to their Her2 status and willing. Patients in validation set were prospectively designed to receive chemotherapy of paclitaxel (80 mg/m^2 of body surface area, day 1, 8, and 15) and carboplatin (area under the curve [AUC] 2, day 1, 8, and 15), or paclitaxel and carboplatin plus trastuzumab (2 mg/kg, day 1, 8, 15, and 22, loading dose 4 mg/kg at week 1) Attending physicians decided the eventual implementation of the NAC regimens.

Histopathological Analysis

Pretherapy core needle biopsy was performed to determine the histological type and assess the expression of ER, PgR, HER2, and Ki67 in primary tumor tissues by using immunohistochemistry. ER and PgR status were considered positive if the proportion of positive cell was more than 10%. HER2 status was evaluated using HercepTest scoring system and fluorescent in situ hybridization was used to confirm the HER2 amplification by a ratio of HER2/CEN17 more than 2.2.[22] Ki67 score was expressed as the percentage of positively nuclear-stained cells among total number of invasive cells in 3 high-power (40× objective) fields. We counted at least 500 malignant invasive cells according to recommendation from the international Ki67 in Breast Cancer Working Group.[23]

FDG-PET/CT Procedures

We controlled the quality of PET/CT and research methodology based on the STARD statement. A SIEMENS Biography 16 HR PET/CT scanner (Siemens Medical Solutions USA, Inc. Malvern, USA) was used in our research. Before scanning, patients were instructed to fast at least for 4 h and serum glucose concentrations were tested $<$10 mmol/L. Sixty minutes after intravenous injection of ^{18}F-FDG (7.4 MBq/kg), CT scans from thyroid cartilage to costophrenic angle were implemented, followed PET emission scans for 2 to 3 min per bed and images reconstruction on the basis of CT images with attenuation correction.

Two experienced nuclear physicians were blinded to evaluate the PET/CT images independently, without knowledge of the patients' clinical, imaging, or pathological information. A region of interest (ROI) was designed for 5 to 10 mm in the malignant breast lesions by adjustment and the SUV from ROIs was calculated as: SUV = [mean activity (ROI) (MBq/mL)/injected dose (MBq)]/total body weight (g).

In our study, SUVmax (maximum standardized uptake value) of primary lesion in breast were acquired, and the targeted lesions were defined as SUVmax $\geq$2.5 in the baseline PET/CT images and the maximum diameter $\geq$1 cm in CT images. All patients in validation set were designed to receive ^{18}FDG-PET/CT scans at baseline and after 2 and 4 cycle of NAC. Metabolic response to NAC for 2 or 4 cycles was calculated as: ΔSUVmax% = (baseline SUVmax − interim SUVmax)/baseline SUVmax × 100 (%).[24]

Pathological Response to NAC

After 4 cycles of NAC, primary tumors were surgically removed and post-therapy surgical excision specimens were evaluated for pathological response to NAC via Miller-Payne Grading System (MP Grading System),[25] which contains 5 hierarchical grades. pCR Grade 5 in MP Grading System was defined as no residual invasive carcinoma in primary tumor tissue but ductal carcinoma in situ was allowed. Grade 1 in MP Grading System was defined as no reduction in overall cellularity. Grades 2 to 4 have different percentage reduction in tumor cells. Pathological response rate was the primary endpoint of our study.

Statistical Analysis

IBM SPSS 22.0 (IBM Corp. New York, USA) and MedCalc 12.7 (MedCalc Software bvba, Ostend, Belgium) were used for statistical analyses. Data were described as numbers

(percentages) or means ± standard deviation (SD). The predictive performance of parameters was evaluated by receiver-operating characteristic (ROC) curve analysis. Logistic regression model was used to analyze different predictive parameters and calculated predictive probability that was used to ROC analysis. Optimal cut-off values to predict chemotherapy response were calculated by Youden index using MedCalc12.7 software, and $P < 0.05$ (2-sided) was considered to be statistically significant.

RESULTS

Patients' Characteristics in Training Set

Table 1 summarizes the characteristics of patients in the training set. Three hundred one patients with available Ki67 and MP scores were retrospectively analyzed, among of whom 282 patients (93.7%) were treated with NAC based on PC, and the median treatment course was 4 cycles. Of patients, 20.6% (62/301) achieved pCR in primary tumor.

