Influence of the Heterogeneity of P-Glycoprotein Expression on Technetium-99m-MIBI Uptake in Breast Cancer

Lale Kostakoglu, Sevket Ruacan, Eser L. Ergün, Iskender Sayek, Nazenin Elahi and Coskun F. Bekdik
Departments of Nuclear Medicine, Pathology and Surgery, Hacettepe University Medical Faculty, Ankara, Turkey

We prospectively studied a total of 30 patients with breast cancer to evaluate the relationship between the degree of accumulation of ^{99m}Tc-sestamibi (MIBI) and the heterogeneity of p-glycoprotein expression in tumor tissues. **Methods:** Twenty patients during initial presentation and 10 patients during post-therapy evaluation underwent contemporaneous ^{99m}Tc-MIBI imaging and surgery or biopsy. Immunohistochemical studies were performed on multiple nonconsecutive sections of the same tumor using a p-glycoprotein-specific monoclonal antibody, JSB-1. Tumor-to-background (T/B) ratios were correlated with the level and heterogeneity of p-glycoprotein expression determined by immunohistochemical studies. **Results:** The T/B ratios were lower for those tumors with strong p-glycoprotein expression (Group 1) than those with strong-to-weak expression (Group 2) or those with weak-to-no expression (Group 3) (1.32 ± 0.19 and 1.85 ± 0.56 and 2.86 ± 1.06, respectively). There was statistically significant difference in T/B ratios between all 3 groups ($p < 0.005$). Although T/B ratios for Group 1 and Group 3 were clearly distinct from one another with no overlapping values, the values for Group 2 overlapped with those of Group 1 and Group 3. When we evaluated the entire patient group with excluding those with strong-to-weak expression, although the p value remained the same ($p < 0.001$), we obtained a stronger correlation between T/B ratios and p-glycoprotein expression ($r = 0.808$ versus 0.735). **Conclusion:** Due to the heterogeneous expression of p-glycoprotein, both immunohistochemistry and ^{99m}Tc-MIBI scintigraphy may yield confounding results by contrasting with one another if the presence or absence of p-glycoprotein is not extensively explored. Although our data confirmed that ^{99m}Tc-MIBI imaging is useful in the determination of the presence of multidrug resistance in patients with breast cancer, the issue of heterogeneous expression of the antigen should be further investigated when unexpected results are obtained.

Key Words: multidrug resistance; heterogeneity; p-glycoprotein, technetium-99m-sestamibi

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Overexpression of p-glycoprotein is an important determinant of inherent or acquired multidrug resistance, which represents a major impediment to effective chemotherapy of cancer (*1–2*). The observed association between p-glycoprotein expression and worse prognosis as well as the possibility of modulating the p-glycoprotein mediated multidrug resistance (MDR) phenotype have stimulated the development of various methods to increase the sensitivity and accuracy of detection techniques (*3–9*). In this regard, a multitude of factors appear to influence the detection of p-glycoprotein in clinical specimens, including its low and heterogeneous expression, use of immunological reagents with variable p-glycoprotein specificity and differences in methods of sample preparation and analysis (*10*). Theoretically, all these variables can be formulated or standardized except the inherent heterogeneity of the antigenic distribution, a feature whose biological and clinical implications deserve further investigation.

On the other hand, ^{99m}Tc-MIBI has been reported to be a transport substrate for p-glycoprotein pump mechanism (*11,12*). Although its accumulation rates are driven by negative transmembrane potentials, the accumulation and retention of ^{99m}Tc-MIBI is reduced in cells expressing multidrug-resistant phenotype because of the energy-dependent p-glycoprotein efflux pump, which expels its substrates from the cell and causes a concomitant decrease (*11–16*). In this context, previous studies have shown an inverse relationship between the levels of p-glycoprotein and the magnitude of ^{99m}Tc-MIBI uptake and washout in the tumor cells (*15,17,18*). However, contrasting results have been reported justifying further analysis of the influence of other factors such as simultaneous presence of other resistance mechanisms and heterogeneity of antigenic distribution on the ^{99m}Tc-MIBI uptake (*17*). The shortcoming of all current methods used in the detection of p-glycoprotein including those at the protein and RNA level is the fact that none allows thorough evaluation throughout the entire tissue in solid tumors unless the samples are extensively sectioned, therefore the influence of the antigenic heterogeneity on the sensitivity and specificity of the methods could not be accurately determined. As antigenic heterogeneity might have important clinical implications, our intent in this particular study was to investigate the significance of heterogeneous p-glycoprotein expression on the magnitude of tumoral ^{99m}Tc-MIBI uptake to determine the contribution of heterogeneity to the misleading results that could be obtained from ^{99m}Tc-MIBI imaging.

