Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

Prognostic Value of ^{99m}Tc-Sestamibi Washout in Predicting Response of Locally Advanced Breast Cancer to Neoadjuvant Chemotherapy

Rosa Sciuto, MD[1]; Rosella Pasqualoni, MD[1]; Serenella Bergomi, MD[1]; Germana Petrilli, MD[1]; Patrizia Vici, MD[2]; Franca Belli, MD[2]; Claudio Botti, MD[3]; Marcella Mottolese, PhD[4]; and Carlo L. Maini, MD[1]

[1]Department of Nuclear Medicine, Regina Elena Cancer Institute, Rome, Italy; [2]Second Department of Medical Oncology, Regina Elena Cancer Institute, Rome, Italy; [3]First Department of Surgical Oncology, Regina Elena Cancer Institute, Rome, Italy; and [4]Department of Pathology, Regina Elena Cancer Institute, Rome, Italy

This study evaluated the role of ^{99m}Tc-sestamibi washout in the prediction of pathologic tumor response to neoadjuvant chemotherapy in 30 patients with locally advanced breast cancer. **Methods:** Two ^{99m}Tc-sestamibi studies were performed before and after chemotherapy for each patient. Early (10 min) and delayed (240 min) planar breast views were acquired after a 740-MBq ^{99m}Tc-sestamibi intravenous injection, and the washout rate (WOR) was computed. All patients underwent radical mastectomy with pathologic evaluation of the residual tumor size. **Results:** The pretherapy ^{99m}Tc-sestamibi WOR ranged from 14% to 92% (mean ± SD, 50% ± 18%). At pathologic examination, 15 patients showed no tumor response to chemotherapy and 15 patients showed an objective response to chemotherapy. The pretherapy ^{99m}Tc-sestamibi study predicted chemoresistance (WOR > 45%) in 18 of 30 patients and no chemoresistance (WOR ≤ 45%) in 12 of 30 patients. When the WOR cutoff was set at >45%, the prognostic performance of the test was indicated by a sensitivity of 100%; a specificity of 80%; positive and negative predictive values of 83% and 100%, respectively; and a likelihood ratio of 5. The repeatability of the test was good, with 80%–93% interreader agreement (κ = 0.57–0.85). Posttherapy ^{99m}Tc-sestamibi studies confirmed the pretherapy study prediction in 29 of 30 patients. **Conclusion:** ^{99m}Tc-Sestamibi WOR is a reliable test for predicting tumor response to neoadjuvant chemotherapy. In fact, negative findings (WOR ≤ 45%) rule out chemoresistance and positive findings (WOR > 45%) indicate a high risk of chemoresistance.

Key Words: ^{99m}Tc-sestamibi; multidrug resistance; neoadjuvant chemotherapy; breast cancer

J Nucl Med 2002; 43:745–751

Biochemical resistance to chemotherapy is the major cause of treatment failure in patients with breast cancer. In fact, an intrinsic chemoresistance is present in 18%–51% of untreated cancer, whereas resistance is acquired later during the treatment in up to 75% of patients (*1–3*).

Chemoresistance is a multifactorial phenomenon including several nonspecific and specific mechanisms (*4–7*). The term *multidrug resistance* (MDR) is commonly used to indicate an overexpression of transmembrane glycoproteins, the P-glycoprotein (Pgp), and the MDR-associated glycoprotein (*1,8*). These proteins allow outward transport of the most important antineoplastic chemotherapeutic drugs, such as anthracyclines, and are responsible for the clinical manifestation of the MDR phenotype in breast cancer (*7–12*). Knowledge of the MDR pattern before and during the treatment can improve therapy planning, including selection of drug-resistant patients for chemorevertant drugs.

The available in vitro techniques to measure MDR gene expression in tumor samples are clinically ineffective to achieve this aim (*13–15*) because they do not necessarily provide information about the dynamic function of drug efflux pumps (*16*). ^{99m}Tc-Sestamibi [hexakis (2-methoxyisobutylisonitrile) technetium (I)], a radiopharmaceutical widely used as a tumor-seeking agent for diagnostic imaging (*17,18*), has been recently used as a general probe for functional imaging of these 2 MDR pumps (*19–21*). Several clinical studies have therefore been designed to study Pgp functionality with ^{99m}Tc-sestamibi in cancer patients (*22–24*), and a correlation between the efflux rate of ^{99m}Tc-sestamibi and quantitative Pgp expression in breast carcinoma patients was clearly evident in a pilot study (*25*).

This study evaluated the prognostic value of ^{99m}Tc-sestamibi washout for predicting the outcome of neoadjuvant chemotherapy in locally advanced breast cancer using pathologic tumor response as the gold standard.

MATERIALS AND METHODS

Study Design

This phase II clinical trial was a prospective cohort study on a sample of 30 patients with untreated locally advanced breast cancer. The patients were evaluated before neoadjuvant chemo-

Received Aug. 14, 2001; revision accepted Feb. 4, 2002.

For correspondence or reprints contact: Rosa Sciuto, MD, via della Giuliana 83/a, 00195, Rome, Italy.

E-mail: sciuto@ifo.it

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

therapy and were followed until surgery to verify the chemotherapy outcome.

