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ORIGINAL ARTICLE

HER2-positive breast cancer: ^{18}F-FDG PET for early prediction of response to trastuzumab plus taxane-based neoadjuvant chemotherapy

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Abstract
Purpose To investigate the value of ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET/CT) to predict a pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) in women with human epidermal growth factor receptor 2 (HER2)-positive breast cancer.
Material and methods Fifty-seven consecutive women with HER2-positive breast cancer, treated with trastuzumab plus taxane-based NAC, were prospectively included. Maximum Standardized Uptake Value of the primary tumor and axillary nodes were measured at baseline ($PET_1.SUV_{max}$) and after the first course of NAC ($PET_2.SUV_{max}$). Tumor metabolic volumes were assessed to determine Total Lesion Glycolysis (TLG). The tumor metabolic response (ΔSUV_{max} and ΔTLG) was calculated.
Results In univariate analysis, negative hormonal receptor status (p=0.04), high tumor grade (p=0.03), and low tumor $PET_2.SUV_{max}$ (p=0.001) were predictive of pCR. Tumor ΔSUV_{max} correlated with pCR (p=0.03), provided that tumors with low metabolic activity at baseline were excluded. ΔTLG did not correlate with pCR. In multivariate analysis, tumor $PET_2.SUV_{max}$<2.1 was the best independent predictive factor (Odds ratio =14.3; p=0.004) with both negative and positive predictive values of 76 %. Although the metabolic features of the primary tumor did not depend on hormonal receptor status, both the baseline metabolism and early response of axillary nodes were higher if estrogen receptors were not expressed (p=0.01 and p=0.03, respectively).
Conclusion In HER2-positive breast cancer, very low tumor residual metabolism after the first cycle of NAC (SUV_{max}< 2.1) was the main predictor of pCR. These results should be further explored in multicenter studies and incorporated into the design of clinical trials.

Keywords Breast cancer · Neoadjuvant chemotherapy · PET · HER2 · Response monitoring

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Introduction

Neoadjuvant chemotherapy (NAC) is used more and more in order to increase conserving surgery of breast cancer by reducing tumor size [1, 2]. Studies have demonstrated that NAC does not improve survival when compared with adjuvant chemotherapy [3], but women who achieve a pathological complete response (pCR) in the breast and axillary nodes at the end of NAC have significantly improved survival [4].

Breast cancer includes several molecular entities that differ in their clinical behavior, biological characteristics and

outcomes [5–7]. The standard of care in the overexpression and/or amplification of human epidermal growth factor receptor 2 (HER2)-positive subtype is characterized by the use of trastuzumab (Herceptin®), which selectively targets HER2 oncoprotein. During neoadjuvant treatment, the synergy between trastuzumab and cytotoxic therapy is very effective. It induces a high rate of complete pathological response at the end of treatment [8] and improves outcomes [9]. However, the features of this HER2-positive subtype are heterogeneous and depend on hormonal receptor (HR) expression [10, 11].

Fluorine-18 fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET/CT) is the gold standard for in vivo evaluation of tumor glucose metabolism. Studies regarding the use of PET/CT to monitor early tumor response to NAC have shown promising results in predicting the final pCR, all tumor subtypes included [12–14]. Nevertheless, because the metabolic behavior of tumors varies considerably among breast cancer subtypes, breast cancer cannot be considered a single entity [15]. Few studies have evaluated the relevance of metabolic response to predict pCR in the HER2-positive subtype specifically, and results are contradictory because of small and heterogeneous patient series [15–18].

The objective of this prospective study was to demonstrate the value of early tumor metabolic changes to predict pCR at surgery in a large group of women with exclusive HER2-positive invasive carcinoma. Different ^{18}F-FDG PET parameters were evaluated, including tumor metabolic volumes. The correlation between PET parameters and the molecular markers of HER2-positive breast cancer, including hormonal receptor status, was also analyzed.

Materials and methods

Patients and treatments

From November 2006 to October 2012, 195 women that were referred to our institution (Centre Georges-François Leclerc, Dijon, France) because of an indication for NAC and clinical stage II or III breast cancer were consecutively evaluated in this prospective study. Only those with non-inflammatory HER2-positive tumors treated with six cycles of standardized trastuzumab and docetaxel-based regimen were included. Patients with high glycemia (> 9 mmol/l) were excluded. The institutional review board approved this prospective study and all women gave their informed consent.