MATERIALS AND METHODS

Patients

A total of 30 patients (age range 25 and 76 yr; mean age 49 ± 12 yr) were included in the study. Of the 30 patients, 26 had infiltrating ductal and 4 had medullary carcinoma of the breast. Twenty patients were evaluated before radio- and/or chemotherapy and 10 of 30 patients were previously treated with various chemotherapy regimens consisting of cisplatin, methotrexate, 5-fluorouracil, doxorubicin, taxol and etoposide. The interval between completion of chemotherapy and relapse ranged from 6 mo to 32 mo (mean 17.2 ± 9.2). All tumors were detectable by radiological modalities such as CT/MRI or mammography and/or ultrasound. All patients had excisional biopsy or surgery after ^{99m}Tc-MIBI imaging. All tumor specimens were obtained within 2 wk of imaging studies for immunohistochemical analysis.

Imaging

A dual-head ADAC Genesys (ADAC, Milpitas, CA) camera with a LEHR collimator interfaced with an ADAC 3300 computer was used for image acquisition. Thirty minutes after the injection of 740 MBq ^{99m}Tc-MIBI 10-min spot images of the thorax were

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For correspondence or reprints contact: Lale Kostakoglu, MD, The New York Hospital-Cornell Medical Center, Nuclear Medicine Division, 525 East 68th St., Starr Bldg. 221, New York, NY 10021.

