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ORIGINAL ARTICLE

Staging of locally advanced breast cancer and the prediction of response to neoadjuvant chemotherapy: complementary role of scintimammography and 18F-FDG PET/CT

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ABSTRACT

BACKGROUND: The primary endpoint of the study was to established the role of sestamibi scintimammography and PET/CT findings in locally advanced breast cancer (LABC) before neoadjuvant systemic therapy (NST) in different histological subtypes. The secondary endpoint was to determine the role of FDG PET/CT as multi-drug resistance marker.

METHODS: From January 2012, we prospectively enrolled 51 consecutive women (median age: 49 years; range: 27-76 yrs) with a biopsy-proven LABC. All patients underwent both sestamibi scintimammography and FDG PET/CT within one week before to start NST. Both examinations were qualitatively and semiquantitatively analysed. For scintimammography we calculated the tumor to background ratio (T/B) and the most intense uptake of the tumor to background ratio (I/B) according the following formula: T/B=[cntsT-cntsB]/ [cntsB] and I/B [cntsI-cntsB]/ [cntsB]. Furthermore, the percentage washout index (WO) for T and I were obtained, according to: WOT,I= [cntsT,I]early image-[cntsT,I] delayed image/[cntsT,I]early image. Maximum and average (avg) standardized uptake value (SUV) was computed by PET/CT, using a region of interest. Patients who had an evidence of systemic metastases or a second active cancer at imaging scans, were excluded. At the end of preoperative therapy, the response to therapy was assessed by the analysis of surgical specimen and then correlated with both scintimammographic and PET/CT data.

RESULTS: Based on the inclusion criteria, the final analysis was performed in 49 patients. Scintimammography and PET/CT showed a sensitivity of 100% for the evaluation of primary cancer, while PET/CT showed a slightly higher detection rate for axillary lymph node than scintimammography. According to the biological pattern, SUVmax and SUVavg resulted significantly different among histological subtypes, whereas scintimammographic data did not. At the end of neo-adjuvant therapy, pathological complete response was obtained in 12 (24.4%) patients, while 37 had a partial or no response to NST (identified as no-responders). On the basis of histopathological response to NST, median WOI resulted significantly lower in responders than non-responders (30.5% *vs.* 44%; P=0.027). Conversely, SUVmax and SUVavg were significantly higher in responders than non-responders (all P<0.05). In this latter subset of patients, high WOTs were associated with low SUVs. On the contrary, in responder group, high SUVs were reported particularly for high WOT values.

CONCLUSIONS: Scintimammography with sestamibi did not accurately determine the responsiveness to therapy. FDG PET/CT is more accurate in the prediction of response to therapy, particularly in the aggressive LABC subtype. Moreover, semiquantitative data by FDG PET seems to be linked with the chemosensitivity to NST.

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Key words: Ultrasonography, mammary - Positron-emission tomography - Tomography, X-ray computed - Breast neoplasms - Neoadjuvant therapy

Through the years, the goal of neoadjuvant systemic therapy (NST) in locally advanced breast cancer (LABC) has been changed. It was initially used in order to convert a previously unresectable cancer into an operable one,[1-3] while nowadays it has been widely administered in primarily operable breast cancer (BC) to reduce tumor volume and thus allow conservative surgery.[4, 5] The down-staging of the primary tumor and the increase in breast conservation rates seems to be the only clinical benefit of NST, given that several studies failed to demonstrate an improvement in overall survival compared with postoperative adjuvant chemotherapy.[3, 6-8] The response to standard therapies and the relative outcomes of BC depend on its biological heterogeneity that reflects the complexity and variability of the vast array of somatic mutations acquired during oncogenesis. This heterogeneity is concretely apparent in tumor with expression of hormone receptor (HR) or human epidermal receptor 2 (HER2).[9, 10] As reported by a recent meta-analysis from Houssami *et al.*,[11] different subtype-specific pathological complete response rate (pCR%) was proven: positive HR/negative HER2=8.3%, positive HER2/positive HR=18.7%, triple negative (no HR expression and negative HER2)=31.1% and positive HER2/negative HR=38.9%. These findings had both clinical and biological implications; firstly they can aid clinicians in selecting appropriate candidates for NST *vs.* adjuvant therapy and secondly the majority of positive HR/negative HER2 tumor are generally resistant to chemotherapy.

Through the years, the goal of neoadjuvant systemic therapy (NST) in LABC has been changed. It was initially used in order to convert a previously unresectable cancer into an operable one,[1-3] while nowadays it has been widely administered in primarily operable BC to reduce tumor volume and thus allow conservative surgery.[4, 5] The down-staging of the primary tumor and the increase in breast conservation rates seems to be the only clinical benefit of NST, given that several studies failed to demonstrate an improvement in overall survival compared with postoperative adjuvant chemotherapy.[3, 6-8] The response to standard therapies and the relative outcomes of BC depend on its biological heterogeneity that reflects the complexity and variability of the vast array of somatic mutations acquired during oncogenesis. This heterogeneity is concretely apparent in tumor with expression of hormone receptor (HR) or human epidermal receptor 2 (HER2).[9, 10] As reported by a recent meta-analysis from Houssami *et al.*,[11] different subtype-specific pathological complete response rate (pCR%) was proven: positive HR/negative HER2=8.3%, positive HER2/positive HR=18.7%, triple negative (no HR expression and negative HER2)=31.1% and positive HER2/negative HR=38.9%. These findings had both clinical and biological implications; firstly they can aid clinicians in selecting appropriate candidates for NST vs. adjuvant therapy and secondly the majority of positive HR/negative HER2 tumor are generally resistant to chemotherapy.