Patients' Characteristics in Validation Set

From March 2012 to August 2014, 80 patients were enrolled into our study and underwent baseline PET/CT scan and Ki67 assessment, but 20 of them were excluded because of metastasis at baseline PET/CT (n = 16) and postoperated pathological reports were unavailable (surgery was operated in other cancer center, n = 4). Finally, 60 patients with evaluable PET-CT and pathological data were included for statistical analysis. Table 1 shows the baseline characteristics of validation patients.

TABLE 1. Baseline Characteristics of Patients in Training Set and Validation Set

	No. of Patients (%)	
Characteristics	**Training Set (n = 301)**	**Validation Set (n = 60)**
Age, y		
Median	49 (21–80)	49 (26–65)
Menopausal status		
Premenopausal	157 (52.2%)	33 (55%)
Postmenopausal	144 (47.8%)	27 (45%)
Clinical T classification		
T1	20 (6.6%)	2 (3.3%)
T2	202 (67.1%)	39 (65%)
T3	28 (9.3%)	18 (30%)
T4	51 (16.9%)	1 (1.7%)
Clinical nodal status		
N0	36 (12.0%)	15 (25%)
N1	195 (64.8%)	35 (58.3%)
N2	31 (10.3%)	9 (15%)
N3	39 (13%)	1 (1.7%)
Tumor subtype		
Luminal A	36 (12.0%)	4 (6.7%)
Luminal B	171 (56.8%)	25 (41.7%)
TNBC	50 (16.6%)	9 (15%)
HER2 overexpress	44 (14.6%)	22 (36.6%)
Ki-67 expression		
Mean ± SD	41 ± 23%	45 ± 23%
Neoadjuvant chemotherapy		
Paclitaxel + carboplatin	223 (74.1%)	29 (48.3%)
Paclitaxel + carboplatin+ trastuzumab	36 (12.0%)	20 (33.3%)
Paclitaxel + carboplatin + others	23 (7.6%)	/
Others	19 (6.3%)	11 (18.3%)
Number of course		
<4	38 (12.6%)	7 (11.7%)
4	234 (77.7%)	46 (76.7%)
5–6	14 (4.7%)	7 (11.6%)
7–8	11 (3.7%)	/
Unknown	4 (1.3%)	/
Surgery		
Modified radical mastectomy	277 (92%)	55 (91.7%)
Breast-conserving surgery	24 (8.0%)	5 (8.3%)
Pathologic response		
pCR	62 (20.6%)	22 (36.7%)
Non-pCR	239 (79.4%)	38 (63.3%)

pCR = pathological complete response, SD = standard deviation, TNBC = triple negative breast cancer.

All patients were treated with paclitaxel-based chemotherapy. Of them, 48.3% (29/60) received PC, 33.3% (20/60) received paclitaxel plus carboplatin plus trastuzumab (PCH). The NAC courses were designed at least 4 cycles, but finally 76.7% (46/60) of them underwent 4 cycles NAC, 11.7% (7/60) and 11.6% (7/60) received <4 cycles and 6 cycles, respectively, according to patients' performance condition. Overall, pCR was achieved in 36.7% of patients (22/60).

Ki67 Predicts pCR in Training Set

In training set, Ki67 expression was 41 ± 23% (mean ± SD). We used ROC curve to evaluate the value of Ki67 in predicting the pCR to NAC independently (Figure 1), then obtained an AUC of 0.624 (95% confidence interval [CI] 0.544–0.704, $P = 0.003$). It indicated that pretherapeutic Ki67 was a pCR predictor to NAC in patients with LABC. Cut-off value of 60% for Ki67 offered the best accuracy in predicting pCR with the sensitivity of 33.87%, specificity of 85.36%, positive predictive value (PPV) of 37.5%, and negative predictive value (NPV) of 83.3%.

Ki67 and ΔSUVmax% Predict pCR in Validation Set

In validation set, Ki67 expression was 45 ± 23%. All 60 patients underwent PET-CT scans at baseline and after 2 cycles of NAC, and 48 patients underwent the post-4 cycles PET-CT scan. Baseline SUVmax (SUV1max, mean ± SD) was 9.4 ± 5.3. After 2 and 4 cycles of NAC, mean SUVmax (SUV2max and SUV3max) were 3.9 ± 2.8 and 2.4 ± 1.7, respectively (Figure 2). Mean ΔSUVmax% after 2 and 4 cycles (ΔSUV1max% and ΔSUV2max%) were 53.7 ± 27.9% and 71.8 ± 18.6%, respectively. The correlation between ΔSUV1max% and ΔSUV2max% was significant with correlation coefficient of 0.708 ($P = 0.001$; Figure 3).