Docetaxel was administered as an intravenous infusion every 3 weeks, at the dose of 100 mg/m^2 with a concomitant dose of trastuzumab. About 1 month after the last course of NAC, the tumors were surgically removed, and pCR was defined as no residual invasive cancer in the breast and nodes, though in-situ breast residuals were allowed (ypT0/is ypN0) [10].

Histopathological analysis

Pre-treatment core biopsies from the primary tumor were used to determine the histological type, tumor grade [19], architectural differentiation, nuclear polymorphism and rate of mitosis. The molecular markers examined included estrogen receptor (ER), progesterone receptor (PR) and HER2 expression.

Tumor samples were fixed on buffered formalin, embedded in paraffin and cut with a microtome. Immunohistochemistry was performed with an indirect immunoperoxydase method using antibodies directed against HER2 oncoprotein, ER and PR (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 performed on an automated immunostainer (Ventana XT, Tucson, AZ, USA). ER and PR status were considered positive if the tumor showed at least 10 % of positive cells [20]. HER2 status was graded according to the HercepTest scoring system modified by ASCO/CAP recommendations (0, 1+, 2+ or 3+) [21]. Scores of 3+ were considered positive. In the case of 2+ scores, fluorescent in situ hybridization (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, by a ratio of HER2/CEN17>2.2 [21].

^{18}F-FDG PET/CT procedures

A first ^{18}F-FDG PET/CT study was performed at baseline. Two different PET/CT imaging systems were used: a Gemini GXL PET/CT scanner from November 2006 to December 2010, and a Gemini TF PET/CT scanner from December 2010 to October 2012 (Philips Medical Systems, Eindhoven, The Netherlands). Patients were instructed to fast for at least 6 h before the intravenous injection of 5 MBq/kg of ^{18}F-FDG for Gemini GXL studies and 3.5 MBq/kg for Gemini TF studies. Emission and transmission scans from the brain to mid-thigh were acquired 60 min later. Scans restricted to the chest with patients in the prone position were started 90 min after the injection of ^{18}F-FDG. Emission data were corrected for dead time, random and scatter coincidences, and attenuation before reconstruction with the RAMLA iterative method. A second ^{18}F-FDG PET/CT study was performed just before the second course of NAC. A chest-restricted acquisition was done 90 min after the injection of the ^{18}F-FDG. For each patient, the same imaging system, ^{18}F-FDG activity and time from injection to acquisition were used for both studies.

A spheroidal volume of interest (VOI) encompassing the primary tumor or nodes was manually drawn on the chest-restricted acquisitions, to measure the Standardized Uptake

Value maximal index (SUV_{max}) at baseline ($PET_1.SUV_{max}$) and after the first course of NAC ($PET_2.SUV_{max}$). Measured SUV_{max} was systematically corrected for body surface area (BSA) and glycemia, as detailed in our previous studies [12, 15].

The metabolic response to NAC was calculated:

$$\Delta SUV_{max}(\%) = 100 \times (PET_1.SUV_{max} - PET_2.SUV_{max})/PET_1.SUV_{max}.$$

Metabolic tumor volume (MTV) was automatically measured inside the tumoral VOI, which had been previously drawn using a dedicated software package (Tumor-tracking; Philips) with margin thresholds set at 41 % of SUV_{max}, after correction for the breast background activity [threshold= 0.41*(Tumor SUV_{max}- Contralateral breast background SUV_{max})]. Total lesion glycolysis (TLG) was then calculated as SUV_{mean} x MTV, which considers both the metabolic activity and tumor burden. Tumor ΔTLG (%) was calculated.

Three other VOIs were drawn to assess different background SUV_{mean} and SUV_{max}:

- on the contralateral breast glandular tissue (breast background)
- inside the ascending aorta (mediastinal background)
- on the liver parenchyma, at mid height (hepatic background)

The mediastinal and hepatic SUV_{max} were used as thresholds on the PET2 examination to distinguish between tumors with low or high residual metabolism.