Resistance of BC cells to several structurally unrelated classes of natural products, including anthracyclines, taxanes, and epipodophyllotoxines, is often referred as multidrug resistance (MDR).[12] In tumor cell lines, MDR is often associated with an ATP-dependent decrease in cellular drug accumulation and is attributed to the over-expression of certain ATP-binding cassette (ABC) transporter proteins. ABC proteins that confer drug resistance include (but are not limited to) P-glycoprotein (Pgp), the multidrug resistance protein 1 (MRP1), MRP2, and BC resistance protein (BCRP). Multidrug resistance and specific ABC transporters can be imaged using radiopharmaceuticals that are themselves MDR substrates or inhibitors. The first and most studied of these is 99mTc-metossiisobutilisonitrile or sestamibi, which is a substrate for Pgp, MRP1, MRP2 and BCRP and can therefore be used to image their expression *in vivo*.[13] Although scintimammography with sestamibi is readily available and provides useful and well established tumor information, it is underutilised in clinical practice. Differently, 18F-fluorodeoxyglucose (FDG) hybrid positron emission tomography (PET)/computed tomography (CT) is gaining importance for the staging of patients with large or LABC.[14] The evaluation of tumor glucose metabolism let to determine tumor vitality estimating glycolytic metabolism of the cancer cells. Since cancer growth is characterised by an increased rate of aerobic glycolysis,[15] FDG would be appropriate to indicate and to predict the tumor response to treatment. Moreover, FDG uptake of a Pgp-positive tumor has been shown to be lower than that of a Pgp-negative tumor in a mouse model [16] and an *in vitro* study showed that 2-deoxy-D-glucose accumulation is reduced in MDR cell line with strong Pgp expression and reduced GLUT-1 expression.[17]

The primary endpoint of the study was to established the role of scintimammographic and PET/CT findings in LABC before NST in different histological subtypes. The secondary end-point was to determine the predictive value for chemosensitivity by sestamibi scintimammography and FDG PET/CT in the same setting of patients.

Materials and methods

Recruitment of population

From January 2012, we prospectively enrolled 51 consecutive women (median age: 49 years; range: 27-76 years) with a biopsy-proven LABC. Clinical stage was determined in accordance with the American Joint Committee on Cancer (AJCC). All patients underwent both scintimammography and FDG PET/CT within one week. The exclusion criteria were: 1) a not suitable performance status (according to the criteria of the World Health Organization); 2) a previous hormonal treatment or chemotherapy or radiotherapy; 3) the evidence of systemic metastases or a second active cancer at imaging scans; 4) the inability to sign the consensus. The research project was authorized by the Committee for Ethical Research of our Institute. Written informed consent to perform both the imaging modalities was obtained by all subjects. The study was conducted according to the Declaration of Helsinki (2000).

Histology, immunohistochemistry and molecular biology

One breast consultant pathologist (E.O.) evaluated all patients. In each cases evaluation of histotype, nuclear grade, HR expression, proliferative activity (Ki67) and HER2 status was completed before treatment. Breast lesions were classified into five molecular subtypes according to the 12th International Breast Conference recommendations.[18] We used a panel of immunohistochemical markers for estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 to categorize our patients into one of the five molecular subtypes: 1) luminal A: ER-positive and/or PR-positive, HER2-negative and Ki-67 low (<14%); 2) luminal B-HER2(-): ER-positive and/or PR-positive, HER2-negative and Ki-67 high (≥14%); 3) luminal B-HER2 enriched: ER-positive and/or PR-positive, HER2-positive and any Ki-67 index; 4) HER2 enriched: ER-negative, PR-negative and HER2 positive; 5) basal or triple negative: ER, PR, and HER2 negative. Ki-67 index is primarily used to differentiate between luminal A and luminal B-HER2(-) subtypes.

In the post-treatment specimen, the surgeons (S.M. or T.S. or F.B.) identified tumor bed by a marker to allow the identification of residual tumor. A pCR was defined as complete absence of residual tumor cells at microscopy both in the breast and in the axillary lymph nodes. The Sataloff criteria was considered for assessing the response to NST.[19] All tumors showing progression, stable disease, or partial response to NST were classified as residual disease.

