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ORIGINAL ARTICLE

^{99m}Tc -MIBI scintigraphy as a functional method for the evaluation of multidrug resistance in breast cancer patients

S.B. Sergieva[1], K.V. Timcheva[2], N.D. Hadjiolov[3]

[1]Department of Nuclear Medicine, Sofia Cancer Centre, Sofia; [2]Department of Chemotherapy and [3]Department of Pathology, National Cancer Centre, Sofia, Bulgaria

Summary

Purpose: *To evaluate the clinical application of ^{99m}Tc-methoxyisobutylisonitrile (MIBI) scintigraphy as a functional method for assessment of multidrug resistance (MDR) in breast cancer patients and the correlation of these results with P-glycoprotein (P-gp) overexpression and objective response to chemotherapy.*

Patients and methods: *22 women, 35-68 years old with breast cancer, suitable for neoadjuvant chemotherapy were included onto this study. Two or three cycles of neoadjuvant chemotherapy were administered (FEC in 15 and CMF in 7 patients). Planar and SPECT ^{99m}Tc-MIBI scintigraphy was carried out before and after neoadjuvant chemotherapy. Focal ^{99m}Tc-MIBI uptake in breast cancer lesions was used as a scintigraphic criterion of abnormality. Tumor/background uptake (T/B Index) was calculated. Immunohistochemistry was carried out after surgery for P-gp detection in all cases. The degree of expression was evaluated according to semi-quantitative score analysis from 0 to 4.*

Results: *Planar imaging was true positive in 20 patients, false positive in 1 (with breast cancer and mastopathy), and false negative in 1 (with wide tumor necrosis and deep location in the breast). SPECT imaging was true positive in 21 patients and false positive in 1. In 3 patients with multifocal disease additional tumour masses were visualized using SPECT. Sensitivity was 95% (21/22) and 100% (22/22), respectively, for planar and SPECT detection of breast cancer. P-gp expression was positive in 40.8% of the patients and negative in 59.2%. Intense ^{99m}Tc-MIBI uptake was shown on the planar images in 21 patients independently of the P-gp expression. There was no significant relationship between T/B Index and P-gp detection. Objective response included 2 clinical complete remissions, partial response in 1 patient, minimal response in 12, and no change in 7. Some clinical results corresponded to ^{99m}Tc-MIBI scintigraphic data: after neoadjuvant chemotherapy T/B Index was reduced ≥ 20% in 9 patients with objective response.*

Conclusion: *SPECT is an important diagnostic approach for identification of breast cancer with deep location and satellite tumour spots in multifocal disease. T/B Index did not correlate with P-gp overexpression on baseline ^{99m}Tc-MIBI scan. Objective clinical results after neoadjuvant chemotherapy corresponded to scintigraphic results in 75% of the patients with minimal response.*

Key words: breast cancer, multidrug resistance, neoadjuvant chemotherapy, ^{99m}Tc-MIBI

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Author and address for correspondence:

Sonia B. Sergieva, MD, PhD
Department of Nuclear Medicine
Sofia Cancer Centre
Blvd "Andrey Saharov"1
PO Box 54
Sofia 1784
Bulgaria
E-mail: sergieva_s@yahoo.com

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Introduction

Neoadjuvant chemotherapy of breast cancer is a treatment method aiming at reducing tumour volume and realizing conservative surgery, as well as reaching early elimination of micrometastases [1]. This is determined by the fact that many tumours with a size more than 2 cm are metastatic at the time of diagnosis: more than 60% in the regional lymph nodes and more than 50% with distant micrometastases [2]. Good response during neoadjuvant chemotherapy has prognostic

significance concerning the disease outcome [3]. One of the reasons of treatment failure is MDR. MDR is a widely studied cellular transport – mediated resistance. This phenomenon, described as classic MDR, is characterised by [4-7]:

1. Cross-resistance to chemically unrelated drugs, mostly of natural origin, such as anthracyclines, vinca alkaloids, taxanes etc.
2. Decreased intracellular drug accumulation
3. Overproduction of plasma membrane glycoproteins (P-gps), due to overexpression of the MDR genes.
4. Susceptibility to reversal by certain agents (MDR modulators).

P-gps are plasma membrane glycoproteins of about 170 kDa and belong to the superfamily of ATP-binding cassette (ABC) transporters. They directly bind cytotoxic compounds and reduce intracellular drug accumulation through an energy-depended efflux mechanism. Technetium-99m MIBI is a substrate of the P-gp multidrug transporter encoded by the MDR-1 gene [8,9].