Statistical analysis

Statistical analysis was performed with the use of WinSTAT software (Microsoft, Redmond, Washington, USA) and Systat software (Systat Inc., Evanston, IL). Data were described by frequency (percentage) or mean and standard deviation (SD).

Correlations between metabolic tumor parameters and the different clinical, biological and pathological variables were assessed with either the Mann–Whitney or the Kruskall-Wallis test.

Tumor metabolic characteristics according to the PET/CT system used and to achievement of pCR were compared with the Mann–Whitney test.

The Chi-square test was used to compare the rates observed.

The predictive value of ΔSUV_{max} was first evaluated in the whole population. But because previous studies reported ΔSUV_{max} to be less accurate in response assessment for women with low-metabolic tumors (tumor-to-background ratio of less than 5) [22–24], this analysis was secondly restricted to women with hypermetabolic tumors at baseline. In the present study, a tumor-to-background tumor ratio≥5 was comparable to a tumor $PET_1.SUV_{max} \geq 3.7$ (mean contralateral breast tissue SUV_{mean}=0.74): this threshold was therefore used.

Receiver operating characteristic curves (ROC) were performed to define the optimal threshold of ΔSUV_{max} and $PET_2.SUV_{max}$ for the prediction of pCR.

Univariate analyses of the different clinical, histopathological and metabolic parameters to predict pCR were performed using a logistic regression model. Multivariate analyses with backward variable selection were done to identify prognostic variables of independent statistical significance. P values < 0.05 were considered significant in all tests.

Results

Patients' characteristics (Table 1)

Among the 195 women evaluated, 72 had HER2-positive breast tumors. Seven of them were excluded because they had undergone a NAC regimen that did not include trastuzumab and docetaxel, and eight were excluded because of obvious upstaging after the first PET exam (stage IV), and in whom NAC was no longer indicated. In the remaining 57 patients, three missed the second PET exams because they declined to continue or because of problems with the equipment. All tumors were invasive ductal carcinoma; none of them belonged to the lobular histological subtype.

The mean tumoral SUV_{max} (± SD) at baseline was 8.3±4.4 (Table 2) and the mean lymph node SUV_{max} was 5.9±3.9. After the first cycle of NAC, the mean tumoral $PET_2.SUV_{max}$ was 2.9±1.5. The mean tumoral ΔSUV_{max} was 58.5±22.7 %. There were no significant differences between patients imaged with the Gemini GXL (n=39) and the Gemini TF PET/CT (n=18) systems for mean $PET_1.SUV_{max}$, $PET_2.SUV_{max}$ and ΔSUV_{max}.

The mean tumoral TLG1 and ΔTLG (± SD) were calculated at 31.4±38.3 and 84.9±12.8 %, respectively.

The pCR rate was 43.9 % (25/57).

Correlation between metabolic features and clinical/histopathological parameters (Table 2)

Regarding primary tumor metabolism, both higher SUV_{max} and higher ΔSUV_{max} were associated with a higher mitotic count (respectively P=0.001 and P<0.001). The correlation between ΔTLG and mitotic count was lower (P=0.02). Baseline TLG was only correlated with PR status: TLG_1 was 37.0±40.1 in tumors with negative PR status and 25.7± 36.2 in tumors with positive PR status (P=0.04).

Regarding axillary node metabolism, higher $PET_1.SUV_{max}$ and greater ΔSUV_{max} were only associated with negative tumor estrogen receptor status. In negative ER tumors, mean

Table 1 Population characteristics

Characteristics	Number of patients: N (%)
Total patients	57
Age (years)	
≤50	36 (63 %)
>50	21 (37 %)
Menopause	
No	35 (61 %)
Yes	21(37 %)
Unknown	1 (2 %)
Tumour size (T)	
≤5 cm	50 (88 %)
>5 cm	7 (12 %)
Lymph node involvement	
Negative	19 (33 %)
Positive	38 (67 %)
Tumour grading	
Grade I	2 (4 %)
Grade II	24 (42 %)
Grade III	28 (49 %)
Missing	3 (5 %)
Architectural differentiation	
Score I	1 (2 %)
Score II	12 (21 %)
Score III	37 (65 %)
Missing	7 (12 %)
Nuclear pleomorphism	
Score I	0 (0 %)
Score II	21 (37 %)
Score III	29 (51 %)
Missing	7 (12 %)
Number of mitoses	
Score I	14 (25 %)
Score II	27 (47 %)
Score III	11 (19 %)
Missing	5 (9 %)
HER2 status	
++ and FISH +	8 (14 %)
+++	49 (86 %)
Estrogen receptor status	
Negative	18 (32 %)
Positive	39 (68 %)
Progesterone receptor status	
Negative	29 (51 %)
Positive	28 (49 %)
Hormonal receptor status	
Negative (ER and PR negative)	15 (26 %)
Positive (ER or PR positive)	42 (74 %)
pCR (ypT0/is ypN0)	
Yes	25 (44 %)
No	32 (56 %)