The primary endpoint of the study was determination of the prognostic value of ^{99m}Tc-sestamibi scintigraphy in predicting tumor response to neoadjuvant chemotherapy. The predictor variable of chemoresistance was considered ^{99m}Tc-sestamibi washout in the pretherapy study (prognostic test). The outcome variable was considered the residual tumor size evaluated on pathologic samples.

The tests and the outcome results were read in a standardized and masked fashion. The local Ethics Committee approved the study, and all patients gave written informed consent before entering the study.

Patients

Thirty patients (age range, 31–70 y) with locally advanced breast cancer entered the study on the basis of the following eligibility criteria: clinical stage, according to the system of the Union Internationale Contre le Cancer, of T2 $\geq$ 3 cm N0–N1 M0, T1–T3 N2 M0, or T4 N0–N2 M0 and adequate hematologic function (baseline total white blood cell count $\geq 4 \times 10^9$/L, neutrophil count $\geq 2 \times 10^9$/L, and total platelet count $\geq 100 \times 10^9$/L). The following exclusion criteria were also considered: a performance status $\geq$ 1 according to the criteria of the World Health Organization; previous hormonal treatment, chemotherapy, or radiotherapy; evidence of systemic metastases or a second active malignant tumor; and severe heart disease or insulin-dependent diabetes.

Protocol

Before starting chemotherapy, all patients underwent a baseline evaluation that included a clinical examination, bilateral mammography, fine-needle aspiration cytology or histologic biopsy of the lesion, bone scintigraphy, standard chest radiography, liver sonography, and a basal ^{99m}Tc-sestamibi study. The clinical tumor size was determined by measuring the 2 largest perpendicular diameters evidenced on clinical and mammographic examinations.

Two chemotherapy regimens were used. Schedule A, administered to 7 patients, included 3 cycles of chemotherapy (cyclophosphamide, 400 mg/m^2; epirubicin, 50 mg/m^2; and fluorouracil, 500 mg/m^2, on days 1 and 8) every 3 wk. Schedule B, administered to 23 patients, included 4 cycles of high-dose epirubicin (80 mg/m^2 plus docetaxel, 75–80 mg/m^2, on day 1) every 3 wk. Granulocyte colony-stimulating growth factor as support therapy was administered when required.

At the end of chemotherapy, the patients were reevaluated clinically, mammographically, and with ^{99m}Tc-sestamibi. All patients underwent radical mastectomy and axillary lymphadenectomy, with pathologic examination of the removed tumor and nodes.

^{99m}Tc-Sestamibi Scintigraphy

Two ^{99m}Tc-sestamibi scintigraphic studies, with different aims, were performed for each patient before and after chemotherapy. The study performed before chemotherapy investigated the risk of intrinsic chemoresistance and was aimed at predicting tumor response to chemotherapy (prognostic test). The study performed after the end of chemotherapy and immediately before surgery investigated the effect of the just-completed chemotherapy. This latter study was aimed at assessing the actual tumoral mass (diagnostic test) and at confirming the pretherapy study prediction. Both studies were performed with the following criteria.

Image Acquisition. ^{99m}Tc-sestamibi (Cardiolite; DuPont Pharmaceuticals Co., Billerica, MA), 740 MBq, was injected intravenously in the arm contralateral to the lesion. Early (10 min after the injection) and delayed (240 min after the injection) acquisitions were obtained with the patient carefully placed in the same position. In both acquisitions, digital planar images of the breast region (256 $\times$ 256 matrix, 10-min preset time) were acquired with the patient prone (lateral views) and supine (anterior views). A large-field-of-view gamma camera (PRISM 2000 XP; Picker Medical Systems, Cleveland, OH) equipped with a high-resolution low-energy parallel-hole collimator and a dedicated foam rubber mattress was used.

Image Reading and Processing. Tumoral and background regions of interest were drawn on the lateral views of the early images and then translated to the delayed images to compute mean ^{99m}Tc-sestamibi uptake counts. The uptake index was calculated both for early and for delayed images by the ratio of tumor to background mean count, with decay correction for delayed images. The washout rate (WOR) was computed by the ratio of delayed to early uptake index, with decay and background correction as follows: $\mathrm{WOR} = [(\mathrm{T} - \mathrm{B})_{10\ \mathrm{min}} - (\mathrm{T} - \mathrm{B})_{240\ \mathrm{min}}]/(\mathrm{T} - \mathrm{B})_{10\ \mathrm{min}}$, where T is the tumoral region of interest and B is the background region of interest. Film reading and processing were performed in clinically masked fashion. Intra- and interobserver agreements for WOR estimation were evaluated.

Pathologic Studies

Mastectomy specimens from each patient were evaluated. The pathologic residual tumor size was estimated as the residual tumor burden and quantitated from the 2 largest perpendicular diameters, measured in centimeters. The number of metastatic nodes was also evaluated for each patient.

Hormone receptor status (estrogen and progesterone receptors) was determined immunohistochemically on frozen sections using commercially available kits. The cutoff value was nuclear staining of 10% of neoplastic cells.

Data Evaluation

Prognostic Test Results. Prediction of tumor response to neoadjuvant chemotherapy was based on the results of the pretherapy ^{99m}Tc-sestamibi WOR. Accordingly, the test was defined as a positive prognostic test when it predicted high expression of intrinsic chemoresistance and, consequently, a high risk of no tumor response to the therapy. The test was conversely defined as a negative prognostic test when it predicted low expression of intrinsic chemoresistance and, consequently, a low risk of no tumor response to the therapy. The criteria for defining a test as positive or negative were based on the WOR cutoff point identified by the likelihood ratio method.

