

P-Glycoprotein and Multidrug Resistance-related Protein Expressions in Relation to Technetium-99m Methoxyisobutylisonitrile Scintimammography Findings¹

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ABSTRACT

The purpose of this study was to retrospectively study 48 patients with infiltrating ductal breast cancer to evaluate the relationship between the degree of accumulation of technetium-99m methoxyisobutylisonitrile (Tc-MIBI) and P-glycoprotein (Pgp) or multidrug resistance-related protein (MRP) expression in breast cancer tissues. Before surgery or biopsy, all 48 patients underwent scintimammography started 10 min after the injection of Tc-MIBI. Tumor:background (T:B) ratios were calculated from the Tc-MIBI scintimammography. Immunohistochemical analysis was performed on the pathological specimens of the 48 breast tumors to determine Pgp and MRP expression. According to the results of immunohistochemical analysis, the 48 breast cancers were separated into four groups: (a) group 1, 12 cancers with both positive Pgp expression and positive MRP expression; (b) group 2, 12 cancers with positive Pgp expression and negative MRP expression; (c) group 3, 12 cancers with negative Pgp expression and positive MRP expression; and (d) group 4, 12 cancers with both negative Pgp expression and negative MRP expression. Among the four groups, the T:B ratio was lowest in group 1 (1.13 ± 0.10) and highest in group 4 (2.17 ± 0.14), respectively ($P < 0.05$). The T:B ratios of groups 2 (1.30 ± 0.25) and 3 (1.32 ± 0.26) were between those of groups 1 and 4. Our data confirmed that Tc-MIBI scintimammography is useful for determining Pgp and MRP expression in patients with breast cancers.

INTRODUCTION

Determining the mechanisms of drug resistance is imperative to the development of rational therapeutic strategies. Because the causes of chemotherapy failure are multifactorial, a major advance in the treatment of patients with neoplastic disease would be the identification of specific markers predictive of drug resistance. Recent studies have reported that the multidrug resistance gene 1 (*MDR1*) encoding human Pgp³ and the *MRP* gene may play an important role in the multidrug resistance of breast cancer (1–4). It has been suggested that determinations of Pgp and MRP expression at the time of diagnosis may provide valuable information for the design of treatment protocols. Therefore, before initiating chemotherapy, it is important to accurately determine the presence of Pgp and MRP expression in breast cancer to achieve a satisfactory response to chemotherapy.

Tc-MIBI has been reported to be a transport substrate for the Pgp and MRP pump mechanism (5, 6). Not surprisingly, resistant cancer

cells showing Pgp or MRP expression were found to transport Tc-MIBI outside the cells. Thus, information from Tc-MIBI scintimammography may be useful in the prediction of breast cancer response to chemotherapy and could guide the design of the most effective therapy protocols. However, widespread clinical use of Tc-MIBI scintimammography for noninvasive determination of Pgp and MRP expression is hampered by the lack of complete clinical studies. The need for extended clinical studies to supplement the existing data provided the basis for this study, which was an attempt to determine whether or not there is any association between Pgp or MRP expression and Tc-MIBI cancer uptake ratios calculated from Tc-MIBI scintimammography in patients with breast cancer.

PATIENTS AND METHODS

Patients. Twenty-four female patients (age range, 39–62 years) diagnosed with infiltrating ductal breast cancer were randomly selected and included in this study before biopsy, surgery, radiotherapy, and/or chemotherapy. The patients' cancers were graded according to Bloom and Richardson's scale and staged according to the International Union against Cancer TNM classification (7). All tumors were detectable by radiological modalities such as mammography or ultrasound. All patients had excisional biopsy or surgery after Tc-MIBI scintimammography, and all tumor specimens were obtained for IHA within 2 weeks of the imaging studies.