pCR is defined as ypT0/is ypN0: no invasive residual in the breast and nodes; in-situ breast residuals allowed

$PET_1.SUV_{max}$ was 9.2±4.5 in the nodes and mean ΔSUV_{max} was 76.6±11.6, whereas these values were 4.7±3.0 and 60.1±19.0 in positive ER tumors (P=0.01 and P=0.03, respectively).

Relationship between tumor metabolic parameters and pCR (Tables 3 and 4)

There was a strong correlation between tumor residual SUV_{max} at PET2 and pathological response: mean $PET_2.SUV_{max}$ was 3.2±1.6 in women without pCR and 2.3±1.1 in those with pCR (P=0.003) (Fig. 1, Table 3).

ROC analyses were performed for SUV2 for the early prediction of pCR; the area under the curve (AUC) was 0.73 (P=0.003) (Fig. 2). With an optimal cut-off determined at 2.1, both the negative predictive value (NPV), and the positive predictive value (PPV) of a low $PET_2.SUV_{max}$ to predict pCR were 76 %. Other cut-offs could be used. For example, if the aim rather was to best identify the non-complete responding women, a higher cut-off might be favored to improve the NPV of $PET_2.SUV_{max}$, but at the expense of the PPV. Indeed, the accurate identification of non-responding women may also be an important point in the design of treatment intensification trials for poor-responding women.

Instead of a fixed value, intra-subject background cut-offs (mediastinal or hepatic SUV_{max}) were used on interim PET to distinguish between tumors with low or high residual metabolism, but this did not predict pCR (P=0.58 in univariate logistic analysis, both).

In the first analysis, ΔSUV_{max} did not correlate significantly with pathological response (P=0.17). But when ΔSUV_{max} was compared with baseline SUV_{max} (Fig. 3), we reported ΔSUV_{max} to be less accurate in response assessment for the subset of five patients with low ^{18}F-FDG uptake at baseline ($PET_1.SUV_{max}$<3.7) because of lower metabolic response, despite a 80 % rate of pCR in this subgroup. Therefore a second analysis of ΔSUV_{max} was restricted to the subset of 52 women with hypermetabolic tumors ($PET_1.SUV_{max}$≥3.7): there was a correlation between the early percentage decrease in SUV_{max} and the pathological response at surgery (ΔSUV_{max}=58.6±17.6 % in women without pCR vs. 71.3±14.2 % in those with pCR, P=0.02; Table 3). Using ROC analysis, the AUC was 0.70, 95 % IC=[0.55–0.85], P=0.02. When an optimal cut-off of 60 % was used, the sensitivity, specificity, positive predictive and negative predictive value of ΔSUV_{max} to predict pCR were 83 % (15/18), 52 % (16/31), 50 % (15/30), and 84 % (16/19), respectively.

By univariate logistic analysis, tumor $PET_1.SUV_{max}$ (P=0.03), ΔSUV_{max} if baseline SUV_{max}≥3.7 (P=0.03), tumor $PET_2.SUV_{max}$ (P=0.001), SBR grade (P=0.03) and hormonal receptor status (P=0.04) correlated with pCR (Table 4). By multivariate analysis, tumor $PET_2.SUV_{max}$ was the best independent predictive factor of pCR: a decrease in SUV_{max} below

Table 2 Metabolic characteristics of the primary tumor according to the clinical and histopathological status