The purpose of this study was to evaluate the clinical application of 99mTc-MIBI scintigraphy as a functional method for assessment of MDR in breast cancer patients and to correlate these results with P-gp overexpression and objective response to chemotherapy.

Patients and methods

Twenty-two women, 35-68-year-old with mammographic and cytological tests positive for breast cancer, suitable for neoadjuvant chemotherapy were included onto this study. Their characteristics are listed in Table 1. TNM classification showed that 10 patients had T_2 lesions, 7 had T_3 and 5 had T_4 lesions; 19 of them were with axillary nodal involvement. In 3 patients distant metastases were diagnosed during neoadjuvant chemotherapy.

Neoadjuvant chemotherapy (2 or 3 cycles) was administered to all 22 patients using standard regimens: FEC: 5-fluorouracil 500 mg/m^2 + epirubicin 75 mg/m^2 + cyclophosphamide 500 mg/m^2, i.v. push day 1, every 3 weeks (15 patients); or CMF: cyclophosphamide 500 mg/m^2 + methotrexate 40 mg/m^2 + 5-fluorouracil 500 mg/m^2, i.v. bolus days 1 and 8, every 3 weeks (7 patients).

Planar and SPECT ^{99m}Tc-MIBI scintigraphy was carried out before and after neoadjuvant chemotherapy (740-925 MBq, administered i.v. in the arm ipsilateral to the normal breast). Focal tracer uptake in the breast lesions was used as the scintigraphic criterion of abnormality. T/B Index of early (10 min) images was calculated before and after neoadjuvant chemotherapy.

Immunohistochemistry was carried out after surgery for P-gp detection. Tissue samples from all 22 cases of invasive lobular and ductal primary breast cancer, as well as the respective axillary (I-III level) and internal mammary lymph nodes were examined by standard hematoxylin–eosin staining. In all patients immunohistochemistry was carried out using mouse monoclonal antibody p170 (p-Lipoprotein/MDR (Human) AB-2 (clone F4, Research Diagnostic Inc.) for the P-gp detection on formalin-fixed, paraffin-embedded tissues. The tissue sections (4mm) were rehydrated. The endogenous peroxidase activity was blocked by 3% H_2O_2 for 10 min. The tissue sections were washed with PBS 0.05 M (Tris-EDTA buffer). After washing with PBS, the sections were incubated for 10 min with primary antibody, which was diluted 1: 50 in 0.05 M Tris-EDTA buffer with pH 7.2-7.6. Then, incubation followed with biotinylated anti-mouse immunoglobulins in PBS for 10 min. Another incubation followed with streptavidin-peroxidase conjugate for 10 min; 3% 3-amino-9-ethylcarbasol in N,N-dimethyl- formamide was used as chromogene for 10 min. The cell nuclei were rehydrated and examined. The sections were incubated with PBS instead of primary antibody as negative control.

Microscopic analyses and immunohistochemical staining were evaluated semi-quantitatively by the number of the stained cells and formed the following categories:

1. (–) negative result: no stained cells or stained up to 5%.
2. (+) stained from 5% to 25%.
3. (++) stained from 25% to 50%.
4. (+++) stained more than 50%.

All negative controls appeared negative. All ambiguous controls were considered negative.

Results

Pretreatment planar imaging was true positive in 20 patients, false positive in 1 case (with breast cancer and mastopathy), and false negative in 1 patient (with wide tumour necrosis and deep location in the breast). SPECT imaging was true positive in 21 patients and false positive in 1 case. Additional tumour lesions were visualised in 3 patients with multifocal breast cancer on the tomographic slides (Figure 1). Sensitivity was 95% (20/21) and 100% (21/21), respectively, for baseline planar and SPECT primary cancer detection. Axillary lymph node metastases were visualised