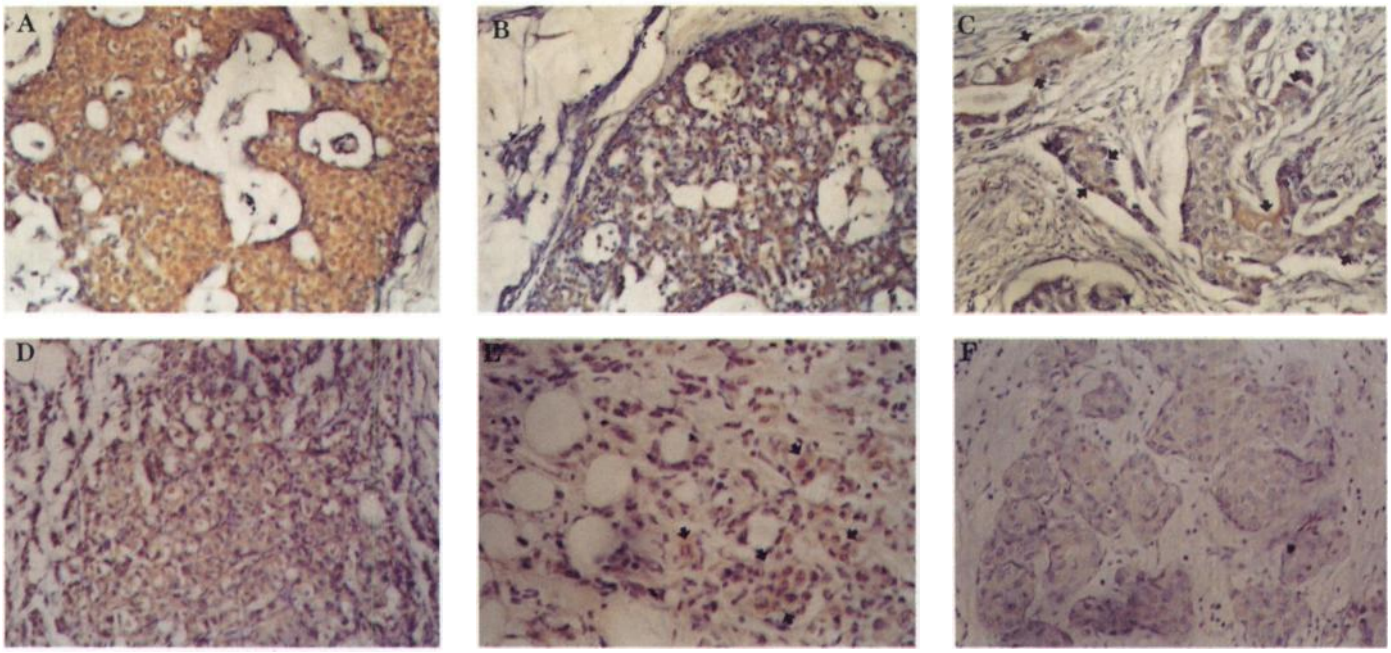

FIGURE 1. Immunohistochemistry performed on nonconsecutive sections of the tumor in two different Group 2 patients reveals Patient 1: (A) +++ strong; (B) ++ strong; and (C) F+ (arrows) focal p-glycoprotein staining. Corresponding ^{99m}Tc-MIBI imaging on this patient is shown in Figure 3A: Patient 2: (D) ++ strong; (E) + weak; and (F) F+ (arrows) focal p-glycoprotein staining. Corresponding ^{99m}Tc-MIBI imaging on this patient is shown in Figure 3B (all magnifications ×230).

obtained in supine position. A SPECT study was performed starting at approximately 45 min after injection. SPECT was performed in supine position using a matrix size of 64 × 64 × 16 for 64 projection and an imaging time of 30 sec per projection. Tomographic images were reconstructed using a Butterworth filter with a cutoff frequency of 0.35 and an order of 6. Attenuation correction was applied to all frames to assess the lung parenchyma for the presence or absence of any metastatic foci. We obtained both planar and SPECT images to avoid any possible false-negative results that could be obtained by either method. However, quantitative analyses were done using only consecutive transverse sections of the SPECT study to achieve quantitation throughout the entire tumor volume. The uptake ratios were taken in the regions of interests drawn over the tumor and the contralateral site. When planar images were compared with SPECT data, they were in complete agreement with SPECT images. Technetium-99m-MIBI scans were interpreted by two nuclear medicine physicians blinded to the patients' clinical information and immunohistochemistry findings.

Immunohistochemistry

We used a well-characterized p-glycoprotein-specific monoclonal antibody (MAb), JSB-1, detecting spatially distinct epitopes on the cytoplasmic site of p-glycoprotein. The technique has been described elsewhere in detail (*19*). Briefly, 5-μm-thick, formalin fixed, paraffin-embedded tissue samples were obtained and placed on poly-L-lysine-coated slides (Sigma, St. Louis, MO). For each patient, sections were obtained in duplicate from four nonconsecutive sections of the same tumor with 80–100 μm apart from one another. After treatment with 0.1% methanol-hydrogen peroxide the sections were incubated with normal horse serum (Vector, Burlingame, CA) for 30 min at 37°C and incubated with primary MAb, JSB-1 (Novocastra Lab, Cornwall, UK) overnight in a moist chamber at 4°C at a dilution of 1:20. The tissue sections were incubated with secondary biotinylated antimouse antibody and with avidin-biotin-peroxidase complex. The final reaction product was exposed to 0.03% diaminobenzidine and hydrogen peroxide. The nuclei were counter-stained with Mayer's hematoxylin. A negative control was obtained by staining the sample with secondary antibody and a positive control by inclusion of a tumor section with known positivity for p-glycoprotein. The results of p-glycoprotein immunostaining were independently interpreted by two pathologists who were blinded to the results of imaging studies. On initial analysis, the tumors were classified under three groups according to the distribution of p-glycoprotein expression and the degree of immunostaining as follows (*19*):

Group 1: Tumors strongly positive for p-glycoprotein. This group consisted of two subgroups: (a) diffuse positivity with strong staining, more than 10% of the specimen referred to as +++ (Fig. 1A) or (b) diffuse positivity with weak staining, more than 10% of the specimen referred to as ++ (Fig. 1B).

Group 2: Tumors weakly or focally positive for p-glycoprotein. This group consisted of two subgroups: (a) weak staining in scattered positive cells, involvement of less than 10% of the specimen referred to as + (Fig. 1E) or (b) focal positivity with strong staining referred to as F+ (Figs. 1C and 1F).