Sestamibi imaging

For the evaluation of appropriateness, 99mTc-Sestamibi (sestamibi; Technemibi Italy®, Mallinckrodt Medical B.V., the Netherlands; 740 MBq intravenous-i.v.) was injected before the start of NST performing a conventional scintimammography. The scintimammographic study started 5 minutes after the i.v. injection of tracer. Radiolabeling and quality control procedures of the radiotracer were carried out according to the manufacturer's instructions. Labelling efficiency was always over 95%. The images were acquired by an expert technician (R.S.) both using a large field of view dual-head gamma cameras (E.Cam, Siemens USA) equipped with high-resolution collimation and positioning the patient in the prone, breast-dependent position as described by Khalkhali *et al.*[20] All the entire breast and its tail were positioned as close as possible to the camera. Moreover, a planar view in the anterior projection with the patient supine and the arms elevated above the head was performed. A second collection of images was provided after 3 hours from the tracer injection. The images were interpreted by two nuclear medicine physicians blinded to the clinical, mammography and histopathology results. The reports included location of the abnormality and presence or absence of focal increased uptake. Moreover, a semiquantitative analysis of the images was performed to assess the time of tracer persistence in the tumor. In particular, tumor to background ratio (T/B)=[cntsT-cntsB]/ [cntsB] and the most intense uptake of the tumor to background ratio (I/B) [cntsI-cntsB]/ [cntsB], were computed by drawing two ROIs

(one comprising the entire tumor and another one comprising only the most intense tumor uptake), in lateral projected images. Furthermore, the percentage washout index (WO) for T and I were obtained, according to: WOT,I= [cntsT,I]early image-[cntsT,I]delayed image/[cntsT,I]early image.[21] Two washout thresholds were considered: 45% and 56%, according to Del Vecchio *et al.*,[22] Sciuto *et al.*[23] and Mezi *et al.*[24]

FDG PET/CT imaging

Whole body FDG PET/CT was performed using a dedicated PET/CT scanner (Biograph 16, by Siemens Medical Solutions, IL, USA), updated by a high-definition software. Emission images ranging from the proximal femur and the base of the skull were acquired for 2-3 min (based on the body weight) per acquisition field of view (AFOV) after 60 minutes from the injection of FDG (3 MBq/kg/body weight). Acquired images were reconstructed using the attenuation weighted-Ordered Subset Expectation Maximization (OSEM) iterative reconstruction, with 2 iterations and 8 subsets. Two experienced specialists (L.E. and A.R.C.) evaluated fused PET/CT images. At visual analysis, increased FDG uptake on the basis of either highly suspicious or definite CT morphologic changes and not corresponding to physiological uptake patterns was recorded as positive. The support of abnormal uptake was given by semiquantitative data. The semi-quantitative evaluation of FDG uptake was measured using maximum standardized uptake values (SUV), obtained by generating a 3D region of interest (ROI) based on region-growing procedures, using a cut-off ≥2.5. In case of a low tumor-to-background ratio, rendering an automated ROI generation unreliable, SUV was derived from a manually drawn 3D volume of interest. Maximum (max)/average (avg)/minimum SUV and metabolic tumour volume (MTV) were computed.

Schedule of treatment

According to the oncologist (C.G)'s indications, patients were treated with pre-operative chemotherapy given in three-week cycles. The following chemotherapeutic regimens were used:

— epirubicin+cisplatinum+fluorouracil in 1 patient;
— doxorubicin+cyclosphamide in 7 patients and the combination with trastuzumab in 8 patients;
— antracyclines+taxanes in 29 patients and the combination with the trastuzumab in 8 patients;
— taxanes+trastuzumab in 1 patient.

At the end of neo-adjuvant therapy, all 49 patients were send to surgery. In particular, 10 patients underwent lumpectomy and axillary lymph node dissection (I and II level) while 39 patients were treated with mastectomy and axillary lymph node dissection (I and II level in 7 patients, I-II-III level in 32 patients). Based on histologic assessment, pCR was obtained in 12/49 patients (24.4%), while 29 (59.2%) had a partial response and 8 (16.4%) no response to NST.

Statistical analysis

The normality of the variables were verified by the Shapiro-Wilk Test. Continuous data were presented as median and range, while categorical data as numbers (percentage). Associations for paired samples were assessed using Mann-Whitney Test. Comparisons between dichotomizes variables were performed by χ^2 Test, or Fischer Exact Test, as appropriate. $P<0.05$ was considered statistically significant. Sensitivity, specificity, positive predictive and negative predictive values (PPV and NPV, respectively) were determined by a person-based analysis. Univariate and multivariate logistic regression analysis were performed to identify the independent predictors of response to therapy. Variables were selected with entry and retention set at a significance level of 0.1. Statistical analysis was performed by using SPSS software (Chicago, IL, USA).

Results

Scintimammography and PET/CT performances

In Table I are reported the characteristics of all 51 patients. In all population, a significant uptake of tracer in the primary tumor was identified by scintimammography and PET/CT. Moreover, scintimammographic studies showed a significant uptake of sestamibi in axillary and both in axillary and supraclavicular lymph nodes in 29 patients and 1 patient, respectively (56.8 and 2%), while PET/CT demonstrated the presence of axillary lymphnodes and both axillary and distant lymphnode involvement in 34 and 9 patients, respectively (63.9% and 17.6%). Therefore, PET/CT showed a slightly