Table 1. Patient characteristics

Pt No.	*Age (years)*	*TNM Stage*	*Histology Tumour grade (G)*	*ER (fmol/mg)*	*PR (fmol/mg)*	*T/B index before CT*	*CT*	*T/B index after CT*	*P-gp*	*Clinical effect on the breast tumour*
1.	54	T3N2M1 supraclav. LN metastases IV	Invasive ductal G2	73	41	1.96	FECx2	1.57	–	35% reduction
2.	57	T4N1M0 III-B	Invasive ductal G2	64	34	1.74	FECx2	1.67	+++	No change
3.	57	T2N1M0 II-B	Invasive lobular G3	negative	negative	1.94	FECx2	1.76	++	25% reduction
4.	38	T2N1M0 II-B	Invasive lobular G3	164	161	1.82	CMFx2	1.79	–	No change
5.	48	T4N1M1 bone mets IV	Invasive ductal G2	negative	negative	1.50	FECx3	1.35	+++	No change
6.	64	T3N2M1 lung mets IV	Invasive ductal G2	19	177	1.83	FECx3	1.73	–	25% reduction
7.	65	T2N2M0 III-A	Invasive lobular G3	46	57	1.37	FECx2	1.26	+++	No change
8.	38	T3N1M0 III-A	Invasive ductal G2	negative	negative	1.29	FECx3	1.08	–	Complete remission
9.	35	T4N1M0 III-B	Invasive ductal G3	negative	negative	2.00	FECx3	1.80	+++	25% reduction
10.	66	T2N0M0 II-A	Invasive ductal G3	51	negative	2.17	CMFx2	1.61	++	30% reduction
11	51	T3N1M0 III-A	Invasive ductal G3	114	56	1.32	FECx3	1.29	–	25% reduction
12.	61	T3N1bM0 III-A	Invasive ductal G3	69	negative	1.89	CMFx2	1.56	–	50% reduction
13.	45	T4bN1M0 III-B	Invasive lobular G3	negative	144	1.16	CMFx2	1.01	+++	No change
14.	45	T2N0M0 II-A	Invasive lobular G2	65	negative	1.55	FECx3	1.20	–	Complete remission
15.	56	T2N0M0 II-A	Invasive ductal G3	negative	115	1.51	CMFx2	1.50	–	20% reduction
16.	41	T2N1M0 II-B	Invasive ductal G2	negative	negative	1.60	FECx2	1.40	–	25% reduction
17.	57	T3N1M0 III-A	Invasive ductal G2	negative	211	1.38	FECx3	1.12	–	20% reduction
18.	49	T2N1bM0 II-B	Invasive lobular G3	negative	negative	1.61	CMFx2	1.60	++	No change
19.	45	T4bN1M0 III-B	Invasive ductal G2	78	120	2.95	FECx3	2.52	–	25% reduction
20.	46	T2N2M0 III-A	Invasive ductal G3	16	162	1.55	FECx2	1.50	++	No change
21.	68	T3N2M0 III-A	Invasive ductal G3	118	34	1.48	CMFx3	1.24	–	35% reduction
22.	48	T2N1M0 II-B	Invasive ductal G3	916	negative	1.60	FECx2	1.30	–	25% reduction

CT: chemotherapy, LN: lymph nodes, ER: estrogen receptor, PR: progesterone receptor, P-gp: P-glycoprotein