Group 3: Tumors completely negative for p-glycoprotein referred to as "−".

Statistical Analysis

The correlation between p-glycoprotein expression levels and T/B ratios obtained from ^{99m}Tc-MIBI imaging was determined using Spearman correlation test. The difference in T/B ratios between three groups was determined using Kruskal Wallis test. Further analyses for the pairwise comparisons of these ratios and different p-glycoprotein levels were performed using Mann-Whitney-U test as an ad-hoc analysis where cutoff value for statistical significance is reduced from 0.05–0.016 due to multiple comparisons.

RESULTS

Immunohistochemistry

The results of immunohistochemical studies are summarized in Table 1. As immunohistochemical evaluation was performed on multiple and nonconsecutive sections of the same tumor, the final interpretation of immunostaining was given as the following expressions:

TABLE 1
Immunohistochemistry Results of Nonconsecutive Tumor Sections

Patient no.	Consecutive tumor sections*				Result	Therapy
	S1	S2	S3	S4		
1	++	+++	+++	++	S	Pre
2	+++ s	+++	++ s	++ s	S	Post
3	+++	+++	+++	++	S	Pre
4	+++	+++	++	++	S	Post
5	+++	+++ s	++	+++	S	Post
6	+++	++	++	++	S	Pre
7	+++	+	++	++	S/W	Pre
8	+	++	F+	++	S/W	Post
9	++	+++	F+	++	S/W	Pre
10	++	+	++	F+	S/W	Pre
11	+	++	+++	++	S/W	Pre
12	+++	F+	F+	F+	S/W	Pre
13	+	+++ s	+++ s	+	S/W	Pre
14	+	+++	++	F+	S/W	Post
15	++	++	F+	F+	S/W	Post
16	F+	++	++	++	S/W	Post
17	+ s	+++ s	++	F+	S/W	Post
18	F+	F+	+	+	W	Post
19	F+	F+	+	F+	W	Pre
20	+	F+	F+	+	W	Pre
21	F+	F+	+	F+	W	Post
22	+	−	F+	−	W/N	Pre
23	−	F+	−	F+	W/N	Pre
24	−	+	F+	F+	W/N	Pre
25	−	+	F+	F+	W/N	Pre
26	−	−	F+	F+	W/N	Pre
27	−	−	−	−	N	Pre
28	−	−	−	−	N	Pre
29	−	−	−	−	N	Pre
30	−	−	−	−	N	Pre

*Sections were 80–100 μm apart.
s = accompanying stromal p-glycoprotein expression; S = strong expression; S/W = strong-to-weak expression; W = weak expression; W/N = weak-to-no expression; N = no expression.

1. Strong expression: Homogeneously diffuse expression throughout all sections, including both subgroups of ++ and +++.
2. Strong-to-weak expression: Occurrence of strong, weak and focal expression on different sections. Presence of weak or strong expression on at least one section was enough for that particular tumor to be classified in this group regardless of the dominant feature when all sections evaluated together.
3. Weak-to-no expression: There are three subgroups in this group: (a) weak = homogeneously weak expression including both subgroups of + and F+; (b) weak-to-no expression = associated with no expression on different sections; and (c) no expression = complete absence of staining throughout all sections (Tables 1 and 2).

Based on our previous study performed on patients with breast cancer, as there was statistically no significant difference between tumors with weak p-glycoprotein expression and those with no expression, in this study, the patients with weak expression or weak-to-no expression or no expression were evaluated as one group under weak-to-no expression, to achieve statistical significance (*17*).

In the group with heterogeneous staining showing both strong and weak expression on different sections (strong-to-weak) we did not attempt to determine the predominant staining pattern as the number of sections we obtained might not have yielded an optimal evaluation of the dominant feature of the entire tumor so that we could introduce significant errors by deriving definite results from an inadequate data based on only four sections taken from the tumor. Therefore, we avoided to subgroup these patients while performing statistical analysis.