TABLE I.—*Characteristics of patient population.*

Characteristics	N. (%)
Median age (range)	49 (27-76)
Clinical stage	
II	30 (58.9)
III	21 (41.1)
Hystological type	
Invasive ductal cancer	43 (84)
Invasive lobular cancer	8 (16)
Ki67	
<14%	3 (6)
>14%	48 (94)
Estrogen receptor	
Negative	24 (47)
Positive	27 (53)
Progesterone receptor	
Negative	32 (63)
Positive	19 (37)
HER2	
Negative	37 (73)
Positive	14 (27)
Triple negative	
No	34 (67)
Yes	17 (33)
Grade	
Gx	4 (8)
G2	11 (22)
G3	30 (59)
Unknown	6 (11)
Luminal categories	
Luminal B	20 (39)
Luminal B/HER2+	8 (16)
HER2+	6 (12)
Triple negative	17 (33)

Gx=indeterminate grade

higher detection rate for axillary lymph node than scintimammography (97% *vs.* 91.7%), in particular for the extra-axillary lymph node stations. At visual assessment, PET/CT recognized suspected liver and skeletal metastases in 2 and 5 patients, respectively. The findings were truly positive only in 2 patients who showed liver and bone metastases at magnetic resonance imaging, later confirmed by histologic and biopsy specimen. Based on the inclusion criteria, the final analysis was performed in 49 patients. According to the biological pattern of BC, only SUVmax and SUVavg resulted significantly different between positive vs. negative HR, no-triple negative vs. triple negative and among luminal subtypes. On the other hand, none of scintimammographic data resulted statistically different among the immunohistochemical features (Tables II-IV). Accordingly to the cut-off values, 41 (83.6%) and 30 (61.2%) patients had a WOT<45% and <56%, therefore the residual 16.4% and 38.8% of subjects had a WOT>45% and >56%, respectively. Median SUVmax and SUVavg were higher in patients with low than high WOT cut-off, although not statistically significant (Figure 1).

Relations among response to neoadjuvant therapy, scintimammography and PET/CT data

Based on the different BC subtypes, the rate of pCR was 10.5% in Luminal B, 25% in Luminal B/HER2 enriched, 83% in HER2 enriched and 18.7% in triple negative cancer (χ^2 Test; P=0.004). In Figure 2 are depicted images relative PET/CT and scintimammographic studies in responder and non-responder patients. On the basis of histopathological response to NST, median WOI resulted significantly lower in responders than no-responders, (30.5% *vs.* 44%; P=0.027). Moreover, MTV, SUVmax and SUVavg were significantly higher in responders than no-responders (all P<0.05). The associations among the cut-off values of responding

TABLE II.—*Correlation among hormone-receptor expression, scintimmagraphic and PET/CT data.*

	No-ER	ER	*P* value	No-PR	PR	*P* value
N.	23	26		31	18	
Early T/B	7.5 (2.1-39.7)	9.5 (2.1-29.6)	0.237	9.8 (2.1-39.7)	7.4 (2.1-29.6)	0.237
Early I/B	1.6 (0.2-4)	1.6 (0.2-4.8)	0.764	1.8 (0.2-4.1)	1.2 (0.2-4.8)	0.254
Late T/B	5.5 (0.7-29.8)	8.2 (1.9-23.6)	0.065	7.1 (0.7-29.8)	7 (1.9-26.3)	0.803
Late I/B	1.3 (0.1-2.7)	40.1 (4.6-74.8)	0.749	1.4 (0.4-3.6)	1.3 (0.2-3.1)	0.481
WOT	43.7 (9.7-73)	42.5 (3.4-79.6)	0.984	44.2 (8.8-74.8)	39.7 (4.6-64.5)	0.419
WOI	36.8 (1.7-56.2)	42.5 (3.4-79.6)	0.262	38.7 (1.7-79.6)	36.4 (17.2-68.4)	0.772
MTV (cm^3)	9.7 (3.1-52.8)	9.9 (0.1-695.4)	0.489	10.9 (0.1-695.4)	7.2 (0.5-34.2)	0.250
SUVmax	13.1 (4.7-36.5)	6.3 (1.7-35.1)	**<0.001**	11.7 (2.6-36.5)	6.4 (1.7-17.1)	**<0.005**
SUVavg	5.2 (3.5-11.6)	3.7 (1.4-9.3)	**<0.001**	5.1 (1.8-11.6)	3.8 (1.4-6.9)	**<0.01**

ER: estrogen receptor; PR: progesterone receptor; T/B: tumor to background ratio; I/B: intense tumor uptake to background ratio; WOT: washout index of the entire tumor; WOI: washout index of the most intense tumor uptake; MTV: metabolic tumor volume; avg: average.