	TUMOR								
	Baseline SUV_{max}			ΔSUV_{max}			ΔTLG		
	N	mean ± S.D.	*P*	N	mean ± S.D.	*P*	N	mean ± S.D.	*P*
All patients	57	8.3±4.4		54	58.5±22.7		54	84.9±12.8	
Age (years)									
≤50	36	8.3±4.5	*NS**	34	59.1±20.6	*NS**	34	85.9±14.0	*NS**
>50	21	8.2±4.2		20	57.5±26.5		20	83.3±10.6	
Tumor size									
≤5 cm	50	8.4±4.5	*NS**	47	59.8±23.5	*NS**	47	85.3±12.5	*NS**
>5 cm	7	7.2±2.6		7	49.7±15.4		7	82.3±15.2	
Tumour Grading (SBR)									
I+II	26	7.5±4.0	*0.05**	25	53.8±19.8	***0.02****	26	87.9±10.4	*NS**
III	28	9.4±4.6		26	64.0±22.7		25	83.0±12.7	
Number of mitosis									
I	14	7.3±4.9	***0.001****	13	44.2±18.9	***< 0.001****	13	77.1±12.2	***0.02****
II	27	7.6±3.7		26	59.9±22.3		26	88.5±11.0	
III	11	12.1±3.8		10	76.6±9.4		10	88.8±8.1	
Estrogen receptor status									
Negative	18	7.9±3.5	*NS**	16	57.6±26.8	*NS**	16	78.9±15.7	*NS**
Positive	39	8.4±4.7		38	58.8±28.2		38	87.5±10.6	
Progesterone receptor status									
Negative	29	8.7±4.7	*NS**	27	58.7±25.7	*NS**	27	85.1±13.9	*NS**
Positive	28	7.8±4.0		27	58.2±19.8		27	84.8±11.9	
Hormonal receptor status									
Negative	15	8.0±3.9	*NS**	13	57.3±28.9	*NS**	13	80.9±16.7	*NS**
Positive	42	8.4±4.5		41	58.8±20.8		41	86.2±11.2	

S.D. = Standard Deviation, NS = not significant, TLG = Total Lesion Glycolysis

*Mann–Whitney test, **Kruskal-Wallis test

Menopausal status, architectural differentiation and nuclear pleomorphism were not significantly correlated with tumor metabolic characteristics

2.1 after the first cycle of NAC had a high odds ratio of 14.3 (95 % CI=[2.3–90.9]; P=0.004).

We found no significant association between the various tumor volume parameters (MTV_1, MTV_2, ΔMTV, TLG_1, TLG_2 or ΔTLG) and a pCR.

No correlation was found between axillary metabolic parameters and nodal or tumoral pCR.

Discussion

Because alternative therapies are now available in HER2-positive tumors (e.g. association of an anti-angiogenic drug, dual anti-HER2 blockage, etc.), the early identification of women who do not respond to a trastuzumab/taxane regimen is an important clinical issue. Neoadjuvant chemotherapy allows the quantifiable in vivo assessment of tumor chemosensitivity and is an excellent setting for the translational evaluation of new predictive biomarkers of tumor response.

The interest of the present study is that it included a relatively large population of women with invasive HER2-positive ductal breast carcinoma whose tumor response to taxane and trastuzumab-based neoadjuvant chemotherapy was monitored early with ^{18}F-FDG PET/CT.

Correlation between early metabolic parameters and pCR

The findings demonstrate that in this specific breast cancer subtype, tumor metabolic response after the first cycle of NAC correlated with pCR. Nonetheless, this correlation was strongly linked to the metabolic parameter studied.

A SUV_{max} lower than 2.1 after the first cycle of NAC was the only independent predictor of pCR (P=0.004), with an accuracy of 76 %. Because HER2-positive cancers are highly sensitive to trastuzumab associated with chemotherapy, this threshold is very low, meaning a nearly complete metabolic response after the first course of NAC is required to predict pCR. A recent study by Groheux et al., which included 30 patients with the same subtype but a different PET timing