Outcome Measures. The gold standard of the study was the objective response of the primitive tumor to neoadjuvant chemotherapy, as evaluated on pathologic specimens obtained at surgery. An objectively determined reduction in the dimensions of the tumor (i.e., reduction of the product of the 2 largest perpendicular diameters of the pathologic residual tumor size vs. the baseline clinical tumor size) was considered the criterion for pathologic response. The pathologic outcome was classified as no response to chemotherapy if the residual tumor size was reduced $<$75% or was increased. The pathologic outcome was conversely classified as a positive response to chemotherapy if the residual tumor size was reduced $>$75%. Accordingly, patients presenting with no response to chemotherapy were considered to be nonresponders, whereas

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

patients presenting with a positive response to chemotherapy were considered to be responders.

Other Results. Tumor response to chemotherapy was also evaluated by determining clinical response and scintigraphic response as additional outcome measures. Clinical response, evaluated through clinical examinations and mammography, was classified as positive or negative according to whether tumor reduction was appreciable or not. Scintigraphic response was evaluated by comparing early ^{99m}Tc-sestamibi uptake in the posttherapy study (diagnostic study) versus the pretherapy study (prognostic study). Scintigraphic response was classified as positive if tracer uptake was markedly reduced and negative if tracer uptake was not reduced or only faintly reduced.

Statistical Analysis

The results of ^{99m}Tc-sestamibi prognostic tests were expressed in terms of sensitivity, specificity, positive and negative predictive values, and likelihood ratio; 95% confidence intervals were also evaluated for sensitivity and specificity. A 2-tailed *t* test or Fisher exact test was used, as appropriate, to evaluate differences between clinical, scintigraphic, and pathologic response; differences between early and delayed uptake in pretherapy studies; and differences in baseline variables between groups of responders and nonresponders. Correlations between outcome variables and baseline tumor size, TNM stage, histologic type, pretherapy early uptake index, and WOR were evaluated by the Spearman ρ test. The level of probability significance was set at an α value of 0.05. The repeatability of the test was assessed by evaluating intrareader and interreader agreement among the institutional reader (a dedicated senior resident) and 2 other masked readers using κ statistics. A κ value > 0.75 was considered an index of strong agreement beyond chance, whereas a κ value of 0.40–0.75 was considered an index of intermediate to good agreement (*26*).

RESULTS

Table 1 lists the clinical and pathologic characteristics, including pathologic response, of each patient.

Prognostic Test Results

Early images (10 min) showed marked uptake of ^{99m}Tc-sestamibi in all tumors. The uptake index ranged from 0.48 to 4.85, with a mean of 2.05 $\pm$ 1.16. Only 16 of 23 node metastases showed ^{99m}Tc-sestamibi uptake. In delayed acquisitions (240 min), the ^{99m}Tc-sestamibi uptake index ranged from 0.33 to 4.85, with a significantly reduced mean value (1.64 $\pm$ 1.08, $P = 0.003$).

WOR ranged from 14% to 92% (mean, 50% $\pm$ 18%). Table 2 lists the scintigraphic results for each patient. The WOR cutoff was set at >45% by likelihood ratio analysis for the best test results. Accordingly, the test was defined as positive for 18 of 30 patients, who had a WOR $>$ 45%, and negative for 12 of 30 patients, who had a WOR $\leq$ 45%. The intrareader coefficient of variation for WOR was <2%, and interreader agreement was high, ranging from 80% to 93% ($\kappa = 0.57–0.85$). The sensitivity, specificity, and predictive values of WOR are reported in Table 3.

Outcome Results

After surgery, 15 patients had a substantially unmodified residual tumor (i.e., no response to chemotherapy) and 15 patients had a positive response to chemotherapy. The pathologic response for each patient is reported in Table 1. No significant difference in baseline characteristics (age, clinical tumor and node stage, and tumor size) between the 2 groups (responders and nonresponders) was observed. Outcome did not correlate with baseline tumor size, histologic type, schedule of treatment, or early ^{99m}Tc-sestamibi uptake index.

Other Results

Clinical response was positive in 15 patients and negative in 15, correctly identifying only 25 of 30 pathologic outcomes (accuracy, 83%). Scintigraphic response was negative in 16 patients and positive in 14, correctly identifying 29 of 30 pathologic outcomes (accuracy, 97%).

Scintigraphic response as evaluated on posttherapy ^{99m}Tc-sestamibi studies also confirmed the prediction of the pretherapy study for 29 of 30 patients (concordance of prognostic and diagnostic results, 97%; Figs. 1 and 2). In the only discordant case (patient 11), the pretherapy scintigraphic test wrongly predicted high MDR expression, whereas the posttherapy test correctly predicted a positive response to chemotherapy.

No tumor characteristic (baseline tumor size, histologic type, or estrogen or progesterone receptor status) or other baseline variables (schedule of treatment or early ^{99m}Tc-sestamibi uptake index) correlated with the pathologic response to chemotherapy.