TABLE III.—*Correlation among HER2 expression, triple negative tumor, scintimmagraphic and PET/CT data.*

	No TN	TN	*P* value	Negative HER2	Positive HER2	*P* value
n	33	16		35	14	
Early T/B	9.9 (2.1-39.7)	7.3 (2.1-21.4)	0.050	7.9 (2.1-29.6)	10.6 (2.1-39.7)	0.353
Early I/B	1.5 (0.1-4.8)	1.7 (0.2-4.1)	0.654	1.7 (0.23-4.8)	1.2 (0.2-4.1)	0.877
Late T/B	8.1 (1.9-29.8)	5.5 (0.7-15.5)	0.033	7.1 (0.7-26.3)	7.1 (2.1-29.8)	0.982
Late I/B	1.5 (0.4-3.6)	1.46 (0.1-2.5)	0.932	1.5 (0.4-3.6)	1.2 (0.1-2.7)	0.493
WOT	39.7 (4.6-74.8)	37.8 (9.7-58.4)	0.782	39.7 (4.6-74.8)	41.8 (14.4-73)	0.626
WOI	36.9 (3.4-79.6)	37.8 (1.7-56.2)	0.848	38.7 (1.7-79.6)	34.5 (5.2-68.4)	0.595
MTV (cm^3)	9.7 (0.1-695.4)	9.7 (3.6-42.8)	0.725	10.10 (0.1-695.4)	9.3 (1.1-52.8)	0.715
SUVmax	6.8 (1.7-35.1)	17.4 (5.7-36.5)	**<0.001**	11 (2.6-36.5)	8.1 (1.7-26.6)	0.493
SUVavg	3.9 (1.4-9.3)	5.7 (3.5-11.6)	**<0.001**	4.9 (1.4-11.6)	3.8 (1.5-8.5)	0.472

TN: triple negative; T/B: tumor to background ratio; I/B: intense tumor uptake to background ratio; WOT: washout index of the entire tumor; WOI: washout index of the most intense tumor uptake; MTV: metabolic tumor volume; avg: average.

TABLE IV.—*Correlation among biological features, scintimmagraphic and PET/CT data.*

	Luminal B	Luminal B/HER2	HER2	TN	*P* value
N.	19	8	6	16	
Early T/B	8.7 (4.0-29.5)	9.5 (2.1-24.1)	14.5 (4.2-39.7)	7.3 (2.1-21.4)	0.186
Early I/B	1.7 (0.3-4.8)	1.2 (0.1-4.1)	1.5 (0.5-3.3)	1.7 (0.2-4.1)	0.961
Late T/B	8.28 (1.95-26.3)	6.1 (2.1-16.8)	9.2 (3.1-29.8)	5.5 (0.7-15.5)	0.094
Late I/B	1.6 (0.4-3.6)	1.1 (0.1-2.4)	1.2 (0.1-2.7)	1.4 (0.1-2.5)	0.823
WOT	39.7 (4.6-74.8)	41.8 (17.0-64.5)	39.2 (14.4-73.0)	44 (9.7-58.4)	0.904
WOI	42.7 (3.4-79.6)	37.6 (22.8-68.4)	34.5 (5.2-54.9)	37.8 (1.7-56.2)	0.758
MTV (cm^3)	17.5 (0.1-695.4)	9.6 (1.1-38.1)	7.7 (3.1-52.8)	9.7 (3.6-42.8)	0.874
SUVmax	6.1 (2.6-35.1)	6.7 (1.7-17.1)	7.7 (3.1-52.8)	17.4 (5.7-36.5)	**<0.005**
SUVavg	4.1 (1.4-9.3)	3.6 (1.5-6.95)	9.1 (4.7-26.6)	5.7 (3.5-11.6)	**<0.01**

TN: triple negative; T/B: tumor to background ratio; I/B: intense tumor uptake to background ratio; WOT: washout index of the entire tumor; WOI: washout index of the most intense tumor uptake; MTV: metabolic tumor volume; avg: average.

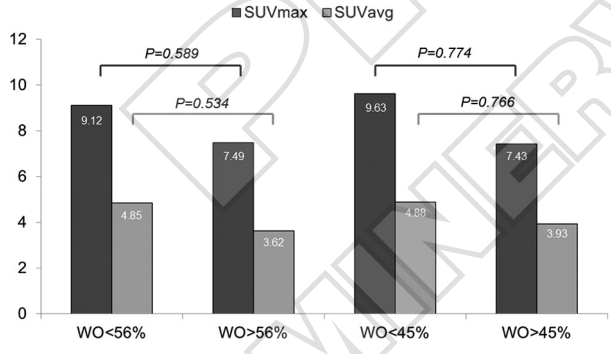

Figure 1.—The correlation among WOT cut-offs, SUVmax and SUVaverage.

and non-responding patients with WOT of 45%-56% and SUVs are reported in Figure 3. As shown, in non-responding patients, high WOTs were associated with low SUVs. Conversely, in responder group, high SUVs were reported, particularly for high WOT values. In accordance with the different subcategories, median SUVmax resulted significantly higher in patients with a pCR as compared to those without response to NST, both in Luminal B and triple negative subsets (25.7 *vs.* 5.9 and 29.4 *vs.* 13.2, respectively, both P<0.05). In HER2 positive tumor, median WOT, WOI, SUVmax and SUVavg were higher in no-responder group although no statistically different than the responder one (Table V). Finally, in non-responding patients both Luminal B and Luminal B/HER2 enriched tumors showed high WOTs and low SUVs, although this difference was not reported for triple negative cancer.