Table 3 Tumor metabolic characteristics according to achievement of pCR

	N	Mean ± S.D.	*P**
$PET_1.SUV_{max}$			
pCR	25	7.9±4.4	*NS*
no-pCR	32	8.6±4.4	
$PET_2.SUV_{max}$			
pCR	22	2.3±1.1	***0.003***
no-pCR	32	3.2±1.6	
ΔSUV_{max}			
pCR	22	59.8±28.4	*NS*
no-pCR	32	57.5±18.3	
ΔSUV_{max} if $PET_1.SUV_{max} \geq 3.7$			
pCR	18	71.3±14.2	***0.02***
no-pCR	31	58.6±17.6	
TLG_2			
pCR	22	3.2±3.3	*NS*
no-pCR	32	4.9±8.3	
ΔTLG			
pCR	22	86.6±12.7	*NS*
no-pCR	32	83.8±12.9	

S.D = Standard Deviation

*Mann–Whitney test

(after two courses), also found that a low $PET_2.SUV_{max}$ was the main determinant to predict pCR early, with an accuracy of 90 % [17]. The predictive threshold in this study was higher (SUV_{max}>3), but may be explained by a different neoadjuvant regimen, which introduced trastuzumab after four cycles of anthracycline-based chemotherapy.

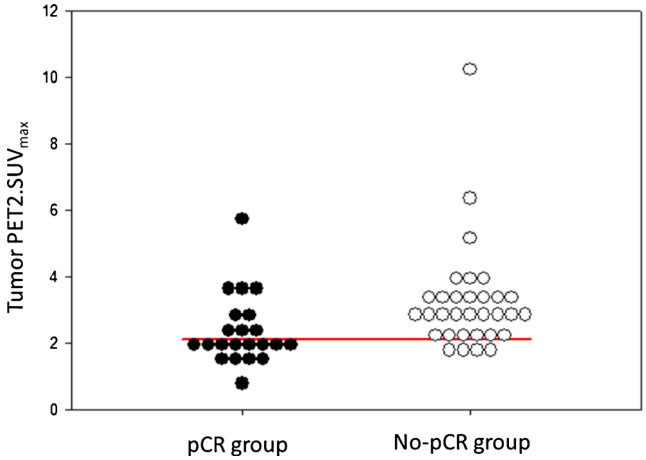

Fig. 1 Distribution of absolute tumor $PET_2.SUV_{max}$ for pCR and non-pCR women. The red line corresponds to the threshold of $PET_2.SUV_{max}$=2.1

The predictive value of ΔSUV_{max} is more conflicting among the few studies. Groheux et al. found an accuracy of 73 % to predict pCR, which is lower than that of $PET_2.SUV_{max}$ [17], but equivalent to the findings of a preliminary study at our institution (76 %) [15]. A study by Koolen

Table 4 Univariate and multivariate logistic analysis of significant predictive factors for pCR

	pCR						
		Univariate analysis			Multivariate analysis		
	N	OR	[95 % CI]	*P* value	OR	[95 % CI]	*P* value
$PET_2.SUV_{max}$							
≥2.1	37	1			1		
<2.1	17	10.1	[2.6–38.4]	***0.001***	14.3	[2.3–90.9]	***0.004***
Missing	3						
$PET_1.SUV_{max}$ and ΔSUV_{max}							
Hypermetabolic with ΔSUV_{max}<65.5 %	24	1			1		
Hypermetabolic with $\Delta SUV_{max} \geq 65.5$ %	25	4.1	[1.2–14.5]	**0.03**	0.9	[0.1–5.6]	NS
Low metabolic ($PET_1.SUV_{max}$<3.7)	5	15.2	[1.4–168.0]	**0.03**	13.7	[1.2–162.1]	**0.04**
Missing	3						
SBR							
I + II	26	1					
III	28	3.6	*[1.2–11.4]*	***0.03***			*NS*
Missing	3						
Hormonal status (ER + PR)							
Positive	42	1		***0.04***			*NS*
Negative	15	3.6	*[1.0–12.5]*				

OR = odds ratio; [95 % CI] = [95 % confidence interval]; NS = not significant

Tumor size and $PET_2.SUV_{max}$ using either the mediastinal or hepatic uptakes as cut-offs were not significant factors in univariate analysis.