We also evaluated the predictive value of Ki67, ΔSUV1max%, and ΔSUV2max% alone using ROC analysis in validation set (Figure 4). There was no significant value for Ki67 in prediction pCR with the AUC of 0.580 (95% CI 0.427–0.733, $P = 0.304$). However, either ΔSUV1max% or ΔSUV2max% could effectively predict the pCR in primary tumor, with the AUC of 0.744 (95% CI 0.616–0.872, $P = 0.002$) and 0.791 (95% CI 0.665–0.918, $P = 0.002$), respectively. At a cut-off of 65%, ΔSUV1max% offered the best accuracy in predicting pCR with the sensitivity, specificity, PPV, and NPV of 68.18%, 76.32%, 62.5%, and 80.6%, respectively; 69% was the optimum cut-off of ΔSUV2%, it clearly discriminates between pCR and non-pCR. The sensitivity, specificity, PPV, and NPV were 100%, 51.43%, 43%, and 100%, respectively.

In order to explore the predictive value of association of Ki67 and ΔSUVmax%, we combined Ki67 with ΔSUV1max% and ΔSUV2max% in logistic analysis model and built the

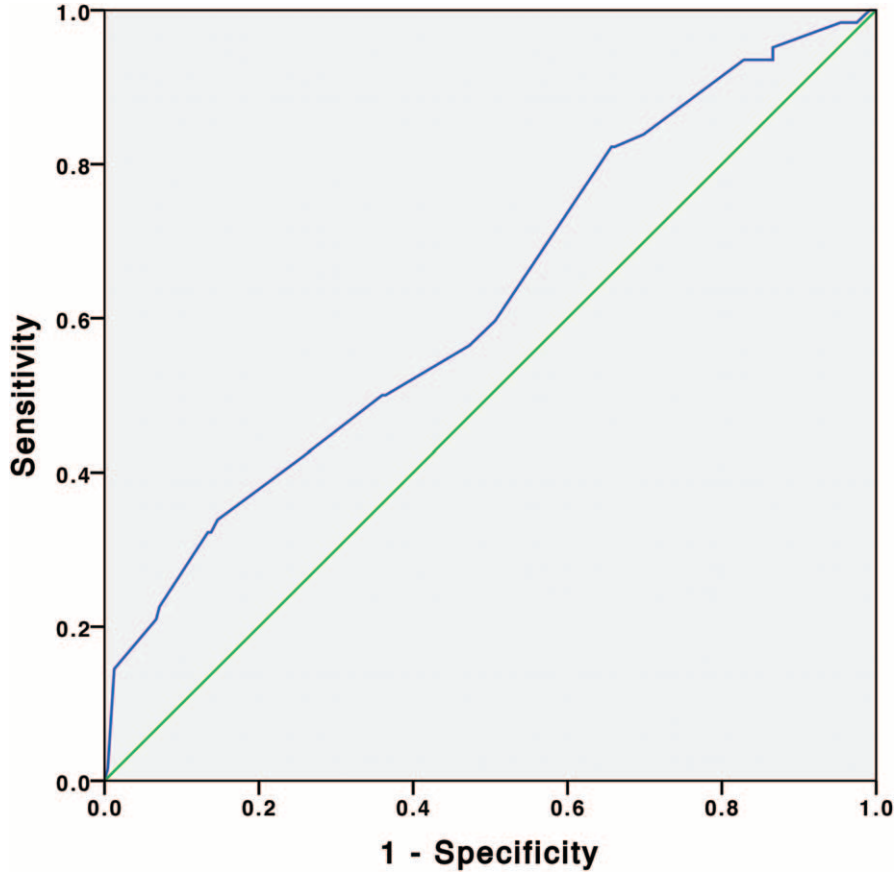

FIGURE 1. ROC analysis for pretherapeutic Ki67 in prediction of pathologic complete response (pCR) in training set (n = 301). Ki67 was a predictive factor for pCR with the area under curve (AUC) of 0.624 (95% confidence interval [CI] 0.544–0.704, $P = 0.003$). ROC = receiver-operating characteristic.