Univariate and multivariate analysis

In all study population, at univariate analysis, the response to NST was significantly associated with WOI (Odds Ratio 1.052, 95%CI 1.006-1.100; P<0.05) SUVmax (Odds Ratio 0.872, 95%CI 0.792-0.961, P<0.005) and SUVavg (Odds Ratio 0.508, 95%CI 0.323-0.800;

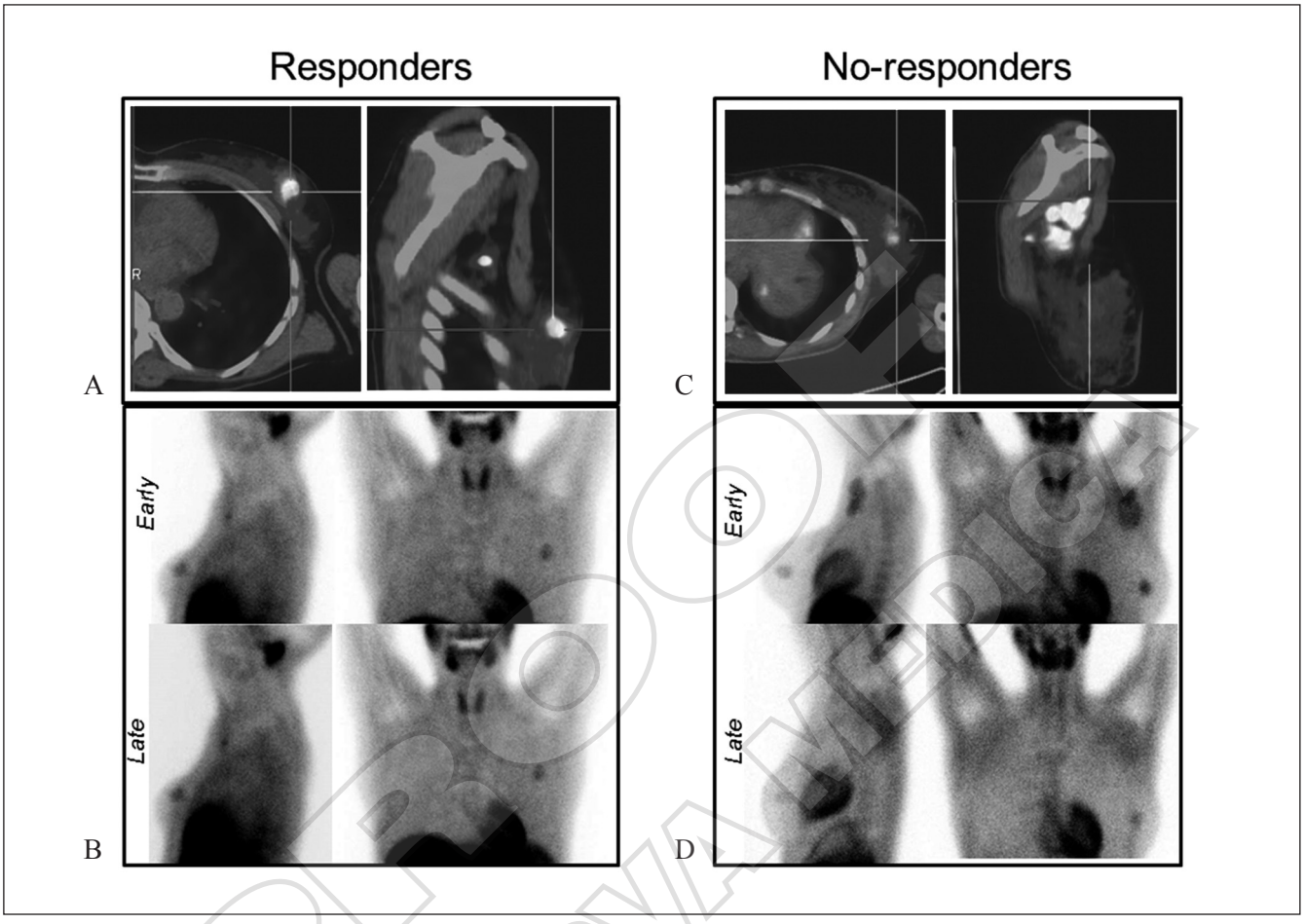

Figure 2.—Images relative PET/CT and scintimammographic studies in responder (A, B) and non-responder (C, D) patients.

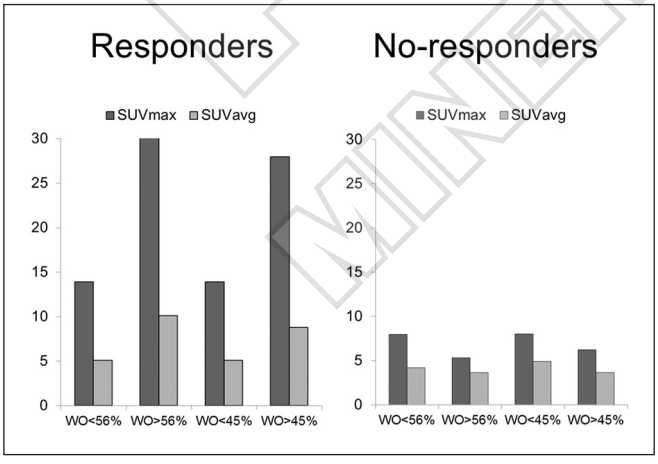

Figure 3.—The comparison between responder and no-responder group based on WOT cut-offs, SUVmax and SUVaverage.