DISCUSSION

Intrinsic or acquired chemoresistance is the major cause of chemotherapy failure in many tumors, including breast cancer (*1–4*). MDR modulators may be used to increase the efficacy of chemotherapy when chemoresistance is identified (*27*). Unfortunately, immunohistochemical measurements of MDR glycoprotein on tumor samples are not effective for this purpose (*13–16,28*). Therefore, noninvasive imaging with ^{99m}Tc-labeled complexes revealing the function of cellular efflux pumps is a topic that raises much interest (*17,19,21*).

^{99m}Tc-sestamibi distribution and kinetics into tumoral cells is determined by mechanisms with different biologic significance. In fact, early ^{99m}Tc-sestamibi uptake is caused by negative potentials across the plasma membrane and the inner mitochondrial matrix and is an expression of tumor viability (*17,29,30*). Conversely, efflux is regulated by MDR glycoproteins and may be considered a marker of chemoresistance (*19–21,31,32*).

Preliminary clinical investigations on patients with breast cancer yielded promising results. A positive correlation was found between the efflux rate of ^{99m}Tc-sestamibi and Pgp expression (*22*) or lack of response to chemotherapy (*25,33*). Retention of ^{99m}Tc-sestamibi appeared to also correlate with chemosensitivity to anthracyclines (*34*).

Unfortunately, the available results are not comparable because of wide differences in study design, tumor histo-

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

TABLE 1
Patient Characteristics Before and After Chemotherapy

Patient no.	Age (y)	Chemotherapy schedule	cTN	Clinical tumor size (cm)	Histologic type	pTN	Pathologic tumor size (cm)	Pathologic node status	Pathologic response
1	45	A	T4b N1	7.5 × 9	Ductal	T2 N1	3 × 2	8	PR
2	52	A	T4d N1	7 × 7.5	Lobular	T4d N1	7 × 6	5	NR
3	33	A	T3 N1	5 × 4.5	Ductal	T2 N1	2 × 2	5	PR
4	32	A	T4b N1	7 × 8	Lobular	T3 N1	5.5 × 2.5	15	PR
5	44	A	T3 N1	4.5 × 5.5	Lobular	T2 N0	3 × 2	0	PR
6	58	A	T4d N2	9 × 7	Lobular	T3 N1	7 × 6	15	NR
7	59	A	T2 N1	3 × 3	Ductal	T1 N1	2 × 2	4	NR
8	47	B	T2 N0	NE	Lobular	T2 N0	4.5 × 4	0	NR
9	47	B	T4d N0	NE	Ductal	T1 N0	2 × 1.5	0	PR
10	62	B	T4b N0	10.5 × 8	Lobular	T2 N1	3.2 × 4.5	5	PR
11	45	B	T3 N1	8 × 7.5	Ductal	T2 N1	2.5 × 3	19	PR
12	37	B	T3 N1	6.5 × 5.5	Ductal	T2 N1	5 × 4	6	NR
13	50	B	T4d N0	NE	Lobular	T4d N0	5.5 × 6	0	NR
14	69	B	T4b N1	17 × 15	Ductal	T4b N1	13 × 12	21	NR
15	31	B	T4d N2	5 × 3.5	Ductal	T4d N2	5 × 3	42	NR
16	73	B	T3 N2	7 × 5	Ductal	T1 N2	2 × 1.5	22	PR
17	65	B	T4b N2	10 × 5.5	Ductal	T1 N1	2 × 1.8	3	PR
18	48	B	T4b N0	10 × 8	Ductal	T2 N1	3 × 4	3	PR
19	47	B	T3 N1	5.5 × 3	Ductal	Tis N1	—	5	PR
20	47	B	T4c N0	9 × 7	Lobular	T4 N0	8 × 7	0	NR
21	40	B	T4d N0	NE	Ductal	T4 N1	9 × 5	12	NR
22	60	B	T2 N1	3 × 2	Ductal	T1 N1	1 × 1	2	PR
23	64	B	T3 N0	6 × 2	Ductal	T3 N1	6 × 2	5	NR
24	47	B	T2 N0	4 × 4	Ductal	T1 N1	2 × 2	6	NR
25	41	B	T3 N1	6.5 × 4	Ductal	T2 N1	3 × 2.5	3	NR
26	49	B	T4d N1	NE	Ductal	T0 N1	—	13	PR
27	64	B	T3 N0	5 × 6	Ductal	T2 N0	3 × 2	0	PR
28	60	B	T2 N1	4 × 4.5	Ductal	T2 N1	4 × 3.5	4	NR
29	50	B	T4 N2	10 × 10	Ductal	T3 N1	3.5 × 3	1	PR
30	48	B	T4d N0	NE	Ductal	T4 N1	7 × 3	11	NR

cTN = clinical tumor and node stage; pTN = pathologic tumor and node stage; A = 3 cycles of cyclophosphamide, epirubicin, and fluorouracil; PR = residual tumor size reduced > 75%; NR = residual tumor size reduced <75% or increased; B = 4 cycles of high-dose epirubicin; NE = diameters of lesion not evaluable because of diffuse infiltration of breast; Tis = carcinoma in situ.

