Changes in $^{18}$F-FDG tumor metabolism after a first course of neoadjuvant chemotherapy in breast cancer: influence of tumor subtypes

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Background: The aim of this study is to evaluate the impact of the different breast cancer subtypes on the tumor $^{18}$F-FDG uptake at baseline and on its changes after the first course of neoadjuvant chemotherapy (NAC).

Patients and Methods: One hundred and fifteen women with newly diagnosed, large or locally advanced breast cancer undergoing NAC were included. Estrogen receptor (ER), progesterone receptor (PR) and HER2 status were used to define three major tumor subtypes: triple negative (TN) (ER$^-$/PR$^-$/HER2$^-$), luminal (ER$^+$ and/or PR$^+$; HER2$^-$) and HER2 positive (HER2$^+$). Using Fluorine-18 fluorodeoxyglucose positron emission tomography, the tumoral standard uptake value (SUV) maximal index was measured at baseline and just before the second course of NAC.

Results: TN tumors presented the highest baseline SUV (11.3 ± 8.5; P < 0.0001). The decrease of SUV after the first course of NAC (ΔSUV) was significantly higher in TN and HER2-positive subtypes (−45% ± 25% and −57% ± 30%,

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demonstrated that 18F-FDG PET is efficient in identifying the highly responding breast cancer after one course, two courses or after completion of NAC [5–8]. This earlier assessment of the tumoral response to NAC could permit to switch to a different regimen of NAC for an individual patient, leading to tailored therapy.

Nonetheless, breast cancer is a heterogeneous disease that comprises several distinct entities with different biological characteristics and clinical behaviors [9–11].

Using immunohistochemistry (IHC) as a surrogate for expression profiling, the aim of this prospective study was to evaluate the impact of these different subtypes both on baseline tumoral metabolism, assessed with 18F-FDG PET, but also on its changes after the first course of NAC. The relationship between this early metabolic response and pCR at time of surgery was then separately evaluated in each subtype of breast cancer.

Introduction

Neoadjuvant chemotherapy (NAC) was first introduced for managing inflammatory or inoperable locally advanced breast cancers. This approach has been extended to earlier tumoral stages in order to increase the rate of breast-conserving surgery [1] and allow for a quantifiable evaluation of the tumoral chemosensitivity. NAC does not improve patient’s survival compared with a conventional adjuvant chemotherapy [2], except for those achieving a pathological complete response (pCR) at the end of the treatment [3, 4].

Fluorine-18 fluorodeoxyglucose positron emission tomography (18F-FDG PET) is the gold standard for in vivo evaluation of tumor glucose metabolism. Many studies, including all immunohistochemical subtypes, have demonstrated that 18F-FDG PET is efficient in identifying the highly responding breast cancer after one course, two courses or after completion of NAC [5–8]. This earlier assessment of the tumoral response to NAC could permit to switch to a different regimen of NAC for an individual patient, leading to tailored therapy.

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Materials and Methods

Patients and Treatments

One hundred and thirty six women with newly diagnosed, non-inflammatory, biopsy proven, large or locally advanced breast cancer (T2/3; N0/N1/N2) were consecutively evaluated in this prospective study. Twenty patients were excluded because of metastases revealed on the baseline 18F-FDG PET and one because of high glycaemia (>8 mmol/l) due to uncontrolled diabetes. The remaining 115 women underwent 18F-FDG PET before and after the first course of NAC. These patients were included in clinical trials with the main objective to determine the pathological response at surgery; an ancillary study with the two 18F-FDG PET was planned and included within the informed consent.

Clinical characteristics included age, tumor size and lymph node involvement. Histological characteristics included histological type, tumor grading using the modified Scarff–Bloom–Richardson system [12], architectural differentiation, nuclear polymorphism and mitotic figure count.

The women benefited of different NAC regimens, described in Table 1. Within 4 weeks after the last course of NAC, tumors were surgically removed and pCR was assessed according to Chevallier’s classification [19]: pCR was defined as no evidence of carcinoma either in the breast or in the lymph nodes, without (grade 1) or with (grade 2) in situ carcinoma.