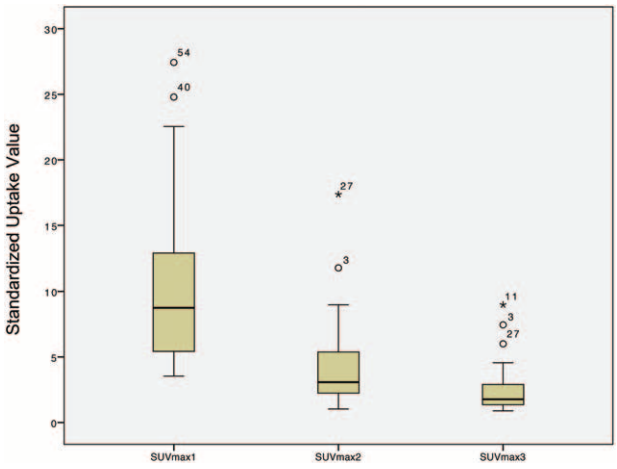

FIGURE 2. Box-plot showed the maximum standardized uptake value (SUVmax) of baseline (SUV1max), post-2 and 4 cycles (SUV2max and SUV3max).

regression equation to calculate pCR probability comprehensively, and subsequently used ROC analysis to calculate the AUC (Figure 5). We found that the combined association predicted pCR more effectively with the highest AUC of 0.824 (95% CI 0.704–0.945, $P = 0.001$). The sensitivity, specificity, PPV, and NPV of the association to predict pCR were 92.31%, 65.71%, 50%, and 95.8%, respectively.

SUVmax Predicts Nonpathologic Response (Grade 1 in MP Grading System)

We also explored the predictive value of SUVmax and found baseline SUVmax (SUV1max), post-2 cycles SUVmax (SUV2max) and post-4 cycles SUVmax (SUV3max) all could not predict pCR (figures were not shown in this paper). However, when we changed the target parameter as totally nonpathologic response (Grade 1 in the MP grading system), the SUV2max and SUV3max showed remarkable predictive value

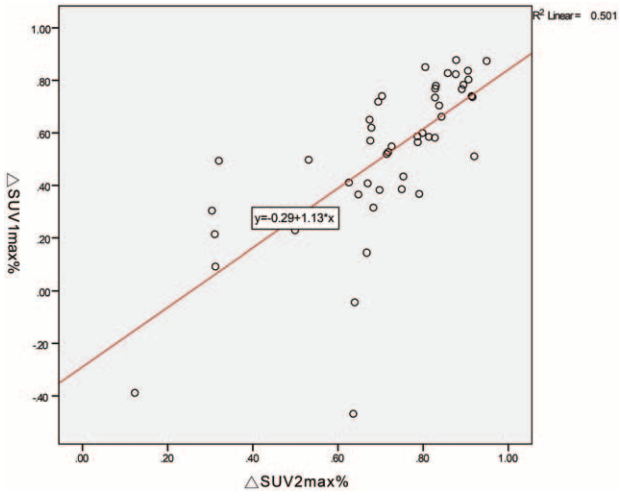

FIGURE 3. Scatterplot showed the correlation between ΔSUV1-max% and ΔSUV2max%. ΔSUV1max% and ΔSUV2max% were significant correlated with coefficient of 0.708. SUVmax = maximum standardized uptake value.