Clinical tumor size was found on clinical examination by determining the 2 largest perpendicular dimensions, in centimeters. Pathologic tumor size was determined by measuring residual tumor burden (the 2 largest perpendicular dimensions, in centimeters). Pathologic node status is number of metastatic nodes at pathologic examination. Pathologic response was evaluated by measuring residual tumor size (product of the 2 diameters of pathologic tumor size) vs. baseline tumor size (product of the 2 diameters of clinical tumor size).

logic type, method of ^{99m}Tc-sestamibi scintigraphy acquisition and processing, and gold standard (*24*,*35*–*37*). The reliability of ^{99m}Tc-sestamibi efflux in predicting MDR remains to be validated, and a method for clinical routine use has not been standardized yet.

A well-designed study on this topic, with a reasonable gold standard (i.e., pathologic evidence of tumor response), was previously reported (*33*). The authors evaluated ^{99m}Tc-sestamibi efflux kinetics by clearance analysis in 39 patients with locally advanced breast cancer and concluded that this functional approach may identify patients at high risk of treatment failure. The method was too complex and time consuming for routine use, requiring both dynamic acquisition and 4 following planar images. In addition, the high rate of false-negative findings (35%) seriously limited the diagnostic performance of the test, considering the high prevalence of disease (59% of patients were nonresponders).

In this study, we validated a simple, reproducible, and reliable method for ^{99m}Tc-sestamibi WOR analysis. The method, requiring only 2 sequential planar images and a quantitative measure of an index (WOR) by simple regions-of-interest design, was more practical for routine examinations. Determination of the WOR cutoff value (>45%) by likelihood ratio analysis for best test results was also a simple method and closely fulfilled the need for a clinically useful criterion.

This method also performed well in predicting chemoresistance (Table 3). In fact, we observed a sensitivity significantly higher than that previously reported by Ciarniello et al. (*33*) (100% vs. 65%, $P < 0.001$) and a similar specificity (80% vs. 87%, $P = 0.6$). The difference in test

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

TABLE 2
Scintigraphic Results of Pretherapy ^{99m}Tc-Sestamibi Study

Patient no.	Uptake index, early	Uptake index, delayed	WOR (%)	Test result
1	0.92	1.03	17	Negative
2	1.41	0.86	69	Positive
3	2.17	1.83	43	Negative
4	0.63	1.09	21	Negative
5	1.06	0.71	54	Positive
6	1.62	0.64	48	Positive
7	1.88	1.46	64	Positive
8	2.26	0.80	77	Positive
9	2.82	2.41	41	Negative
10	1.70	2.54	14	Negative
11	1.09	0.50	81	Positive
12	1.41	0.33	92	Positive
13	1.43	1.09	51	Positive
14	1.73	0.95	78	Positive
15	1.37	1.11	49	Positive
16	1.04	1.40	29	Negative
17	2.16	1.46	42	Negative
18	0.48	0.55	43	Negative
19	3.63	2.18	45	Negative
20	2.27	1.19	70	Positive
21	1.27	1.10	54	Positive
22	4.85	4.85	44	Negative
23	1.74	1.02	64	Positive
24	1.77	2.02	47	Positive
25	4.85	3.71	46	Positive
26	1.30	1.00	56	Positive
27	2.38	2.42	41	Negative
28	3.81	2.64	46	Positive
29	2.07	3.51	30	Negative
30	4.49	3.02	51	Positive

Early uptake index = ^{99m}Tc-sestamibi uptake index in acquisition at 10 min; delayed uptake index = ^{99m}Tc-sestamibi uptake index in acquisition at 240 min; positive test result = WOR > 45%; negative test result = WOR ≤ 45%.

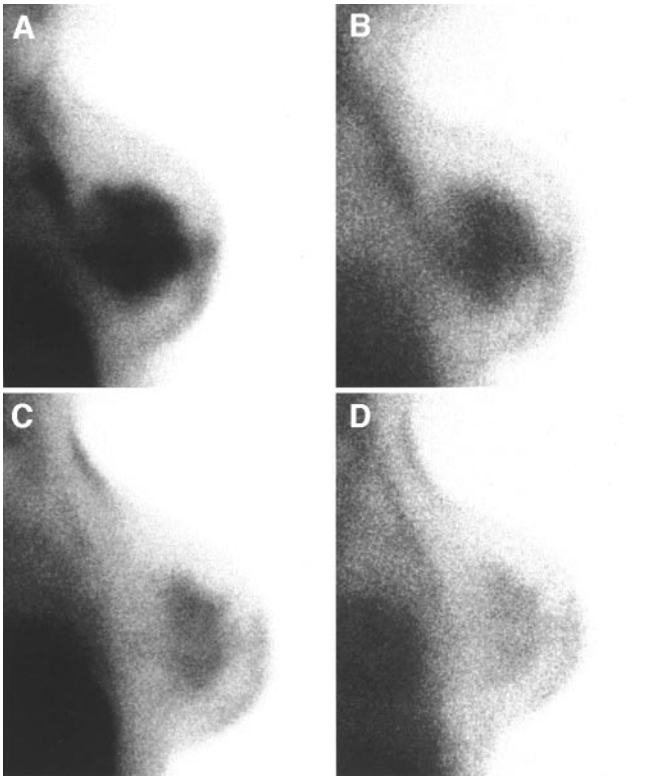


FIGURE 1. Pretherapy (A and B) and posttherapy (C and D) ^{99m}Tc-sestamibi studies (lateral views of right breast in prone position) of patient 30, who showed no response to chemotherapy at pathologic examination. Pretherapy study (A, early image; B, delayed image) evidenced high ^{99m}Tc-sestamibi WOR (51%), predicting high MDR expression. Early posttherapy image (C) confirmed negative response to chemotherapy, because tracer uptake persisted in tumoral region. Corresponding delayed image (D) showed evident decrease in tracer uptake, confirming high WOR and high MDR expression.