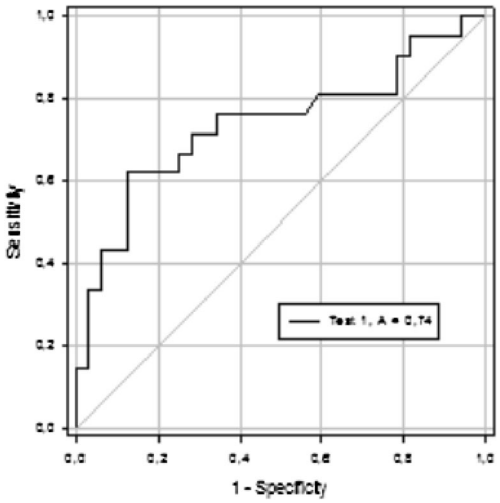

Fig. 2 ROC curve analysis of $PET_2.SUV_{max}$ for prediction of pCR. Area under curve=0.73±0.07; 95 % IC=[0.59–0.88]; p=0.004; threshold= 2.1; sensitivity is 59 % (13/22), specificity is 88 % (28/32), positive predictive value is 76 % (13/17), negative predictive value is 76 % (28 / 37), and accuracy is 76 % (41/54)

et al., however, found that ΔSUV_{max} evaluated after 3 or 8 weeks of treatment was not accurately associated with a pathological response [18]. Differences between results may be explained by the small numbers of patients, and different regimens and timing for the neoadjuvant treatment.

In the first analysis of the present study, ΔSUV_{max} was not an appropriate metabolic parameter to predict histological response in the HER2+ subtype. Nonetheless, we observed that, although tumors with low baseline SUV_{max} frequently reached pCR, their early metabolic response (ΔSUV_{max}) was always weak and therefore not accurate. This is due to the high impact of the breast background metabolic activity on ΔSUV_{max} measurements in low-metabolism tumors. This finding is in keeping with previous studies suggesting that ΔSUV_{max} only be evaluated in cases with a tumor-to-background ratio higher than 5 [22–24]. A statistically significant improvement in the classification of responses was

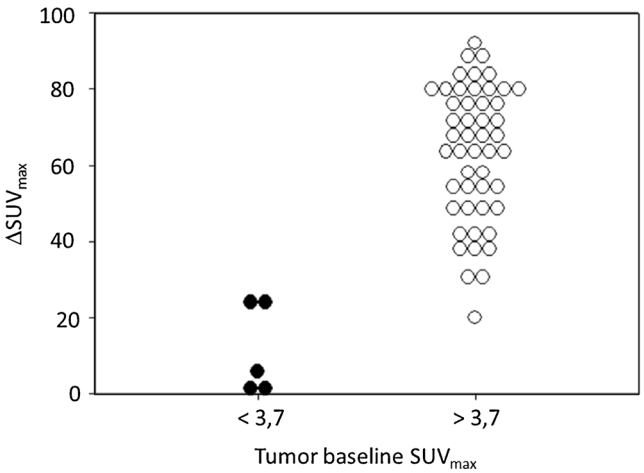

Fig. 3 Distribution of ΔSUV_{max} according to tumor $PET_1.SUV_{max}$

indeed obtained when the predictive value of ΔSUVmax was assessed only in tumors with baseline $SUV_{max} \geq 3.7$ (comparable to a tumor-to-background tumor ratio≥5 because of mean breast tissue SUV measured at 0.74). Similarly, according to the data reported by Groheux et al. [17] , it appeared that none of the four low-metabolism tumors ($SUV_{max} < 3.7$) reached the −62 % cutoff used in the study to predict pCR, even though it was finally achieved by three of them, demonstrating many false negative results. Therefore, ΔSUV_{max} may be an early predictor of pCR, but caution is required when the response of tumors with low tumor-to-background SUV_{max} ratio is evaluated.

Among other PET parameters, the predictive value of early changes in the metabolic tumor volume was assessed, using the TLG. As previously suggested by a pilot study by Hatt et al., which included a small cohort of 12 women [25], this metabolic parameter was not found to correlate significantly with pCR in the HER2-positive subtype.

The use of intra-subject reference background SUV_{max} (mediastinal or hepatic) to distinguish between tumors with a poor and those with a good response on interim PET is more robust than absolute SUV_{max} cut-offs for multicenter settings, and is currently applied in the prognostic stratification of lymphoma [26]. Nevertheless, no predictive value of these thresholds has been found on PET2.