Tc-MIBI Scintimammography. Before biopsy or fine needle aspiration, all patients received Tc-MIBI scintimammography. The imaging procedure was started 30 min after oral intake of 500 mg of perchlorate to prevent abnormal uptake of free Tc-99m pertechnetate. A commercial methoxyisobutylisonitrile preparation [maximum 5.56 GBq (150 mCi) in approximately 1–3 ml] was obtained from Dupont Co. (Cardiolite). Labeling and quality control procedures were carried out according to the manufacturer's instructions. Labeling efficiencies were always higher than 95%. Each patient received an i.v. injection of 740 MBq (20 mCi) of Tc-MIBI in the arm contralateral to the breast with the abnormality. Three planar images were obtained starting 10 min after injection of Tc-MIBI using a gamma camera equipped with a low-energy, high-resolution collimator. The energy peak was centered at 140 keV with a 10% window. The imaging sequence for each patient was as follows: (a) a lateral planar image of the breast with the tumor in a prone position; (b) another lateral planar image of the other breast in the same prone position; and (c) an anterior planar image of the bilateral breasts in a supine position with the patient's arms raised behind her head. Each planar image was recorded on a 256×256 matrix for 5 min, and a total count of at least 1500 kilocounts was collected for each planar image. For the bilateral planar images, all of the patients were imaged in a prone position using a plastic table overlay that allowed the breast being imaged to be freely dependent from the imaging table. The prone planar image of a single dependent breast provided maximal separation of breast tissue from the myocardium and liver, as well as exclusion of any activity present in the opposite breast. The findings of Tc-MIBI scintimammography were evaluated as follows. Based on the prone lateral planar image of the breast with the tumor, a ROI was carefully drawn around the tumor, and then another ROI of the same size was drawn over the surrounding normal breast tissues. The T:B ratio was calculated by the following formula: (total counts in the ROI over the tumor) \div (the total counts in the same size ROI over the surrounding normal breast tissues). Because the T:B ratio was calculated from the same prone lateral image, image decay correction was not necessary.

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³ The abbreviations used are: Pgp, P-glycoprotein; Tc-MIBI, technetium-99m methoxyisobutylisonitrile; MRP, multidrug resistance-related protein; T:B, tumor:background; IHA, immunohistochemical analysis; ROI, region of interest; TNM, tumor-node-metastasis.

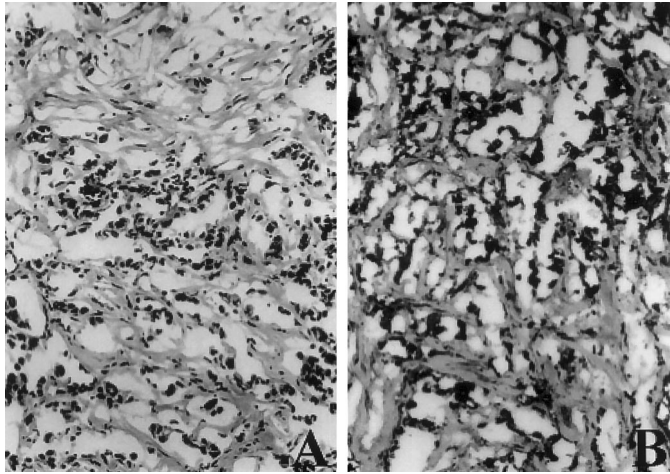


Fig. 1. IHAs were performed on sections of the whole breast cancer specimens in two different groups, revealing (A) negative (<10% stained tumor cells) and (B) positive ($\geq 10\%$ stained tumor cells) Pgp expression.

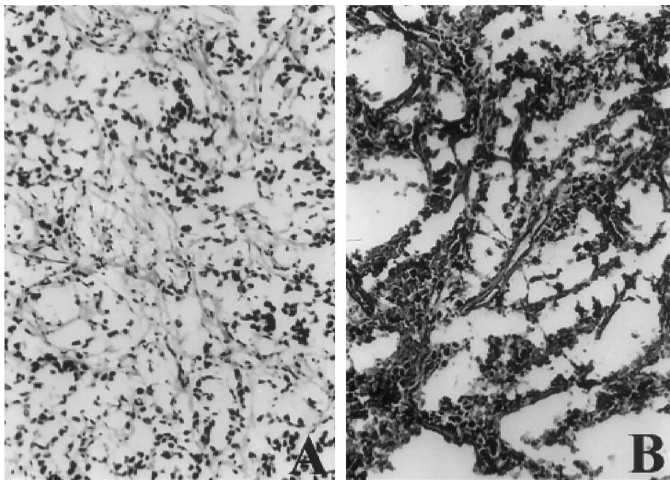


Fig. 2. IHAs were performed on sections of the whole breast cancer specimens in two different groups, revealing (A) negative (<10% stained tumor cells) and (B) positive ($\geq 10\%$ stained tumor cells) MRP expression.