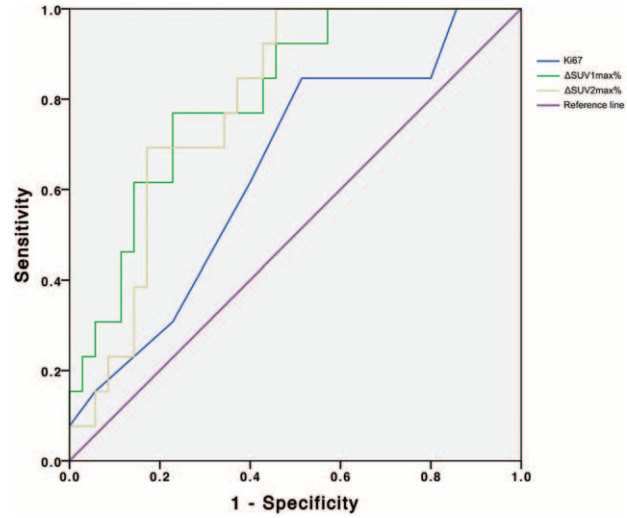

FIGURE 4. ROC analysis for Ki67, ΔSUV1max%, and ΔSUV2-max% in prediction of pCR in validation set (n = 60). Ki67 alone did not show significant value in prediction pCR with the AUC of 0.580 (95% CI 0.427–0.733, $P = 0.304$). But either ΔSUV1max% or ΔSUV2max% could effectively predict pCR, with the AUC of 0.744 (95% CI 0.616–0.872, $P = 0.002$) and 0.791 (95% CI 0.665–0.918, $P = 0.002$), respectively. AUC = area under the curve, CI = confidence interval, pCR = pathologic complete response, ROC = receiver-operating characteristic, SUVmax = maximum standardized uptake value.

with the AUC of 0.898 (95% CI 0.819–0.977, $P = 0.0001$) and 0.801 (95% CI 0.650–0.952, $P = 0.003$; Figure 6), SUV1max still showed nonpredictive value (AUC of 0.638, $P = 0.155$). With the cut-off value at 3.17 of SUV2max, the sensitivity, specificity, PPV, and NPV to predict the Grade 1 were 100%, 73.47%, 45.8%, and 100%, respectively. In addition, 1.9 was the optimum cut-off for SUV3max in predicting the Grade 1 with the sensitivity, specificity, PPV, and NPV of 81.82%, 70.27%, 45%, and 92.9%, respectively.

DISCUSSION

We found that pretherapeutic Ki67 expression in primary tumor could predict pCR in training set. And in validation set, the relative change of maximum standardized uptake value (ΔSUVmax%) in primary lesions results in early prediction for pCR to NAC. Ki67 in validation set did not show significant value in predicting pCR to NAC, but the combination of Ki67 and ΔSUVmax% maximally increased the predictive power. The SUVmax value at baseline and after 2 or 4 courses of NAC did not show predictive value for pCR, but the latter 2 parameters were found to be strong independent predictors for nonpathologic response.

Ki67 as a cellular marker for tumor proliferation was recommended to be used distinguishing between luminal A and luminal B subtypes with a cutoff value of 20% to 30% in the St Gallen International Consensus in 2015.[26] It is routinely assessed with ER, PgR, and HER2 by immunohistochemistry in pretherapy core-needle biopsy in many centers. Sueta et al[15] and Ohno et al[12] both reported that high pretherapeutic Ki67 level was an independent predictive factor for pCR with a cut-off value of 35% and 10%, respectively. In our study, we firstly evaluated Ki67 to sever as predictor for pCR using ROC

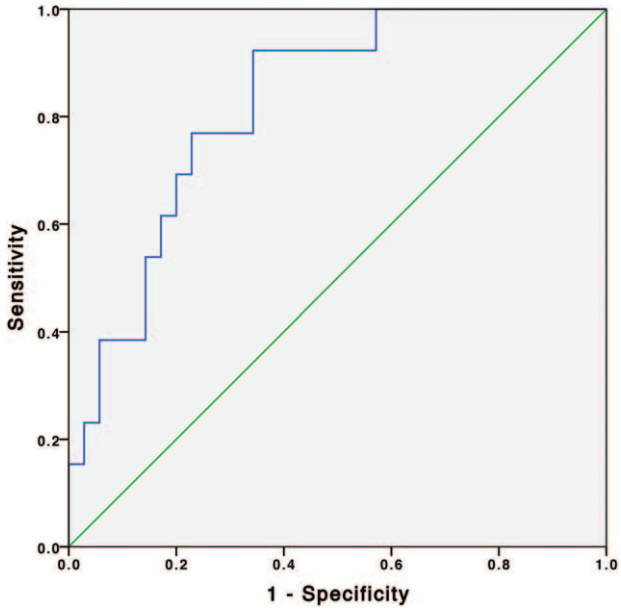

FIGURE 5. ROC analysis for combination of Ki67, ΔSUV1max%, and ΔSUV2max% in prediction pCR in validation set. The combination of Ki67 and ΔSUVmax% maximally increased the predictive power with the highest AUC of 0.824 (95% CI 0.704–0.945, $P = 0.001$). AUC = area under the curve, CI = confidence interval, pCR = pathologic complete response, ROC = receiver-operating characteristic, SUVmax = maximum standardized uptake value.