Histopathological analysis and definition of breast cancer subtypes

Tumor samples were collected by needle core biopsy before NAC. IHC was carried out on buffered formalin fixed, paraffin-embedded tissues with an indirect immunoperoxidase method, using antibodies directed against HER2 oncoprotein, estrogen receptor and progesterone receptor (HER2: rabbit monoclonal prediluted antibody 4B5; ER: rabbit monoclonal prediluted antibody SP1; PR: rabbit monoclonal prediluted antibody 1E2; Ventana Tucson, AZ, USA). All immunostainings were carried out on an automated immunostainer (Ventana XT).

Estrogen receptor (ER) and progesterone receptor (PR) status were considered as positive if the tumor showed at least 10% of positive cells using ER or PR antibody [20]. HER2 status was graded according to the HercepTest scoring system modified by the recommendations of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) (0, 1+, 2+ or 3+) [21]. Tumors with scores of 3+ were considered positive. In case of 2+ scores, FISH was used to confirm HER2 amplification, using the dual color HER2 and CEN17 probes (ZytoLight, SPEC HER2/CEN17 Dual Color Prob kit; Zytovision GmbH, Bremerhaven, Germany). HER2 amplification was defined, according to ASCO/CAP criteria [21], by a ratio of HER2/CEN17 > 2.2.

Using IHC, the tumors were classified as three subtypes:

- Triple negative (TN): ER, PR and HER2 negative (n = 25).
- Luminal: ER and/or PR positive, HER2 negative (n = 53).
- HER2 positive: HER2 positive, ER and PR may be positive or negative (n = 37).

This classification is roughly similar to the prior studies that used IHC as surrogate for molecular classification [11, 22–24].

18F-FDG PET/CT Imaging

Two whole-body PET imaging systems were used: a C-PET Plus scanner and a Gemini GXL scanner (Philips Medical Systems, Eindhoven, The Netherlands). Emission data were corrected for dead time, random and scatter coincidences and attenuation before reconstruction with the RAMLA iterative method. The image voxel counts were calibrated to activity–concentration (Bq/ml) and decay corrected using the time of tracer injection as reference.

All patients were instructed to fast for at least 6 h before the injection of 18F-FDG. Serum glucose level was measured by the hexokinase method [25]. Whole-body emission and transmission scans were acquired 60 min after the i.v. administration of 2 MBq/kg of 18F-FDG for CPET studies (Nal(Tl) detector) and 5 MBq/kg of 18F-FDG for Gemini studies (GSO-Zr detector). Then, for both PET system, emission and transmission scans restricted to the chest with patients in prone position and arms raised started 80–90 min after the administration of 18F-FDG.
Two $^{18}$F-FDG PET studies were carried out: the first one at baseline, at least ten days after core biopsy, and the second one just before the second course of NAC. The same imaging system and the same acquisition parameters (duration and delay from injection) were used for baseline and post-treatment studies.

The standard uptake value (SUV) of the primary tumor was measured on the chest-restricted acquisition at baseline (SUV1) and before the second course of NAC (SUV2). A stack of transaxial attenuation-corrected slices encompassing the upper and lower limits of the tumor was first selected. A region of interest (ROI) was manually drawn on the slice with the highest tumoral radioactivity concentration. The location of this ROI was identical at first and second study. The SUV$_{\text{max}}$ was corrected by body surface area (BSA) and glycemia and normalized to standard BSA ($1.72$ m$^2$) and normal glycemia ($5.6$ mmol/l):

$$SUV_{\text{max}} \cdot \frac{\text{BSA}}{G} = C_{\text{max}} \times 70 \times \text{BSA} \times G / (1A \times 1.72 \times 5.6),$$

where $C_{\text{max}}$ (Bq/ml): activity concentration in the voxel of highest tumor activity; IA: injected activity (Bq); G: blood glucose (mmol/l) and BSA ($m^2$).

For the reader’s convenience, all the SUVs mentioned in this paper represent in fact the SUV$_{\text{max}} \cdot \frac{\text{BSA}}{G}$.