Ultimately, the scintigraphic findings were correlated with three separate patient groups as follows:

1. Group 1 (strong expression): There were six patients with consistently strong p-glycoprotein expression on all sections obtained.
2. Group 2 (strong-to-weak expression): There were 11 patients with heterogeneous p-glycoprotein expression varying from strong-to-weak on different sections of the same tumor (Fig. 1A and 1B).
3. Group 3 (weak-to-no expression): There were a total of 13 patients in this group (4 patients with weak expression, 5 with weak-to-no expression and 4 with no expression).

Ten of 30 patients were evaluated after completion of chemotherapy consisting of chemotherapeutic regimens generally associated with p-glycoprotein dependent MDR (etoposide, doxorubicin, taxol). As the interval between the completion of chemo- or radiotherapy and ^{99m}Tc-MIBI imaging was quite long (mean = 17.2 ± 9.2), separate evaluation of pre- and post-therapy patients was not necessary to avoid any chemotherapy impact on membrane potentials or tumor composition. Although the difference in p-glycoprotein expression between pre and post-therapy

TABLE 2
Correlation Between Tumor-to-Background Ratios and Heterogeneity of P-Glycoprotein Expression

Patient no.	T/B	P-glycoprotein	Size (cm)	Site	Type
1	1.2	S	1.0	Breast	Inf Duc
2*	1.3	S	2.5	Axilla	Inf Duc
3	1.1	S	2.0	Breast	Inf Duc
4*	1.6	S	3.0	Chestwall	Inf Duc
5*	1.5	S	1.5	Breast	Inf Duc
6	1.2	S	1.0	Breast	Inf Duc
7	1.3	S/W	2.0	Breast	Inf Duc
8*	1.3	S/W	2.2	Axilla, breast	Inf Duc
9	2.3	S/W	7.0	Breast	Inf Duc
10	1.5	S/W	2.5	Breast	Inf Duc
11	1.3	S/W	4.0	Breast	Medullary
12	1.8	S/W	2.5	Breast	Inf Duc
13	1.8	S/W	5.0	Breast	Inf Duc
14*	2.5	S/W	5.0	Breast	Inf Duc
15*	2.8	S/W	2.0	SCL	Inf Duc
16*	1.3	S/W	1.8	SCL	Inf Duc
17*	2.4	S/W	1.5	Axilla	Inf Duc
18*	1.9	W/N	4.0	Breast	Inf Duc
19	4.3	W/N	4.0	Breast	Inf Duc
20*	5.3	W/N	5.0	Breast	Inf Duc
21	2.0	W/N	2.0	Breast	Inf Duc
22	1.8	W/N	1.0	Breast	Inf Duc
23	3.8	W/N	6.0	Axilla	Inf Duc
24	3.0	W/N	3.5	Breast	Medullary
25	2.0	W/N	2.5	Breast	Inf Duc
26	2.6	W/N	3.0	Breast	Inf Duc
27	2.3	W/N	2.0	Axilla	Medullary
28	2.6	W/N	2.0	Breast	Medullary
29	2.3	W/N	2.8	Breast	Inf Duc
30	3.3	W/N	3.0	SCL	Inf Duc

*Patients evaluated following therapy.

Inf Duc = infiltrating ductal carcinoma; SCL = supraclavicular lymph node; T/B = tumor-to-background ratio; S = strong expression; S/W = strong-to-weak expression; W/N = weak-to-no expression.

groups could not be statistically evaluated due to the inadequate number of patients, there was a tendency for the tumors to be positive for p-glycoprotein in the post-therapy group (Table 2). In this group of patients (10 patients), 8 had either strong or strong-to-weak expression, whereas only 2 had weak expression (80% versus 20%). On the other hand, in the pretherapy group (20 patients), 9 had strong or strong-to-weak expression whereas 11 had weak, weak-to-no or no expression (45% versus 55%).

Correlation Between Technetium-99m-MIBI Imaging and Immunohistochemistry

The correlation between ^{99m}Tc-MIBI imaging and immunohistochemistry findings was summarized in Table 2. Our results showed an inverse correlation between T/B ratios and p-glycoprotein expression for each immunostaining study performed on four different sections of the tumor ($p < 0.005$; Spearman test) using standard criteria for immunostaining. We correlated the T/B ratios obtained from ^{99m}Tc-MIBI imaging with immunostaining in three main groups as described before (Fig. 2). Group 1 (strong expression): mean T/B ratio: 1.32 ± 0.19 (range: 1.10–1.60); Group 2 (strong-to-weak expression): mean T/B ratio: 1.85 ± 0.56 (range: 1.30–2.80); Group 3 (weak-to-no expression): mean T/B ratio: 2.86 ± 1.06 (range: 1.80–5.30).