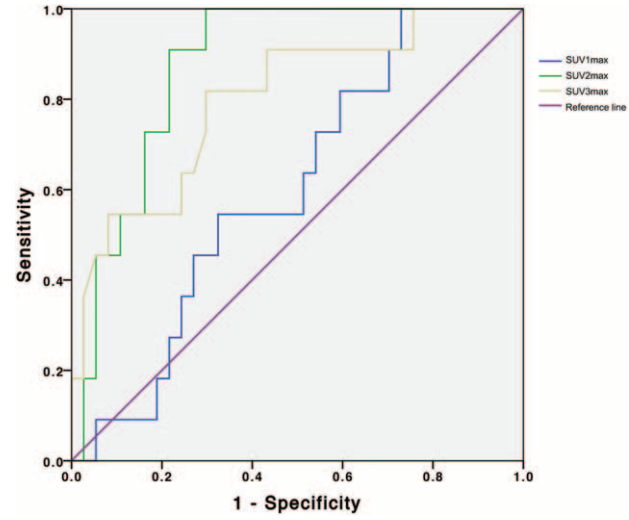

FIGURE 6. ROC analysis for SUV1max, SUV2max, and SUV3max in prediction nonpathologic response. SUV2max and SUV3max showed remarkable value in prediction non-pCR with the AUC of 0.898 (95% CI 0.819–0.977, $P = 0.0001$) and 0.801 (95% CI 0.650–0.952, $P = 0.003$), but SUV1max still showed no predictive value (AUC of 0.638, $P = 0.155$). AUC = area under the curve, CI = confidence interval, pCR = pathologic complete response, ROC = receiver-operating characteristic, SUVmax = maximum standardized uptake value.

analysis in training set and obtained a slightly low but statistical significant AUC of 0.624 ($P = 0.003$), and best cut-off value of 60%. That indicated that Ki67 was a predictor for pCR which is consistent with previous researches, but its predictive value was limited with a relatively low AUC and high cut-off value which might be meaningless in luminal a subtype. Therefore, Ki67 should be combined with more biomarkers to enhance its predictive value.

Our study confirmed the predictive value of Ki67 and ΔSUVmax% of ^{18}FDG-PET/C prospectively. Unfortunately, we did not reach statistical significant value in Ki67 predicting pCR (AUC of 0.580, $P = 0.304$) in validation set because of insufficient patient sample size. ΔSUVmax% of PET/CT was found to predict pCR effectively. When we combined Ki67 with ΔSUV1max% and ΔSUV2max%, the AUC in predicting pCR reached 0.824. These results suggested that Ki67 assessed routinely in pretherapy core-needle biopsy could supplementary increase the value of PET/CT in predicting pCR after NAC. Although ΔSUV2max% seemed better predictive value with AUC of 0.791 compared with ΔSUV1max% with AUC of 0.744, actually initial chemotherapy response is more valuable as a guide to subsequent treatment. Moreover, we found ΔSUV2max% was significantly correlated with ΔSUV1max%, when SUVmax of the 2nd course had a significant decrease; the decrease would last up to the 4th course. It indicated that the chemotherapy regimens continued to be effective in some patients. Therefore, the ΔSUV1max% after 2nd course was efficient to predict the patient's pCR. Many researches have probed the value of PET/CT in early evaluating the efficacy of NAC in breast cancer by the parameter of ΔSUVmax% before.[19–21,27,28] But different cut-off values of ΔSUVmax% were reported. Rousseau et al[21] reported that the ΔSUVmax% of 2nd course was the best time point to predict pathologic response (total or near-total therapeutic effect) with a threshold of 40%. Humbert et al[29] found that ΔSUVmax% after the 1st course could early predict the probability of achieving pCR or not with a cut-off of 50% in triple negative breast cancer (TNBC). Schwarz-Dose et al[30] used 45% as the cut-off of 1st course of NAC to predict pCR, and the sensitivity, specificity, PPV, and NPV were 73%, 63%, 36%, and 90%, respectively. In our study, the 65% cut-off of ΔSUV1max% provided the best performance to predict pCR with sensitivity, specificity, PPV, and NPV of 68.2%, 76.3%, 62.5%, and 80.6%, respectively. The difference of cut-off may because of different endpoint of pathologic response and different performance time of interim PET/CT.