### evaluation of metabolic response to therapy

The metabolic response after the first course of chemotherapy was calculated using the following formula:

$$\Delta \text{SUV}(\%) = 100 \times (\text{SUV2} - \text{SUV1}) / \text{SUV1}.$$ 

### statistical analysis

Correlation between baseline $^{18}$F-FDG uptake and clinical, histopathological and biological parameters was assessed by Mann–Whitney and Kruskal–Wallis tests.

The same tests were used to compare SUV1, SUV2 and $\Delta$SUV according to the different tumoral subtypes and to the final histopathological response.

An optimal threshold of $\Delta$SUV was identified for the prediction of the pCR in HER2 positive subtype. A receiver operating characteristics (ROC) analysis was carried out by incrementally increasing cut-off values of the $\Delta$SUV and recalculating corresponding true-positive and false-negative rates.

Univariate and multivariate logistic regression analyses were employed for each potential predictor variable of pCR.

Values of $P \leq 0.05$ were considered significant.

### results

#### patient’s characteristics and pathological response after NAC

A total of 115 patients were included. Patient’s characteristics are summarized in supplemental Table S1 (available at *Annals of Oncology* online). The mean patient’s age was 51 years; 95% of them had ductal carcinoma and 69% had lymph node involvement. Tumor subtype in most patients was luminal (46%), followed by HER2 positive (32%) and TN (22%). Differences in tumor pathological response after NAC were observed among these subtypes: pCR occurred more often in HER2 positive and TN tumors (37.8% and 36%, respectively) when compared with luminal ones (1.9%, $P < 0.001$).

#### correlation between baseline FDG uptake and clinical/histopathological parameters

Baseline SUV (SUV1) was measured according to different clinical and pathological tumoral characteristics. The mean values of SUV1 are shown in supplemental Table S2 (available at *Annals of Oncology* online). High mitotic activity ($P < 0.0001$), high nuclear pleomorphism ($P < 0.0001$), high tumor grading ($P < 0.0001$) and negative estrogen hormonal receptor status ($P < 0.0001$) were significantly associated with higher tumoral SUV1. SUV1 was $11.3 \pm 8.5$ in TN ($n = 25$), $4.7 \pm 3.3$ in luminal ($n = 53$) and $6.3 \pm 4.5$ in HER2-positive tumors ($n = 37$). The Kruskal–Wallis test showed significant differences between these results (supplemental Table S3, available at *Annals of Oncology* online).

Two different PET systems were used in this study. Differences of mean SUVs were observed between the two Pet scans, with lower values on the C-PET scanner. The Kruskal–Wallis test showed the same differences of SUV1 among the tumoral subtypes in both PET system: Luminal tumors always had the lowest mean SUV1 while TN tumors had the highest one (supplemental Table S3, available at *Annals of Oncology* online).

#### metabolic response to NAC

The metabolic response, corresponding to the relative change of SUV, was much more higher in TN ($-45\% \pm 25\%$) and
HER2-positive tumors (−57% ± 30%) than in luminal ones (−19% ± 35%) (supplemental Table S3, available at *Annals of Oncology* online). ∆SUV did not differ by the PET system used in the study.

**relationship between metabolic response and achievement of pCR**

In TN tumors, SUV1, SUV2 and ∆SUV were not significantly different by the pCR (Table 2).

Among the 53 patients with luminal cancer, only one woman achieved a pCR. Thus, it was not possible to compare the tumoral metabolic characteristics according to the pCR.

In patients with HER2-positive tumors, SUV2 was significantly lower in tumors that achieved pCR than in the other ones (1.5 ± 0.1 versus 2.9 ± 2.4; *P* = 0.01). Similarly, ∆SUV was significantly greater in the pCR group than in the non-pCR group (−71% ± 24% versus −47% ± 29%; *P* = 0.01) (Table 2). With ROC curve analysis, the optimized cut-off of ∆SUV for optimal specificity and sensitivity to predict pCR was determined at −75% with a sensitivity of 64%, a specificity of 83%, a positive predictive value of 69%, a negative predictive value of 79%, and accuracy is 76% (area under curve = 0.73; *P* < 0.05) (supplemental Figure S1, available at *Annals of Oncology* online). By univariate logistic analysis, beyond the clinical, histopathological and metabolic factors, only SUV2 and ∆SUV were predictive of pCR in HER2-positive subtype (Table 3). By multivariate analysis, ∆SUV was the only independent predictive factor of pCR in HER2 subtype. A decrease of SUV over 75% (ΔSUV < −75%) had a high odds ratio of 6.31 (*P* = 0.03).