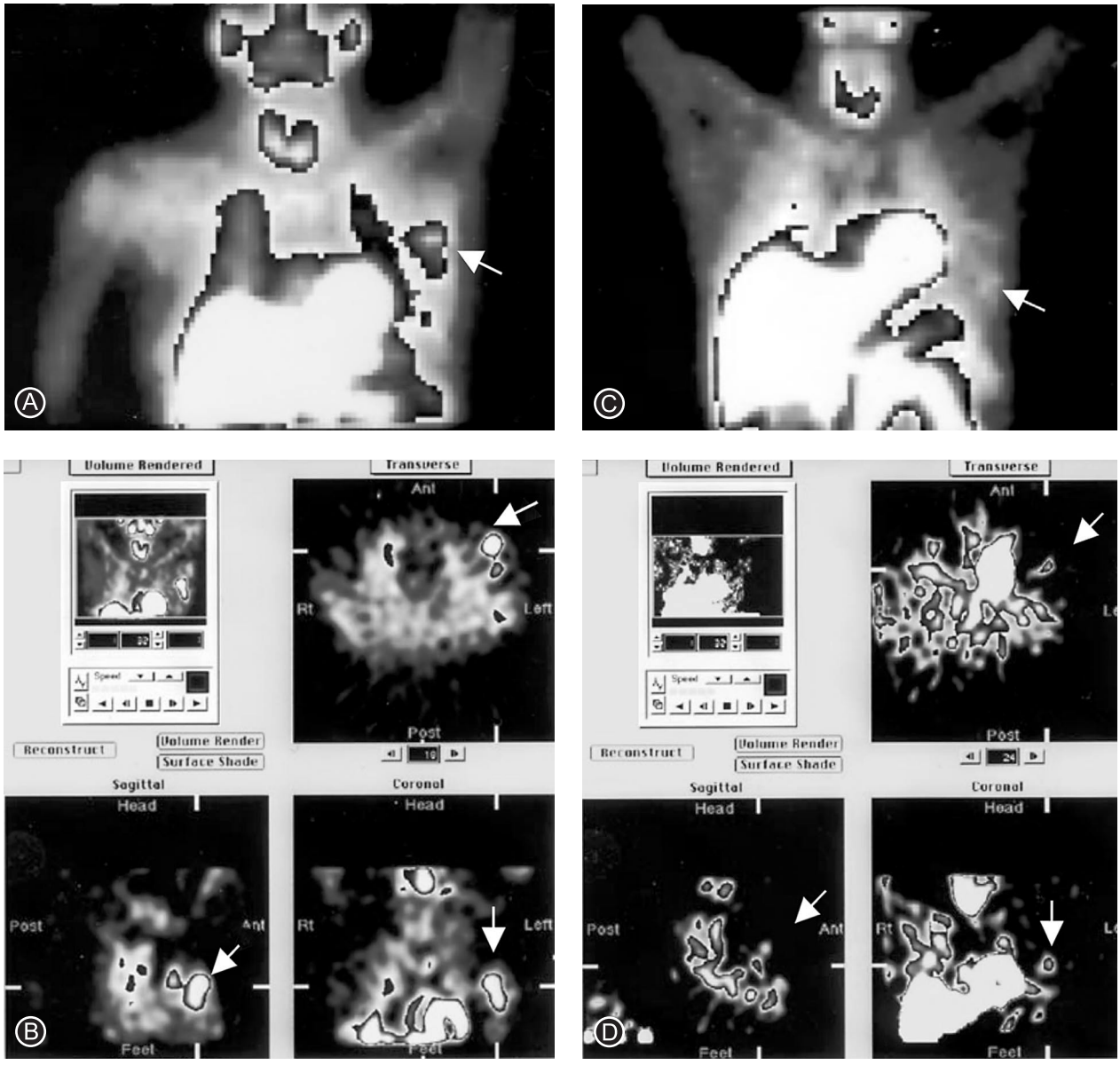

Figure 1. Baseline planar (A) and SPECT (B) ^{99m}Tc-MIBI scintigraphy of one of the patients before neoadjuvant chemotherapy (No.14 in Table 1). Anterior planar and tomographic images demonstrated an intensive focal accumulation of the tracer in the region of the left breast (A and B; arrows). SPECT imaging correctly assessed multifocal breast cancer in this case (B; arrows). Tumour uptake was equal to the normal tissue uptake on repeat planar (C) and SPECT (D) ^{99m}Tc-MIBI scintigraphy (C and D; arrows) after neoadjuvant chemotherapy, significant for the disease complete remission.

together with the primary tumour in 9/19 patients with nodal involvement (Figure 2). Pre-chemotherapy ^{99m}Tc-MIBI uptake in the tumour was intensive in the early planar images and showed marked retention on the tomographic slides in 20 cases independently of the P-gp expression. In 16 cases T/B Index was ≥1.50 (Table 1). P-gp overexpression was positive in 40.8% and negative in 59.2% of the patients. There was no correlation of P-gp expression and type of histology and/or hormonal receptors. Neither P-gp overexpression nor receptor status of tumours showed a significant relationship with T/B Index on the baseline investigations. Objective response included 2 clinical complete remissions (Figure 1), partial remission in 1 patient (Figure 2), minimal response in 12 and no change in 7 (Figure 3).

Some clinical results corresponded with post-therapeutic ^{99m}Tc-MIBI uptake (Table1). Decreased ^{99m}Tc-MIBI uptake was associated with response to therapy while progressive disease was correlated with stable or increasing tracer uptake. In the 2 patients with clinical complete response after 3 cycles of FEC, T/B Index corresponded to a tumour activity uptake equal to the normal tissue uptake; P-gp expression was nega-