The other parameters of PET/CT in predicting pCR were also assessed. SUVmax of baseline and 2nd or 4th course was found not to predict for pCR, but SUVmax of the 2nd or 4th course could effectively predict nonpathologic response, which may hint that tumors still with high metabolism after NAC may have a poor pathologic efficacy. For this population, it is worthy to explore early alteration of chemotherapy regimens to obtain better therapeutic response.

The main limitations of our study were the relative small sample size in validation set. Although 80 LABC patients were enrolled into our study and received the baseline PET/CT scan, unfortunately, 16 of them were diagnosed with distant metastasis at baseline PET/CT and were finally excluded. Four other patients were also excluded because of unavailable postoperated pathological reports in other center. Even though the external validation test consists of 60 patients, the percent of ΔSUVmax still showed significant power in predicting pCR, and the Ki67 enhanced the predictive power. Because of low sample size in validation set, patients in different subtypes were imbalanced between training and validation set. There were

more Her2 overexpressed patients (36.6%) and less Luminal A subtypes (6.7%) in validation set, this may influenced the predictive value of Ki67. In addition, it was well addressed that metabolic changes during therapy in PET/CT are highly dependent on tumor subtype.[31,32] We extended the research by exploring the predictive value of Ki67 and ΔSUVmax% in different subtypes, but there were no patients reached pCR in primary tumor in Luminal A (0/4) and TNBC (0/9) subtypes. Thus, we analyzed ΔSUVmax% in Luminal B and Her2 over-express subtypes and found ΔSUVmax% could predict pCR for Luminal B patients but not for Her2 overexpress ones. Ki67 did not show any predictive value because of small sample size (figures were not shown in the paper). A prospective study with larger simple size to prove the predictive value of Ki67, ΔSUVmax%, and their combination in different subtypes is needed.

CONCLUSIONS

In conclusion, either PET/CT or Ki67 can be used for early prediction of pathologic response to NAC in patients with LABC. PET/CT combined Ki67 could be used as a better predictor of pathologic response to NAC for LABC patients. This may help the physician predict the probability of achieving pCR or not, and facilitate optimization of individual treatment plan by avoiding ineffective and excessive chemotherapy.