**discussion**

Recently, using intrinsic gene set, the molecular classification of breast cancer has demonstrated distinct molecular classes with predictive and prognostic significance [9, 10, 26, 27]. A biology-based breast cancer classification, using ER, PR and HER status is also a reproducible, easy to use, predictive marker of the histological tumoral response to NAC [11, 28, 29]. In the present study, using IHC as a surrogate marker of the gene expression profiling, the tumors were classified as luminal, TN and HER2-positive subtypes. The results confirm that baseline glucidic metabolism is different in these various subtypes of breast cancer because of specific metabolic patterns. Other studies have previously shown that the tumoral uptake of FDG at baseline depends on the biological and pathological characteristics of the tumor [18, 31–33]. But our results also demonstrate a new finding: the change of tumor glucose metabolism, assessed after only once course of NAC, is markedly different in these three subtypes and has to be evaluated differently.

**TN tumors**

In the present study, the highest baseline SUV is found in this subtype. Previous works have also described a high glucidic metabolism in TN tumors [31, 34]. Basu et al. [34] have found an SUV of 7.27 ± 5.6 for TN tumors compared with an SUV of 2.68 ± 1.9 for ER+/PR+/HER2− tumors. The gene expression profiling has demonstrated that TN tumors are the most proliferative and aggressive ones [10, 26, 35], thus explaining their high glucidic metabolism.

In our study, a high metabolic response after the first course of NAC (∆SUV = −45% ± 25%) and a high final pathological response rate (36% of pCR) are observed in TN tumors. The high rate of pCR achievement has already been demonstrated in this subtype [11, 28] but no other study has currently underlined this high metabolic response. Despite this good chemosensitivity, TN subtype has a poor prognosis [24, 28, 36]. In this subgroup, pCR is a good surrogate marker of survival [37], but our results show no significant correlation between the early metabolic and the final pathological response. The heterogeneity of NAC regimens and the switch in some patients to a hypothetically non-cross resistant regimen after three courses (Table 1) can potentially explain why ∆SUV does not predict pCR in TN tumors. Indeed, one hypothesis is that the good metabolic response, induced early after NAC, might be not sustained on the final pathological response in women with a switch at midpoint of NAC. Moreover, ∆SUV is higher in the few women first treated with docetaxel, which can introduce a bias in the correlation between ∆SUV and pCR in TN patients (Table 1). Consistent

**Table 2. Metabolic characteristics according to pathological response in HER2 positive and triple-negative subtypes**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>SUV1</th>
<th>SUV2</th>
<th>∆SUV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>14</td>
<td>6.2 ± 3.5</td>
<td>1.5 ± 0.1</td>
<td>−71 ± 24</td>
</tr>
<tr>
<td>non-pCR</td>
<td>23</td>
<td>6.5 ± 5.2</td>
<td>2.9 ± 2.4</td>
<td>−47 ± 29</td>
</tr>
<tr>
<td><em>P</em> value*</td>
<td>0.67</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Triple negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCR</td>
<td>9</td>
<td>10.7 ± 5.1</td>
<td>5.1 ± 2.6</td>
<td>−45 ± 30</td>
</tr>
<tr>
<td>non-pCR</td>
<td>16</td>
<td>11.7 ± 9.9</td>
<td>6.4 ± 5.3</td>
<td>−45 ± 22</td>
</tr>
<tr>
<td><em>P</em> value*</td>
<td>0.82</td>
<td>0.95</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD. Luminal tumors are not shown due to the very low rate of pCR.

* Mann–Whitney test.
with these results, Schneider–Kolsky et al. [38] have shown that the $\Delta$SUV might be dependent on the NAC regimen used.

luminal tumors

The lowest value of SUV is found in luminal subtype. It corresponds to well-differentiated tumors expressing hormonal receptors. Mavi et al. [32] have also found a baseline SUV of 5.54 in ER and PR negative tumors versus 3.10 in positive ones.