sensitivity was likely caused by the different criteria used to set the cutoff value. In fact, Ciarniello et al. established their cutoff value by correlating ^{99m}Tc-sestamibi clearance (i.e., half-life) with in vitro Pgp expression. On the contrary, in our study the best test result was identified on the basis of objective pathologic tumor response to chemotherapy. Considering that not only Pgp but also MDR-associated glycoprotein is responsible for ^{99m}Tc-sestamibi efflux, systems measuring only Pgp expressions are likely to underestimate clinical chemoresistance and to be characterized by low sensitivity.

TABLE 3
Prognostic Performance of Test

Test result	Disease (no response to chemotherapy)	No disease (positive response to chemotherapy)	Total
Positive	15	3	18
Negative	—	12	12
Total	15	15	30

Sensitivity = 100% (95% confidence interval = 0.78–1.0); specificity = 80% (95% confidence interval = 0.52–0.95); positive predictive value = 83%; negative predictive value = 100%; likelihood ratio = 5.

In our study, the negative predictive value of the test was 100%. This result ensured a good response to neoadjuvant chemotherapy by all patients with a negative test (i.e., WOR < 45%), ruling out all patients with high expression of chemoresistance. On the contrary, a positive test was associated with a high probability of chemoresistance (positive predictive value, 83%, with a 95% confidence interval of 58%–96%). In these patients, the use of chemorevertant or chemomodulator agents can be justified.

The high likelihood ratio observed (i.e., 5) indicated that the test result would greatly raise the prior probability of suspected disease. In fact, by applying Bayes' equation for clinical diagnosis, we could observe a posttest probability of disease of 72%–88%, as opposed to a pretest probability of

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

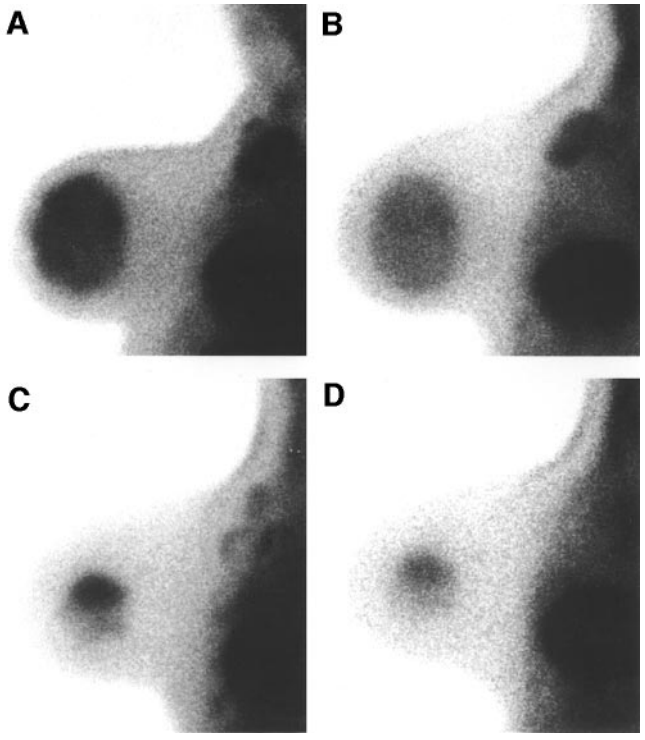


FIGURE 2. Pretherapy (A and B) and posttherapy (C and D) ^{99m}Tc-sestamibi studies (lateral views of left breast in prone position) of patient 29, who showed positive response to chemotherapy at pathologic examination. Pretherapy study evidenced very intense and diffuse tracer uptake both in tumor and in axillary nodes in early image (A) and low ^{99m}Tc-sestamibi WOR (30%) in delayed image (B), predicting low MDR expression. Early posttherapy image (C) confirmed positive response to chemotherapy, showing small area of residual tracer uptake in tumoral region and evident decrease of tracer uptake in axilla. Uptake remained substantially unmodified in corresponding delayed image (D).

35%–59%. This further analysis confirmed that the ^{99m}Tc-sestamibi WOR was clinically useful for assessing the probability of intrinsic chemoresistance in patients with locally advanced breast cancer before neoadjuvant chemotherapy planning.

A false-positive test was observed in 3 patients (20%). Two of these patients presented with strongly inflammatory disease that showed very diffuse and intense uptake. These characteristics probably interfered with the mechanisms of ^{99m}Tc-sestamibi kinetics. In the third patient, the WOR in the prognostic study was high, whereas the diagnostic test showed an evident reduction in ^{99m}Tc-sestamibi uptake in the tumor after chemotherapy. This patient had a positive pathologic response but was found to have progressing disease immediately after surgery.