P<0.005), but at multivariable analysis only WOI (Odds Ratio 1.070, 95%CI 1.007-1.137, P<0.05) remained an independent predictor of response to neoadjuvant therapy.

Discussion

In the present study, we prospectively evaluated 49 patients with LABC who underwent both scintimammography with sestamibi and FDG PET/CT. Visual and semiquantitative analyses were used for the interpretation of data. Our results confirmed primarily that both FDG PET/CT and scintimammography with sestamibi detected primary breast cancer at a sensitivity of 100%, while PET/CT reported a higher detection rate for axillary lymph nodes as compared to scintimammography.

TABLE V.—*Correlations among response to therapy, luminal subcategories, scintimammographic and PET/CT data.*

	Luminal B			Luminal B/HER2			HER2			TN		
	Complete	No com	*P* value	Complete	No com	*P* value	Complete	No com	*P* value	Complete	No com	*P* value
n	2	17		2	6		5	1		3	13	
Early T/B	14.7	8.4	0.232	10.8	9.5	1	11.2	17.8	0.770	7.5	7.1	0.737
Early I/B	2.7	1.5	0.232	2.5	1.2	0.857	1.17	2.0	0.770	1.9	1.6	0.946
Late T/B	13.6	8.1	0.232	8.7	6.1	1	7.75	17.8	0.380	4.8	5.5	0.840
Late I/B	2.73	1.5	0.084	1.7	0.9	0.317	1.24	2.1	0.380	2.5	1.4	0.313
WOT%	21.1	39.7	0.144	28.6	49.7	0.182	32.9	56.9	0.380	47.8	43.7	0.638
WOI%	15.4	46.8	0.111	32.6	41.2	0.505	33.5	54.9	0.143	37	38.7	0.946
MTV	60.9	6.8	<0.05	23.4	8.8	0.317	9.7	4.8	0.380	22.6	7.0	0.069
SUVmax	25.7	5.9	<0.05	11.2	6.7	0.739	9.1	4.7	0.143	29.4	13.2	<0.05
SUVavg	7.1	3.2	0.084	5.1	3.6	0.739	4.9	3.6	0.143	9.0	5.6	<0.05

In lymph node assessment, the sensitivity values were very similar (97% *vs.* 91.7%), especially considering that PET images are tomographic while MIBI images are planar. Anyway, PET/CT was more accurate in the detection of extra-axillary lymph nodes. At semiquantitative analyses emerged that a significant difference in SUVmax and SUVavg were reported among luminal categories (P=0.003 and P=0.006, respectively). In particular SUVmax resulted higher in triple negative and HER2 enriched breast tumors than Luminal B enriched or not by HER2 expression. Conversely, none of scintimammographic findings were different across luminal subsets, although the lowest values were detected in triple negative group. Recently, Yoon *et al.*,[25] have correlated SUVmax and SUVavg with immunostichemical markers, reporting that FDG uptake is significantly higher in ER/PR negative, HER2 negative subgroup compared with non-ER/PRnegative, HER2 negative (ER/PR positive, HER2positive). These latter findings are in line with our data.

Scintimammography, PET/CT findings and prediction of response to therapy

De novo or intrinsic chemoresistance that is the major limitation of NST, refers to cells that are resistant to chemotherapeutic drugs from the very beginning of anticancer drug treatment. This type of chemoresistance originates from cells which have already had capacities of drug-resistance such as limiting drugs uptake, enhancing efflux or activating detoxification of drugs.[26] Approximately 70% of patients demonstrate clinical response after NST, but only 20-30% achieve pCR.[27] In the present study, we tested whether some data by scintimammography or FDG PET performing before to start NST significantly differentiate responder from no responder patients. As extensively stated in literature, a low sestamibi fixation is predictive of high efflux protein expression and therefore of a poor response to therapy.[22, 23, 28, 29] In accordance with this latter concept, in the present report, patients with a pathological partial response showed a higher WOI and WOT as compared to patients with a pCR. Moreover, our results were discordant from those showed by Travaini *et al.*[21] who affirmed that scintimammography cannot be able to give therapeutic information. In our opinion, some limitations are present in this latter report, firstly the scheme of treatments are different (hormone therapy and chemotherapy or their combination), secondly the authors considered a small series of patients for each treatment categories.

In the available literature, different washout rate cut-offs have been considered for predicting the response to NST. Firstly, Zaman *et al.*[30] found that a WO<30% was associated with a good response, considering ultrasound performing after 3 cycles of chemotherapy as the gold standard. Conversely, some Italian authors,[22-24] reported that the rate of washout predictive of responsiveness to therapy was <45%[22, 23] or <56%.[24] Moreover, in accordance with Ciarmiello *et al.*,[31] a 99mTc-sestamibi clearance <204 minutes was a predictive response factor. In the majority of papers, the washout index of the entire tumor was considered, whereas in the present analysis we evaluated both WOT and WOI. Using a cut-off values of 45% and 56% for WOT, we found a low-mild sensitivity and an intermediate-high specificity of 40.5%, 66.6%, and 16.2%, 91.6% respectively, for the prediction of chemosensitivity. However, considering

the tumor immunohistopathological pattern and Ki67 expression, our data demonstrated that the values of both WOT and WOI were unable to predict the chemosensitivity of luminal categories, although they resulted higher in non-responder subset than responder one.