REFERENCES

1. Gralow JR, Burstein HJ, Wood W, et al. Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease. *J Clin Oncol.* 2008;26:814–819.
2. Wolmark N, Wang J, Mamounas E, et al. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr.* 2001;30:96–102.
3. Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet.* 2014;384:164–172.
4. Colleoni M, Viale G, Zahrieh D, et al. Expression of ER, PgR, HER1, HER2, and response: a study of preoperative chemotherapy. *Ann Oncol.* 2008;19:465–472.
5. Colleoni M, Viale G, Zahrieh D, et al. Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment. *Clin Cancer Res.* 2004;10:6622–6628.
6. Rouzier R, Perou CM, Symmans WF, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. *Clin Cancer Res.* 2005;11:5678–5685.
7. Beresford MJ, Wilson GD, Makris A. Measuring proliferation in breast cancer: practicalities and applications. *Breast Cancer Res.* 2006;8:216.
8. de Azambuja E, Cardoso F, de Castro G Jr et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. *Br J Cancer.* 2007;96:1504–1513.
9. Viale G, Regan MM, Mastropasqua MG, et al. Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. *J Natl Cancer Inst.* 2008;100:207–212.
10. Bottini A, Berruti A, Bersiga A, et al. Relationship between tumour shrinkage and reduction in Ki67 expression after primary chemotherapy in human breast cancer. *Br J Cancer.* 2001;85:1106–1112.
11. Estevez LG, Cuevas JM, Anton A, et al. Weekly docetaxel as neoadjuvant chemotherapy for stage II and III breast cancer: efficacy and correlation with biological markers in a phase II, multicenter study. *Clin Cancer Res.* 2003;9:686–692.
12. Ohno S, Chow LW, Sato N, et al. Randomized trial of preoperative docetaxel with or without capecitabine after 4 cycles of 5-fluorouracil-epirubicin-cyclophosphamide (FEC) in early-stage breast cancer: exploratory analyses identify Ki67 as a predictive biomarker for response to neoadjuvant chemotherapy. *Breast Cancer Res Treat.* 2013;142:69–80.
13. Tan QX, Qin QH, Yang WP, et al. Prognostic value of Ki67 expression in HR-negative breast cancer before and after neoadjuvant chemotherapy. *Int J Clin Exp Pathol.* 2014;7:6862–6870.
14. Yoshioka T, Hosoda M, Yamamoto M, et al. Prognostic significance of pathologic complete response and Ki67 expression after neoadjuvant chemotherapy in breast cancer. *Breast Cancer.* 2015;22: 185–191.
15. Sueta A, Yamamoto Y, Hayashi M, et al. Clinical significance of pretherapeutic Ki67 as a predictive parameter for response to neoadjuvant chemotherapy in breast cancer: is it equally useful across tumor subtypes? *Surgery.* 2014;155:927–935.
16. Groheux D, Hindie E, Giacchetti S, et al. Early assessment with ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography can help predict the outcome of neoadjuvant chemotherapy in triple negative breast cancer. *Eur J Cancer.* 2014;50: 1864–1871.
17. Kajary K, Tokes T, Dank M, et al. Correlation of the value of ^{18}F-FDG uptake, described by SUVmax, SUVavg, metabolic tumour volume and total lesion glycolysis, to clinicopathological prognostic factors and biological subtypes in breast cancer. *Nucl Med Commun.* 2015;36:28–37.
18. Kwee RM. Prediction of tumor response to neoadjuvant therapy in patients with esophageal cancer with use of ^{18}F FDG PET: a systematic review. *Radiology.* 2010;254:707–717.
19. Schelling M, Avril N, Nahrig J, et al. Positron emission tomography using [(18)F]Fluorodeoxyglucose for monitoring primary chemotherapy in breast cancer. *J Clin Oncol.* 2000;18:1689–1695.
20. Keam B, Im SA, Koh Y, et al. Early metabolic response using FDG PET/CT and molecular phenotypes of breast cancer treated with neoadjuvant chemotherapy. *BMC Cancer.* 2011;11:452.
21. Rousseau C, Devillers A, Sagan C, et al. Monitoring of early response to neoadjuvant chemotherapy in stage II and III breast cancer by [18F]fluorodeoxyglucose positron emission tomography. *J Clin Oncol.* 2006;24:5366–5372.
22. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol.* 2013;31:3997–4013.
23. Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. *J Natl Cancer Inst.* 2011;103:1656–1664.
24. Pahk K, Rhee S, Cho J, et al. The role of interim ^{18}F-FDG PET/CT in predicting early response to neoadjuvant chemotherapy in breast cancer. *Anticancer Res.* 2014;34:4447–4455.
25. Ogston KN, Miller ID, Payne S, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast.* 2003;12:320–327.
26. Coates AS, Winer EP, Goldhirsch A, et al. -Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann Oncol.* 2015;26:1533–1546.

27. Choi JH, Lim HI, Lee SK, et al. The role of PET CT to evaluate the response to neoadjuvant chemotherapy in advanced breast cancer: comparison with ultrasonography and magnetic resonance imaging. *J Surg Oncol.* 2010;102:392–397.

28. Kim T, Kang DK, An YS, et al. Utility of MRI and PET/CT after neoadjuvant chemotherapy in breast cancer patients: correlation with pathological response grading system based on tumor cellularity. *Acta Radiol.* 2014;55:399–408.

29. Humbert O, Riedinger JM, Charon-Barra C, et al. Identification of biomarkers including ^{18}FDG-PET/CT for early prediction of response to neoadjuvant chemotherapy in triple-negative breast cancer. *Clin Cancer Res.* 2015;21:5460–5468.

30. Schwarz-Dose J, Untch M, Tiling R, et al. Monitoring primary systemic therapy of large and locally advanced breast cancer by using sequential positron emission tomography imaging with [18F]fluorodeoxyglucose. *J Clin Oncol.* 2009;27:535–541.

31. Cheng J, Wang Y, Mo M, et al. ^{18}F-fluorodeoxyglucose (FDG) PET/CT after two cycles of neoadjuvant therapy may predict response in HER2-negative, but not in HER2-positive breast cancer. *Oncotarget.* 2015;6:29388–29395.

32. Koolen BB, Pengel KE, Wesseling J, et al. FDG PET/CT during neoadjuvant chemotherapy may predict response in ER-positive/HER2-negative and triple negative, but not in HER2-positive breast cancer. *Breast.* 2013;22:691–697.