The metabolic response after the first course of NAC is lower than in the two other subtypes ($\Delta$SUV = $-19\% \pm 35\%$) and only one woman exhibit a pCR. These results confirm that, despite their good prognosis, the chemosensitivity of these tumors is low and that pCR is rarely obtained in this subtype [9, 11, 24, 36]. Bhargava et al. [11] have shown on 359 women that pCR is obtained in a very limited number of patients with luminal tumors (1.65%). Therefore, other surrogate markers of survival are required in this subtype. The early metabolic response, evaluated with $^{18}$F-FDG-PET, could be one of these factors but further studies are required.

HER2-positive tumors

Trastuzumab (Herceptin©) selectively targets HER2 oncprotein. During NAC, the synergy between Trastuzumab and cytotoxic therapy [39] is very efficient: Coudert et al. [17] have found a rate of pCR of almost 45%. At baseline, SUV is higher than in luminal subtype. After the first course of NAC, an important decrease of SUV ($\Delta$SUV = $-57\% \pm 30\%$), roughly similar to that found in TN, is observed. At the end of NAC, 14 patients out of 37 achieve a pCR due to the high chemosensitivity of this tumor when treated with trastuzumab [17]. Using a threshold of $-75\%$, the $\Delta$SUV predicts the pCR with an accuracy of 76%. This threshold is different than those found in previous papers studying the predictive value of $^{18}$F-FDG-PET response of all subtypes of tumors included [6–8] and confirms the need to take into consideration the tumoral subtype in the assessment of the metabolic response.

In this tumoral subtype, pCR is considered as a good surrogate of survival [40, 41]. Identifying the poor-responding patients after the first course of NAC might be important for improving outcome by quickly switching to a different preoperative chemotherapy and/or targeted therapy (i.e. lapatinib). In our institution, a prospective study is ongoing: this randomized, open-label study will assess the effect of adding bevacizumab in NAC if the decrease of SUV, evaluated after the first course of trastuzumab and docetaxel, is fewer than 70%.

Our study has some limitations. First, patients were divided into three commonly used subgroups by IHC rather than potentially more precise techniques, such as microarray analysis. But De Ronde et al. [42] have demonstrated a high concordance between the IHC and molecular subtypes. Secondly, our HER2-positive subtype group is not large enough to allow additional stratification into ER-positive and ER-negative tumors in this subset of women as suggested in other studies [11, 22]. Moreover, only static SUV measures were carried out, while Dunnwald et al. [43] have shown that kinetic analysis may hold advantage on static uptake measures for response assessment.

SUV has many drawbacks as it is dependant on parameters such as the delay between injection and measurement, plasma glucose concentration, body weight, instrumental factors and partial volume effect [44]. In the present study, the corrections relying on BSA and serum glycemia limit the impact of their variation during NAC. But the absolute SUV1 and SUV2 measures are affected by the use of two different PET systems, with lower values on the C-PET scanner. It is therefore beyond any doubt that better standardization between PET imaging systems is needed for the use of quantitative $^{18}$F-FDG PET as an imaging biomarker [44, 45]. It is worth to note that the measures of the relative changes in SUV are not different by the two PET systems used in this study because it is less affected by technical factors such as camera calibration. Indeed, when $\Delta$SUV(%) is considered, some factors only need to be consistent across longitudinal scans of the same subject [45]. This intrasubject standardization was applied in the present study. $\Delta$SUV(%) appears as a very reliable measure when analyzing data from various scanners.

In conclusion, our results show that baseline glucidic metabolism, evaluated with $^{18}$F-FDG PET, is higher in TN tumors. The glucidic metabolic response after the first course of NAC also differs among breast cancer subtypes and is efficient in determining the final pCR only in patients with HER2-positive tumor. The metabolic differences found across the distinct immunohistological subtypes bring additional evidence to the fact that they have specific metabolic patterns and have to be evaluated differently. $^{18}$F-FDG PET could offer the opportunity to switch to a different regimen of chemotherapy for an individual patient. These clinical benefits are plausible but have to be confirmed by prospective clinical studies.

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disclosure

The authors have declared no conflict of interest.

references