There was a statistically significant difference in T/B ratios

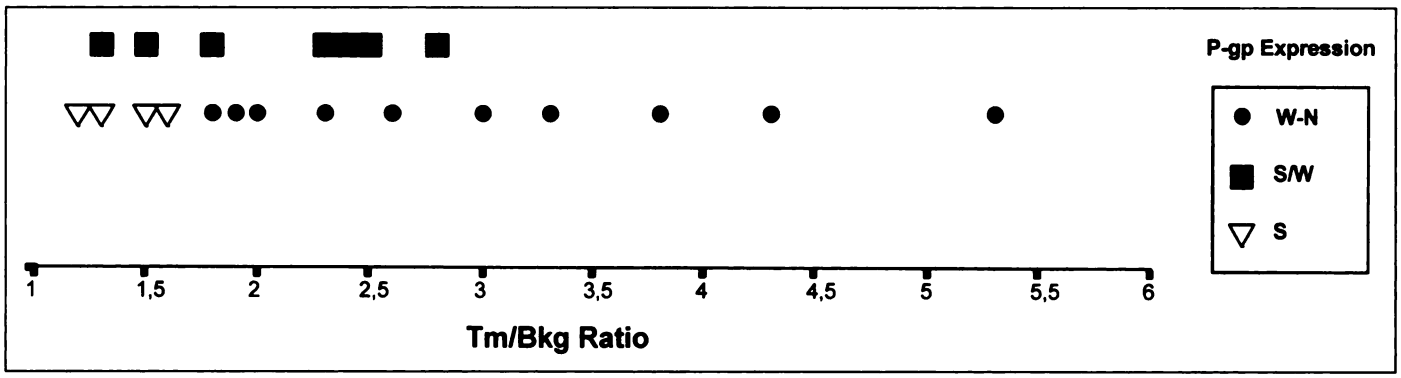

FIGURE 2. The distribution of T/B ratios in relation to the level and heterogeneity of p-glycoprotein expression. Although T/B ratios for Group 1 (S) and Group 3 (W-N) were distinctly different with no overlapping values, the values for Group 2 (S/W) overlapped with those of Group 1 and Group 3. Group 1 = strong expression; Group 2 = strong-to-weak expression and Group 3 = weak-to-no expression.

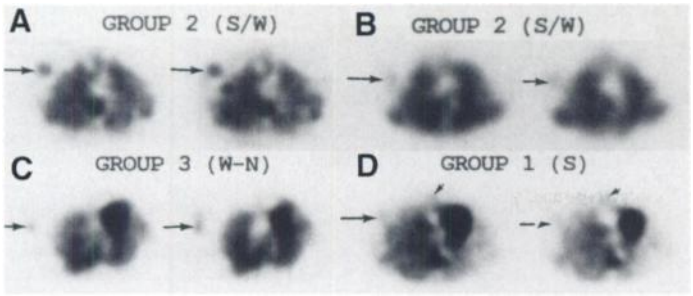

FIGURE 3. (A) Transverse slices of the SPECT image demonstrates an area of intense radiotracer uptake by the tumor in the right upper quadrant of the breast (arrows) in a Group 2 patient (T/B ratio: 2.3, corresponding immunohistochemistry is shown in Figure 1A–1C). (B) Area of faint radiotracer uptake in the right upper quadrant (arrows) in a Group 2 patient (T/B ratio: 1.3, corresponding immunohistochemistry is shown in Fig. 1D–1F). (C) Area of increased radiotracer uptake in the right upper quadrant of the breast in the region of the tumor (arrows) in a Group 3 patient (T/B ratio: 1.9). (D) Areas of faint radiotracer uptake in the right axilla and in the medial aspect of the left breast (arrows) in a Group 1 patient (T/B ratio: 1.2).