Another interesting observation concerns the WOR value obtained in the posttherapy study. All patients with a high WOR on the pretherapy study also had a high value on the posttherapy study, confirming the presence of chemoresistance. In addition, 5 of 12 patients with a low WOR ($<$45%) before therapy showed a high WOR ($>$45%) on the posttherapy study. These patients could have acquired chemoresistance during neoadjuvant chemotherapy. In fact, in addition to the presence of intrinsic chemoresistance, which remains unaffected by the first cycle of chemotherapy, the development of acquired resistance during treatment must be considered. Acquired resistance develops mostly through mutation of gene induction but can also arise when a resistant fraction survives from a tumor that initially responded to the chemotherapy (*27*). The change in WOR pattern from low to high during neoadjuvant chemotherapy can represent survival of a resistant tumor fraction or any other mechanism implied in induction of chemoresistance. Further data on long-term follow-up are clearly needed to confirm this hypothesis.

CONCLUSION

This study showed that ^{99m}Tc-sestamibi WOR, as a test for predicting tumor response to neoadjuvant chemotherapy, is reliable, simple, noninvasive, reproducible, and effective. The clinical role of the test is quite important, because a negative test (WOR $\leq$ 45%) rules out chemoresistance, ensuring the effectiveness of neoadjuvant chemotherapy. Conversely, a positive test (WOR $>$ 45%) indicates a high probability of chemoresistance and suggests that chemorevertant or chemomodulator agents should be used.

ACKNOWLEDGMENT

This study was supported in part by grant 2490/00/CadC from the Fondazione Italiana per la Ricerca Sul Cancro.

REFERENCES

1. Goldstein LJ. MDR1 gene expression in solid tumors. *Eur J Cancer*. 1996;32A: 1039–1050.
2. Goldstein LJ, Galski H, Fojo A, et al. Expression of a multidrug resistance gene in human cancer. *J Natl Cancer Inst.* 1989;81:116–118.
3. Kieth WN, Stallard S, Brown R. Expression of MDR1 and GST-p in human breast tumors: comparison to in vitro chemosensitivity. *Br J Cancer*. 1990;61: 712–716.
4. Lehnert M. Clinical multidrug resistance in cancer: a multifactorial problem. *Eur J Cancer*. 1996;32A:912–920.
5. Epson RA. Drug-induced DNA damage and tumor chemosensitivity. *J Clin Oncol*. 1990;8:2062–2084.
6. Zijlstra JG, de Vries EGE, Mulder NH. Multifactorial drug resistance in an adriamycin-resistant human small cell lung carcinoma cell line. *Cancer Res.* 1987;47:1780–1784.
7. Linn SC, Pinedo HM, van Ark-Otte J, et al. Expression of drug resistance proteins in breast cancer, in relation to chemotherapy. *Eur J Cancer*. 1997;71:787–795.
8. Loe DW, Deeley RG, Cole SPC. Biology of the multidrug resistance-associated protein, MRP. *Eur J Cancer*. 1996;32A:945–957.
9. Fojo AT, Ueda K, Slamon DJ, Poplak DG, Gottesmam MM, Pastan I. Expression of a multidrug resistance gene in human tumors and tissues. *Proc Natl Acad Sci USA.* 1987;84:265–269.
10. Filipits M, Suchomel RW, Dekan G, et al. MRP and MDR1 gene expression in primary breast carcinoma. *Clin Cancer Res.* 1996;2:1231–1237.
11. Ito K, Fujimori M, Nakata S, et al. Clinical significance of the increased multidrug resistance-associated protein (MRP) gene expression in patients with primary breast cancer. *Oncol Res.* 1998;10:99–109.
12. Kostakoglu L, Ruacan S, Ergün ES, Sayek I, Elahi N, Bekdik CF. Influence of the heterogeneity of P-glycoprotein expression on technetium-99m-MIBI uptake in breast cancer. *J Nucl Med.* 1998;39:1021–1026.
13. Noonan KE, Beck C, Holzmayer TA, et al. Quantitative analysis of MDR1