Based on the present data, basal FDG PET/CT seems able to distinguish patients who will have a positive response to NST from those who will not, on the basis of semiquantitative evaluation. Particularly, high SUVmax and SUVavg resulted as significant predictors of pCR in triple negative and Luminal B cancer, while they failed in HER2 positive tumor. Otherwise a recent report by Groheux *et al.*[32] demonstrated that SUVmax at baseline PET/CT was similar between responder and no-responder groups (5.9±3.3 and 6.6±3.5, respectively). Similarly Koolen *et al.*[33] did not find any association between pathological response and SUVmax at baseline (P=0.14 for (near)pCR, P=0.09 for pCR), but the authors showed a significant association between the change in SUVmax and pathological response (P<0.0001 for (near)pCR, P<0.0001 for pCR).

Multidrug resistance and FDG PET/CT data

99mTc-sestamibi has shown to be a general probe for functional imaging of Pgp and MDR-associated glycoprotein. Anyway, the accumulation of sestamibi in tumor tissue depends on various factors [34] including mitochondrial and plasma membrane potentials, intracellular mitochondrial densities and the expression of Pgp. FDG is taken up through glucose transporters followed by phosphorylation.[35] Therefore, a tumor that expresses a high level of Pgp could not accumulate sestamibi [28] and a tumor that expresses a low level of glucose transporter could not take up FDG.[36] *In vitro* studies [17, 37] demonstrated that the accumulation of 2-deoxy-D-glucose was reduced in MDR cell lines. Lorke *et al.*[16] reported that FDG uptake of Pgp positive tumors was reduced when compared with that of Pgp negative tumors in animal studies, and their findings suggested that FDG might be an *in vivo* marker for MDR. A recent preclinical experimental study involving small animals with implanted BC, showed that FDG may be a Pgp substrate, being elevated the glucose request in Pgp positive cells.[38] As abovementioned, the development of MDR is accompanied by enhanced drug efflux [39] and by drug detoxification. Both the mechanisms are energy dependent, primarily glycolysis-dependent.[40, 41] Therefore, the MDR cells have developed an enhanced rate of glycolysis compared to the drug sensitive-line.[42] Several reports have shown that FDG uptake was inversely correlated with the expression of Pgp in lung cancer, hepatocellular carcinoma and intrahepatic cholangiocarcinoma.[43-45] In the present study we found that patients not responding to chemotherapy had a high washout rate and a low FDG-uptake as compared to those with a pCR. These data are consistent with the results from animal model and in vitro studies, and suggest that FDG PET data can be used as a marker for Pgp expression in vivo in breast tumors. We found that in non-responding patients, high WOTs were associated with low SU*Vs*. Conversely, in responder group, high SUVs were reported, particularly for high WOT values. As shown in Figure 3, ah hypothetical cu-off of 10 for SUVmax and of 5 for SUVavg could be extrapolated to distinguish responders from non-responders to NST.

Some data of correlation between the level of SUV and clinicopathological parameters were published with regard to FDG PET.[46-49] As emerged from the study by Ueda *et al.*,[49] the parameters for proliferative activities and aggressiveness of cancer cells were strongly correlated with high levels of SUV in cancer cells. Therefore, high SUVs are correlated with a more aggressive phenotype. From the present report, we suppose that SUVs are correlated not only with aggressiveness but also with Pgp expression. Our results demonstrated that no-responder patients with a triple negative cancer showed similar SUVs, independently from sestamibi washout cut-off value. Therefore, the expression of Pgp or MDR-proteins appears independent from the aggressiveness of cancer cells, in this setting of subjects. Moreover, a low FDG uptake is not necessarily associated with low aggressiveness, but it can be due to a high Pgp expression. These latter result could be used to explain why the majority of positive HR/negative HER2 tumor are generally resistant to neoadjuvant chemotherapy.

Limitations of the study

It should be noted that the present study has limitations. Firstly, a small series of patients was considered, but they were prospectively evaluated. Secondly, we did not evaluate the Pgp expression by immunohistochemical analysis of tumor tissue, but a significant correlation

between the efflux rates of 99mTc-sestamibi and Pgp concentrations in surgical sampling from the same patients was found by Litman *et al.*[50] Both Litman *et al.*[50] and Luker *et al.*[51] reported that functional imaging with 99mTc-sestamibi may potentially provide clinically important information about Pgp status of tumor.

Conclusions

In conclusions, scintimammography with sestamibi did not accurately determine the responsiveness to therapy on the basis of histopathological findings and therefore its utility is limited in clinical practice, although its shows a good performance for the identification of primary lesion. Conversely, FDG PET/CT can provide some information about the extension of disease particularly in triple negative and HER2 enriched LABC, the aggressiveness of cancer and the responsiveness to neoadjuvant chemotherapy. This latter point might be improved by including semiquantitative data that seems indirectly linked with the expression of Pgp.

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