between the three groups ($p < 0.05$; Kruskal Wallis test). Further analysis using Mann-Whitney-U test revealed: (a) There was statistically significant difference between Group 2 (strong-to-weak expression) and Group 3 (weak-to-no expression) ($p = 0.0084$) (Figs. 1 and 3A-C); (b) There was statistically significant difference between Group 1 (strong expression) and Group 3 (weak-to-no expression) ($p = 0.006$) (Fig. 3C and 3D); (c) Although the T/B ratios for Groups 1 (strong expression) and 2 (strong-to-weak expression) were not statistically different ($p = 0.0284$), based on the statistical significance cutoff set at 0.016 for pairwise comparisons, the p value of 0.0284 supports the possibility of obtaining statistical significance when the number of patients is expanded.

Although T/B ratios for Group 1 (strong expression) and Group 3 (weak-to-no expression) were clearly distinct from one another with no overlapping values, the values for Group 2 (strong-to-weak expression) overlapped with those of Group 1 (strong expression) and Group 3 (weak-to-no expression) (Fig. 2). Based on this observation, when we evaluated the entire patient group with excluding those patients with strong-to-weak expression, although the p value remained the same ($p < 0.001$), we obtained a stronger correlation between T/B ratios and p-glycoprotein expression ($r = 0.808$ versus 0.735). There was no visually conceivable necrosis in the tumors (Table 2). The sizes of the tumors ranged from 1.0 cm to 6.0 cm (mean: 3.04 ± 1.58). There was no correlation between tumor sizes and T/B ratios ($p > 0.05$). Four patients had stromal p-glycoprotein expression in their tumors but there were no tumors with stromal expression without accompanying tumoral expression therefore the significance of stromal p-glycoprotein expression could not be investigated (Table 1).

DISCUSSION

Characterization of p-glycoprotein at the RNA and protein level is feasible by various techniques, however, discordant results may emerge when these detection techniques are compared since the sensitivity and specificity of a certain technique are always limited by unpredictable parameters such as the diversity of tumor tissues, simultaneous presence of other resistance mechanisms and heterogeneous expression of p-glycoprotein, all of which could make MDR detection equivocal (*20–23*). In a recent study, concordance between MDR1 expression at RNA level with RT-PCR and dot blot and at the protein level with immunohistochemistry was found in only 47% of the comparable specimens (*24*). It has been reported that majority of these disparities originate from low level and heterogeneous expression of p-glycoprotein, yet the biological significance and implications of the inherent p-glycoprotein heterogeneity has not been clarified by any clinical study (*25*). Notwithstanding the value of quantitative methods such as flow cytometry and PCR, interpretation of MDR1 measurements is complicated by heterogeneity among tumor cells and by a possible contribution from nontumor cells, especially in such tumors as breast carcinomas, which are usually associated with abundant stromal cells. Same levels of p-glycoprotein expression could result from homogeneous expression in all cells or from strong expression in a small population of tumor or stromal cells and no expression in others. No single detection technique provides the ideal test to detect MDR but in this study, we evaluated the heterogeneity of p-glycoprotein expression using immunohistochemistry since immunohistochemical techniques provide specific information on the distribution of p-glycoprotein in different tumor sections along with the definition of morphology and the localization of the p-glycoprotein expressing tumor and stromal cells (*26*). However, immunohistochemistry is more subjective than are bulk methods, therefore to provide a paradigm for investigations, immunostaining data should be reported using a consistent scoring system by an experienced pathologist as done in this study.

Our current clinical data were in complete agreement with the results of our previous study revealing an inverse relationship between the T/B ratios obtained from ^{99m}Tc-MIBI imaging and the density of p-glycoprotein expression ($p < 0.005$) (*17*). On immunohistochemistry, the tumors displayed two different immunostaining patterns reflecting heterogeneous (Fig. 1) and homogeneous distribution of antigenic expression. This finding was in line with the results of a recent study (*18*). Not surprisingly, the coexistence of p-glycoprotein positive and negative cells diminished the strength of correlation between T/B ratios and p-glycoprotein expression. The difference of T/B ratios between the Groups 1 (strong expression) and 3 (weak-to-no expression) was more significant with no overlapping values than that between Groups 1 (strong expression) and 2 (strong-to-weak expression) or between Groups 2 (strong-to-weak) and 3 (weak-to-no expression). On the other hand, the T/B ratios for Group 2 (strong-to-weak expression) overlapped with those of both other groups, strong expression and weak-to-no expression, most likely depending on the dominance of either strong or weak expression throughout the entire tumor section. In light of these observations, heterogeneity constitutes a fundamental concept that could provide an explanation for false-negative or contradictory immunohistochemistry results that may be generated depending on the section on which the immunohistochemistry was performed (*23*) (Figs. 1 and 3). By the same token, although providing in situ measurements of antigen density in mass units using quantitative autoradiography (QAR) is considered a superior method to immunohistochemistry, antigenic heterogeneity might still influence the accuracy of QAR by yielding false results (*18*).