Metabolic characteristics of tumors and lymph nodes according to the clinical and histopathological status

In this HER2-positive subtype, only a higher mitotic activity correlated with both higher baseline tumor metabolism and a greater early decrease in metabolism. These findings corroborate our previous results in a general population of breast cancers [15]. Hormone receptor (HR) status is a major driver of breast cancer clinical phenotype and tumor features, even among HER2-positive patients [10, 11, 27]. The pathological response of tumors to HER2-directed neoadjuvant regimen differs according to ER expression, and a study of Von Minckwitz et al. has reported pCR not to be a prognostic marker in HER2+/HR+ breast cancers [10]. Surprisingly, no significant difference was found in the metabolic behavior of HER2+/HR+ and HER2+/RH- primary tumors, thus confirming the results of Groheux et al. in a smaller cohort of patients [17].

The metabolism of synchronous axillary nodes raises an interesting point: both the baseline metabolism (SUV1) and the early metabolic decrease (ΔSUV) were greater in HER2+/HR- lymph nodes. To our knowledge, this is the first study to evaluate the metabolism of axillary lymph nodes according to the HR status in HER2+ tumors. Several hypotheses could explain these differences. Firstly, breast cancer is a heterogeneous tumor made up of different cell clones [28] that derive from genetic instability acquired by cancer cells during the

multi-step process of tumor progression [29]. The few cells that acquire metastatic behavior and present in the involved nodes may have higher proliferation and apoptosis rates in the HER2+/HR- subtype than in the HER2+/HR+ subtype, thus explaining their higher metabolism. Secondly, crosstalk between the ER and HER2 pathways has been shown to play a role in both intrinsic and acquired resistance to endocrine agents and HER2-directed agents in previous studies [30]. Vaz-Luis et al. found a 49 % discordance rate of one of the markers (ER, PR, HER2 status) between HER2-positive primary tumors and metastatic samples, including a switch from HER2-positive to HER2-negative in 17 % of cases [11]. Even though this discordance may be lower between primary and synchronous axillary nodes, the down-regulation of HER2 overexpression in lymph nodes could explain the higher metabolic activity usually observed in the triple-negative subtype [15, 31]. The expression pattern of markers involved in cell proliferation, differentiation and apoptosis in primary and synchronous axillary node metastasis should be compared in the HER2-positive subtype to confirm or rule out these hypotheses.

Limitations of the study

Our study has some limitations. Firstly, only static SUV measurements were performed, while kinetic analysis may present advantages for response assessment [32]. Secondly, due to the small number of women included, we cannot draw any definitive conclusions about the predictive value of PET with an additional stratification of the HER2 subtype into ER-positive and ER-negative tumors. Moreover, the best metabolic surrogate marker of pCR and the optimal threshold may still differ from one single-center trial with small subgroups of patients to another. There is now a crucial need for multicenter prospective trials. One was recently performed (Neo-ALTTO) to determine the value of ^{18}F-FDG PET for the early prediction of response to neoadjuvant lapatinib and trastuzumab, without associated chemotherapy [33]. The results are encouraging: ΔSUV_{max} was significantly higher in patients who achieved a pCR. Because there had been no prior PET harmonization program, absolute SUV_{max} were not comparable among cameras and therefore not evaluated. Indeed, the absolute SUV_{max} measurements can be affected by the use of different PET systems which is currently a limitation for its use as an imaging biomarker in multicentric studies. In contrast, ΔSUV (%) is more reproducible and reliable when data from various scanners with strict intra-subject standardization are analyzed. Guidelines for the Standardization of PET imaging systems have recently been issued and should be applied in multicenter trials using ^{18}F-FDG PET as an imaging biomarker to improve the reproducibility of SUV_{max} measurements in the future [34, 35].

Conclusion

Because alternative therapies are now available in HER2-positive tumors, the early identification of responding and non-responding women is an important clinical issue. The metabolic response after the first cycle of NAC, assessed with ^{18}FDG-PET, is predictive of the final histological response. Clinical trial now needed to determine whether tailoring early the neoadjuvant drug regimen to the metabolic response could improve the pCR rate in non-responding women and induce a survival benefit.

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