IHA. The surgical breast specimens were laid on ice, embedded in Tissue-Tek OCT (Miles Scientific), and maintained at -70°C . The specimens were kept in liquid nitrogen. For Pgp immunohistochemical staining (8, 9), endogenous peroxidase was blocked by 3% hydrogen peroxide for 15 min. Antigen retrieval was performed by treatment with enzyme digestion in 0.1% trypsin in PBS for 5 min at room temperature and inhibited with 10% skim milk in PBS for 5 min. The sections were incubated for 2 h in a moist chamber at 37°C with primary antibody JSB-1 (50 $\mu\text{g}/\text{ml}$; Boehringer Mannheim Biochemica, Germany) at a 1:50 concentration. After three 5-min washes in PBS buffer, detection of the primary antibody was performed with a link antibody according to the manufacturer's instructions (DAKO LSAB 2 System, Peroxidase; Dako Corp., Carpinteria, CA). For MRP immunohistochemical staining (1, 3, 4), antigen retrieval was performed by treatment in a microwave oven for 5 min in citrate buffer at 700 W. Endogenous peroxidase was blocked by 3% hydrogen peroxide for 15 min followed by 5 min in PBS buffer. The sections were incubated overnight in a moist chamber at 4°C with primary antibody MRP QCRL-1 (10 $\mu\text{g}/\text{ml}$; Signet Laboratories, Inc., Dedham, MA) at a 1:100 concentration. Pgp and MRP expression was interpreted as follows by an experienced pathologist blinded to the clinical outcome: negative, <10% stained tumor cells; and positive, $\geq 10\%$ stained tumor cells (Refs. 10–13; Figs. 1 and 2).

Statistical Analysis. The results of T:B ratio calculations were expressed as the mean ± 1 SD. The differences in T:B ratios between the four groups were determined using ANOVA and *post hoc* tests. The differences in case

distribution among the four groups of patients for different clinicopathological parameters were assessed by χ^2 tests. If the P was < 0.05 , the difference was considered to be significant.

RESULTS

The patients' characteristics are summarized in Table 1. The case distribution among the four groups of patients for different clinicopathological parameters (menopausal status, TNM stage, grading, estrogen receptor content, and progesterone receptor content) are summarized. No significant differences in case distribution among the four groups were found based on the clinicopathological factors (all P s > 0.05). The T:B ratios of the groups were as follows: (a) group 1, 1.13 ± 0.10 (range, 1.01–1.30); (b) group 2, 1.30 ± 0.25 (range, 1.05–1.80); (c) group 3, 1.32 ± 0.26 (range, 1.03–1.78); and (d) group 4, 2.17 ± 0.14 (range, 1.90–2.40). Based on the ANOVA test, there was a statistically significant difference in T:B ratios among the four groups (F value = 65.951; $P < 0.001$). Based on *post hoc* tests, there were statistically significant differences in T:B ratios between group 1 and group 2 ($P = 0.038$), group 1 and group 3 ($P = 0.022$), group 1 and group 4 ($P < 0.001$), group 2 and group 4 ($P < 0.001$), and

Table 1 Characteristics of the four groups of patients with breast cancer

Clinicopathological parameters	Patients		Group			
	No.	Percentage	1	2	3	4
Menopausal status						
Premenopausal	27	67.5%	7	7	6	7
Postmenopausal	21	52.5%	5	5	6	5
T stage						
T ₁₋₂	24	60.0%	5	6	6	7
T ₃₋₄	24	60.0%	7	6	6	5
N stage						
N ₀₋₁	23	57.5%	6	5	6	6
N ₂	25	62.5%	6	7	6	6
M stage						
M ₀	33	82.5%	8	8	8	9
M ₁	15	37.5%	4	4	4	3
Grading						
I–II	28	70.0%	7	8	7	6
III	20	50.0%	5	4	5	6
Estrogen receptor						
Negative	26	65.0%	7	6	5	6
Positive	22	55.0%	5	6	7	6
Progesterone receptor						
Negative	23	57.5%	6	6	5	6
Positive	25	62.5%	6	6	7	6

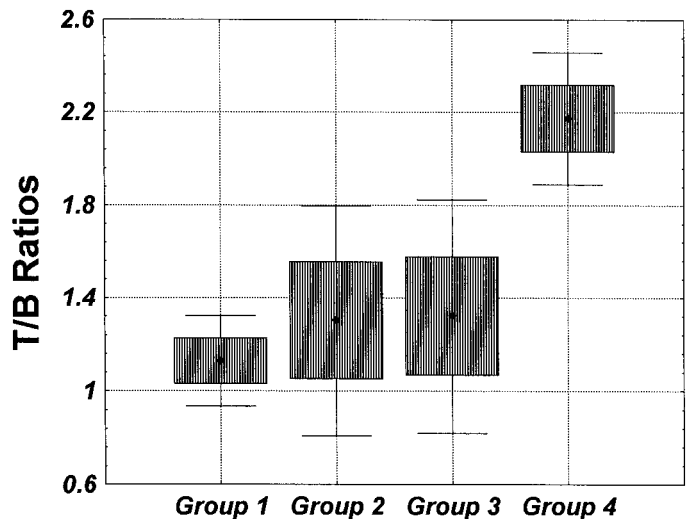


Fig. 3. The distribution of T:B ratios calculated from Tc-MIBI scintimammography in the four groups of patients. Bars, 1.96 SD; \square , 1.00 SD; \bullet , mean.