In concordance with our premise, if immunohistochemistry was not performed on multiple tumor sections, conflicting results would be generated by ^{99m}Tc-MIBI imaging due to the concurrence of p-glycoprotein negative and positive sections of the same tumor tissue representing heterogeneous expression (Figs. 1 and 3A, 3B). In our data, four patients with T/B ratios of ≥ 2.3 and seven with ratios of ≤ 1.8 were detected to have either strong or weak or no p-glycoprotein expression on various sections of the sample. If no further immunohistochemistry was performed on multiple sections of the same tumors, these patients would be reported to have either strong or weak expression instead of heterogeneous expression, which would

give rise to contrasting results diminishing the sensitivity of functional imaging with ^{99m}Tc-MIBI (Table 2). In these patients quantitative methods might also fall short in elucidating the disparity as heterogeneity of the antigenic expression, which might have different biological implications than the total amount of the antigen.

Aside from the issue of antigenic heterogeneity, other factors such as subsets of cells expressing multiple resistance mechanisms that may well coexist (*16*), varying function capacities of the p-glycoprotein efflux pump depending on the ATP content of the cells (*5,6,8*) capillary permeability and poor penetration of the tracer into the tumor due to the necrosis, if any could also be held accountable for discrepancies obtained between p-glycoprotein expression levels and ^{99m}Tc-MIBI imaging.

As observed in the current data, the presence of increased p-glycoprotein levels in untreated breast carcinomas is an inherent characteristic of tumor cells, which is most likely the consequence of the concomitant activation of the human MDR1 gene promoter activity by genes associated with oncogenic development (*25–27*). However, the patients we evaluated after therapy expressed p-glycoprotein in their tumors at a rate of 80% whereas p-glycoprotein presence was observed in only 45% of the patients evaluated before therapy. The higher proportion of p-glycoprotein positive patients among those analyzed after therapy suggests that cytotoxic drug therapy induce p-glycoprotein expression in tumors.

Study Limitations

Considering nonspecificity of the antibodies, use of two or more vendor-standardized anti-p-glycoprotein antibody reagents that recognize different epitopes would improve the reliability of immunological detection of p-glycoprotein. Also various methods of determining MDR1 expression often yield discordant results, therefore, formulation of a standard assay and the use of at least two methods for assessing p-glycoprotein expression are advisable before extended clinical trials are started.

We evaluated the immunohistochemistry sections according to the grading system we adopted in which both the extent and the intensity of the immunostaining were assessed. We did not quantitate the immunostaining in terms of percentage of antigen expression based on the visual interpretation as we believe this method is a rough estimate and does not reflect the intensity of p-glycoprotein expression per se. To precisely quantitate the antigenic expression either autoradiography should be performed or the ratio between positive and total cell surface should be evaluated with the mean optical density using an image analysis system (*18,25*). As the number of patients and the sections we obtained from each tumor might not have represented the entire tumor characteristics as to the dominant feature, we did not favor to subgroup these patients to avoid potential errors.

CONCLUSION

This clinical study further extended our understanding of functional imaging with ^{99m}Tc-MIBI in relation to the heterogeneity of p-glycoprotein expression in the tumor samples in patients with breast cancer. Due to the heterogeneous expression of p-glycoprotein, both immunohistochemistry and ^{99m}Tc-MIBI scintigraphy could yield confounding results as we observed in this study. However, conclusions derived from this study should be considered provisional until they are confirmed by further quantification methods at the molecular level.

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