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

(multidrug resistance) gene expression in human tumors by polymerase chain reaction. *Proc Natl Acad Sci USA.* 1990;87:7160–7164.
14. Charpin C, Vielth P, Duffaud F, et al. Quantitative immunocytochemical assays of p-glycoprotein in breast carcinomas: correlations to messenger RNA expression and to immunohistochemical prognostic indicators. *J Natl Cancer Inst.* 1994;86:1539–1545.
15. Beck WT, Grogan TM, Willman CL, et al. Methods to detect p-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. *Cancer Res.* 1996;53:977–984.
16. Bailly JD, Muller C, Jaffrezou JP, et al. Lack of correlation between expression and function of P-glycoprotein in acute myeloid leukemia cell lines. *Leukemia.* 1995;9:799–807.
17. Schomacker K, Schicha H. Use of myocardial agents for tumor diagnosis: a success story? *Eur J Nucl Med.* 2000;27:1845–1863.
18. Khalkali I, Villanueva-Meyer J, Edell SL, et al. Diagnostic accuracy of ^{99m}Tc-sestamibi breast imaging: multicenter trial results. *J Nucl Med.* 2000;41:1973–1979.
19. Piwnica-Worms D, Chiu ML, Budding M, Kronauge JF, Kramer RA, Croop JM. Functional imaging of multidrug-resistant P-glycoprotein with an organo-technetium complex. *Cancer Res.* 1993;53:997–984.
20. Vergote J, Moretti JL, De Vries EGE, Garnier-Suillerot A. Comparison of the kinetics of active efflux of ^{99m}Tc-MIBI in cells with P-glycoprotein-mediated and MRP-associated multidrug resistant phenotypes. *Eur J Biochem.* 1998;252:140–146.
21. Hendrikse NH, Franssen EJF, van der Graaf WTA, Vaalburg W, de Vries EGE. Visualization of multidrug resistance in vivo. *Eur J Nucl Med.* 1999;26:283–293.
22. Kostakoglu L, Elahi N, Kiratli P, et al. Clinical validation of the influence of the P-glycoprotein on ^{99m}Tc-sestamibi uptake in malignant tumors. *J Nucl Med.* 1997;38:1003–1008.
23. Cwicla JB, Buscombe JR, Kolasinska AD, Pabhoo SP, Thrakhar DS, Hilson AJ. Correlation between uptake of ^{99m}Tc-sesta-MIBI and prognostic factors of breast cancer. *Anticancer Res.* 1999;19:2299–2304.
24. Moretti JL, Azaloux H, Boisseron D, Kouyoumdjian JC, Vilcoq J. Primary breast cancer imaging with technetium-99m-sestamibi and its relation with P-glycoprotein overexpression. *Eur J Nucl Med.* 1996;23:980–986.
25. Del Vecchio S, Ciarmiello A, Pace L, et al. In vivo detection of multidrug resistance (MDR1) phenotype by ^{99m}Tc-sestamibi scan in untreated breast cancer. *Eur J Nucl Med.* 1997;24:150–159.
26. Landis JR, Koch GG. The measurements of observer agreement for categorical data. *Biometrics.* 1997;33:159–165.
27. Sarkadi B, Muller M. Search for specific inhibitors of multidrug resistance in cancer. *Semin Cancer Biol.* 1997;8:171–182.
28. Robert J. Resistance to anticancer drugs: are we ready to use biologic information for the treatment of patients with cancer? *Ther Drug Monit.* 1998;20:581–587.
29. Arbab AS, Koizumi K, Toyama K, Araki T. Uptake of technetium-99m-tetrofosmin, technetium-99m MIBI and thallium-201 in tumor cell lines. *J Nucl Med.* 1996;37:1551–1556.
30. Maini CL, Tofani A, Sciuto R, et al. Technetium-99m-MIBI scintigraphy in the assessment of neoadjuvant chemotherapy in breast carcinoma. *J Nucl Med.* 1997;38:1546–1551.
31. Trock JB, Leonessa F, Clarke R. Multidrug resistance in breast cancer: a meta-analysis of MDR/gp 170 expression and its possible functional significance. *J Natl Cancer Inst.* 1997;89:917–931.
32. Hendrikse NH, Franssen EJF, van der Graaf WTA. ^{99m}Tc-sestamibi is a substrate for P-glycoprotein-mediated and multidrug resistance associated protein. *Br J Cancer.* 1998;77:353–358.
33. Ciarniello A, Del Vecchio S, Silvestro P, et al. Tumor clearance of technetium-99m-sestamibi as a predictor of response to neoadjuvant chemotherapy for locally advanced breast cancer. *J Clin Oncol.* 1998;16:1677–1683.
34. Fujii H, Nakamura K, Kubo A, et al. ^{99m}Tc-MIBI scintigraphy as an indicator of the chemosensitivity of anthracyclines in patients with breast cancer. *Anticancer Res.* 1998;18:4601–4605.
35. Moretti J, Caglar M, Boaziz C, Caillat-Vigneron N, Morere JF. Sequential functional imaging with technetium 99m hexakis-2-methoxy-isobutilisonitrile and indium-111-octreotide: can we predict the response to chemotherapy in small cell lung cancer? *Eur J Nucl Med.* 1995;22:177–180.
36. Dimitrakopulou-Strauss A, Strauss LS, Goldschmidt H, Lorenz WJ, Maier Borst W, van Kaick G. Evaluation of tumor metabolism and multidrug-resistance in patients with treated malignant lymphomas. *Eur J Nucl Med.* 1995;22:434–442.
37. Cwicla JB, Buscombe JR, Barlow RV, et al. The effect of chemotherapy on the uptake of technetium sestamibi in breast cancer. *Eur J Nucl Med.* 1997;24:1175–1178.

SOCIETY OF NUCLEAR MEDICINE

Downloaded from jnm.snmjournals.org by on March 14, 2017. For personal use only.

JNM The Journal of NUCLEAR MEDICINE

Prognostic Value of ^{99m}Tc-Sestamibi Washout in Predicting Response of Locally Advanced Breast Cancer to Neoadjuvant Chemotherapy

Rosa Sciuto, Rosella Pasqualoni, Serenella Bergomi, Germana Petrilli, Patrizia Vici, Franca Belli, Claudio Botti, Marcella Mottolese and Carlo L. Maini

J Nucl Med. 2002;43:745-751.

This article and updated information are available at:
http://jnm.snmjournals.org/content/43/6/745

Information about reproducing figures, tables, or other portions of this article can be found online at:
http://jnm.snmjournals.org/site/misc/permission.xhtml

Information about subscriptions to JNM can be found at:
http://jnm.snmjournals.org/site/subscriptions/online.xhtml

The Journal of Nuclear Medicine is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

© Copyright 2002 SNMMI; all rights reserved.

SNMMI SOCIETY OF NUCLEAR MEDICINE AND MOLECULAR IMAGING