group 3 and group 4 ($P < 0.001$). However, no significant difference was found between group 2 and group 3 ($P = 0.816$; Fig. 3).

DISCUSSION

The incidence of breast cancer has been increasing recently in Taiwan. In patients with breast cancers, combination chemotherapy achieves high response rates and prolongs survival. Unfortunately, some patients become resistant to chemotherapeutic agents. Pgp and MRP are two important mechanisms operating as drug efflux pumps that are involved in breast cancer drug resistance to chemotherapy (1, 4). Because it is difficult to extract RNA from surgical breast specimens embedded and maintained in Tissue-Tek OCT (Miles Scientific; Ref. 3), we did not attempt to quantify Pgp or MRP RNA in these tumors. Many studies have analyzed Pgp or MRP expression by IHA. They provide specific information on the Pgp or MRP expression levels in breast cancer (1, 3, 4, 8, 9). IHA is subjective in interpretation; however, as we did in this study, evaluation of IHA by the same experienced pathologist may provide better comparisons, although the quantity of the antigen cannot be determined.

Nuclear medicine techniques that rely on the biochemical and physiological characteristics of a tumor have been used to evaluate breast cancer. Recently, Tc-MIBI has been suggested as a radiopharmaceutical with a great deal of promise in imaging breast cancer (14). Pgp and MRP, which together function as ATP-dependent efflux pumps, recognize certain chemotherapeutic agents as substrates and prevent the accumulation of lipophilic and cationic radiopharmaceuticals such as Tc-MIBI (5, 6). There have been reported differences in the methods of data acquisition and analysis between evaluation of Tc-MIBI washout (efflux rate constants; Refs. 15–17) and calculation of Tc-MIBI uptake (T:B ratios; Refs. 10 and 11), as in this study; however, there is no definite conclusion as to which one is better for correlation to Pgp expression. Because calculation of T:B ratios is less time-consuming and more easily available than evaluation of efflux rate constants in clinical practice, we selected semiquantitative T:B ratios from Tc-MIBI scintimammography to compare Pgp and MRP expression in human breast cancer. In addition, it is very time-consuming and almost impossible to calculate the actual percentage of Pgp and MRP cells by visual interpretation on IHA. Therefore, we could not evaluate the correlation with the actual percentage of Pgp or MRP cells and the T:B ratio of Tc-MIBI scintimammography.

In the current study, we found that tumor size, nodal status, and presence of metastatic disease (TNM stage); receptor status (estrogen receptor content); and histological grade did not correlate with Pgp or MRP expression at diagnosis (Table 1). Our findings were similar to and supported by those of some previous studies (3, 4). However, in the study by Filipits *et al.* (1), positive MRP staining was more frequent in T₃ and T₄ tumors than in T₁ and T₂ tumors. Although Pgp expression was reported to be associated with a shorter duration of overall survival of breast cancer patients (18), the prognostic and predictive significance of Pgp and MRP expression in breast cancers is still unknown. Thus, additional studies are required to determine whether Pgp and MRP expression in breast cancers affected disease-free survival and the response to chemotherapy. In addition, among the four groups, we found that the T:B ratios calculated from Tc-MIBI scintimammography were lowest in group 1 patients and highest in group 4 patients. The T:B ratios of groups 2 and 3 were between those of groups 1 and 4 (Fig. 3). Therefore, our results indicate that there is

both MRP and Pgp expression in primary breast cancers. This finding was also reported in a previous study (1).

In light of this consistent finding, Tc-MIBI scintimammography could be used as a noninvasive test for guiding treatment when Pgp or MRP expression is pertinent. However, some patients did not receive chemotherapy, and most of the cases were still alive when the study was completed; therefore, we did not calculate the correlation between disease-free survival or response to chemotherapy and Tc-MIBI scintimammography. We believe that the accuracy of our findings should be reproduced by additional studies with large numbers of patients.

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