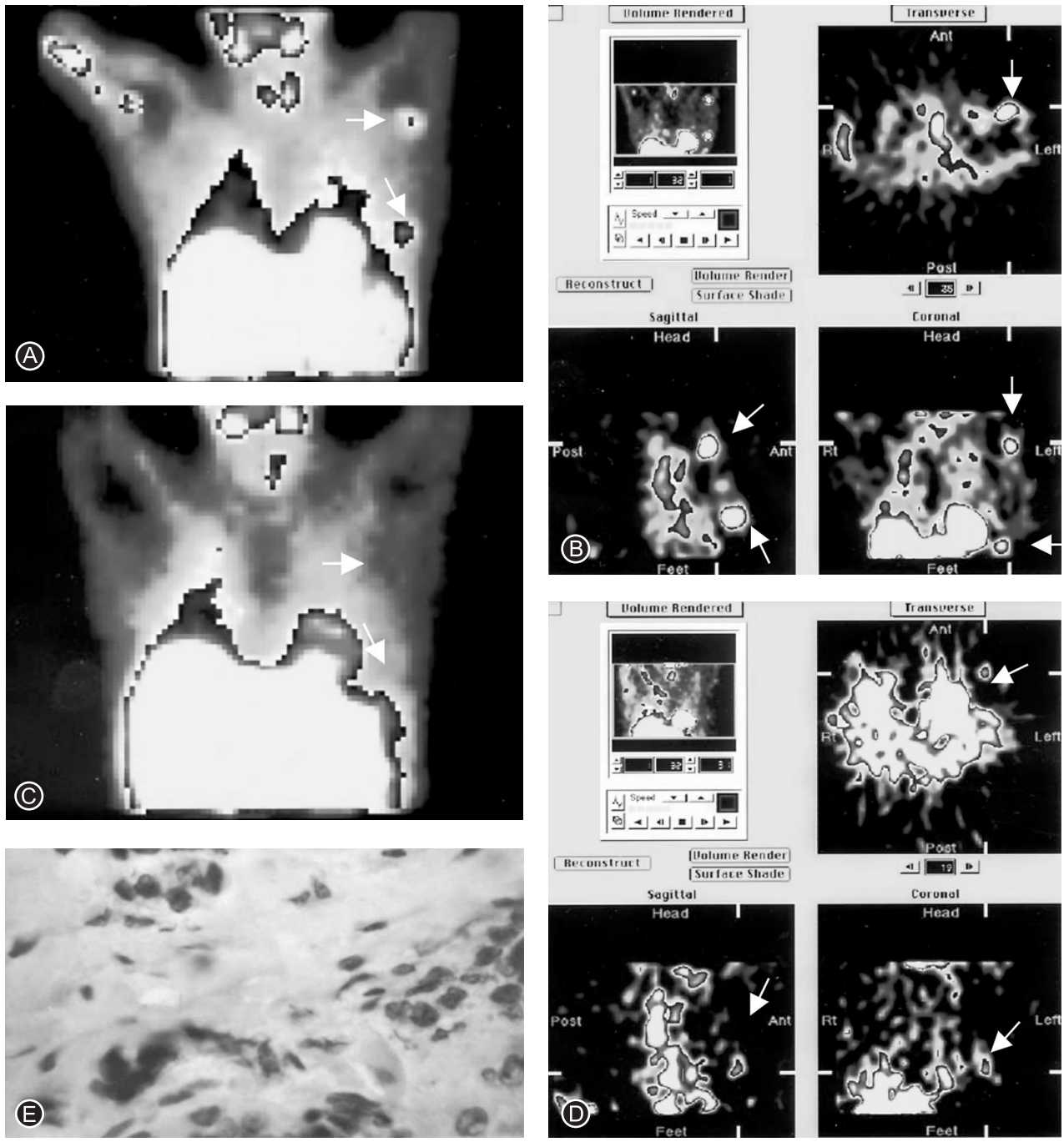

Figure 2. Baseline planar (A) and SPECT (B) ^{99m}Tc-MIBI scintigraphy of one of the patients before neoadjuvant chemotherapy (No.12 in Table 1). Anterior planar and tomographic images demonstrated an intensive focal accumulation of the tracer in the region of the lower lateral segment of the left breast and in the left axillary lymph nodes (A and B; arrows). In the region of the right axilla, high background of non-specific tracer uptake was visualised because of the i.v. application in the right arm vein. There was reduction more than 50% of the tumour and negative image of the axillary lymph node on the repeat planar (C; arrows) and SPECT (D; arrows) ^{99m}Tc-MIBI scintigraphy after neoadjuvant chemotherapy, significant for the partial remission of the disease. Immunohistochemistry was negative for P-gp overexpression (E).

tive (Figure 1). In 7 patients with no change of disease (Figure 3) and 3 patients with minimal response, T/B Index was the same or with minimal reduction: ≤ 0.15. It is more difficult to interpret the results of the remaining 10 patients with partial (Figure 2) and minimal response after neoadjuvant chemotherapy – in 7 cases P-gp expression was negative, in 3 cases P-gp was positive and T/B Index was reduced ≥0.20.

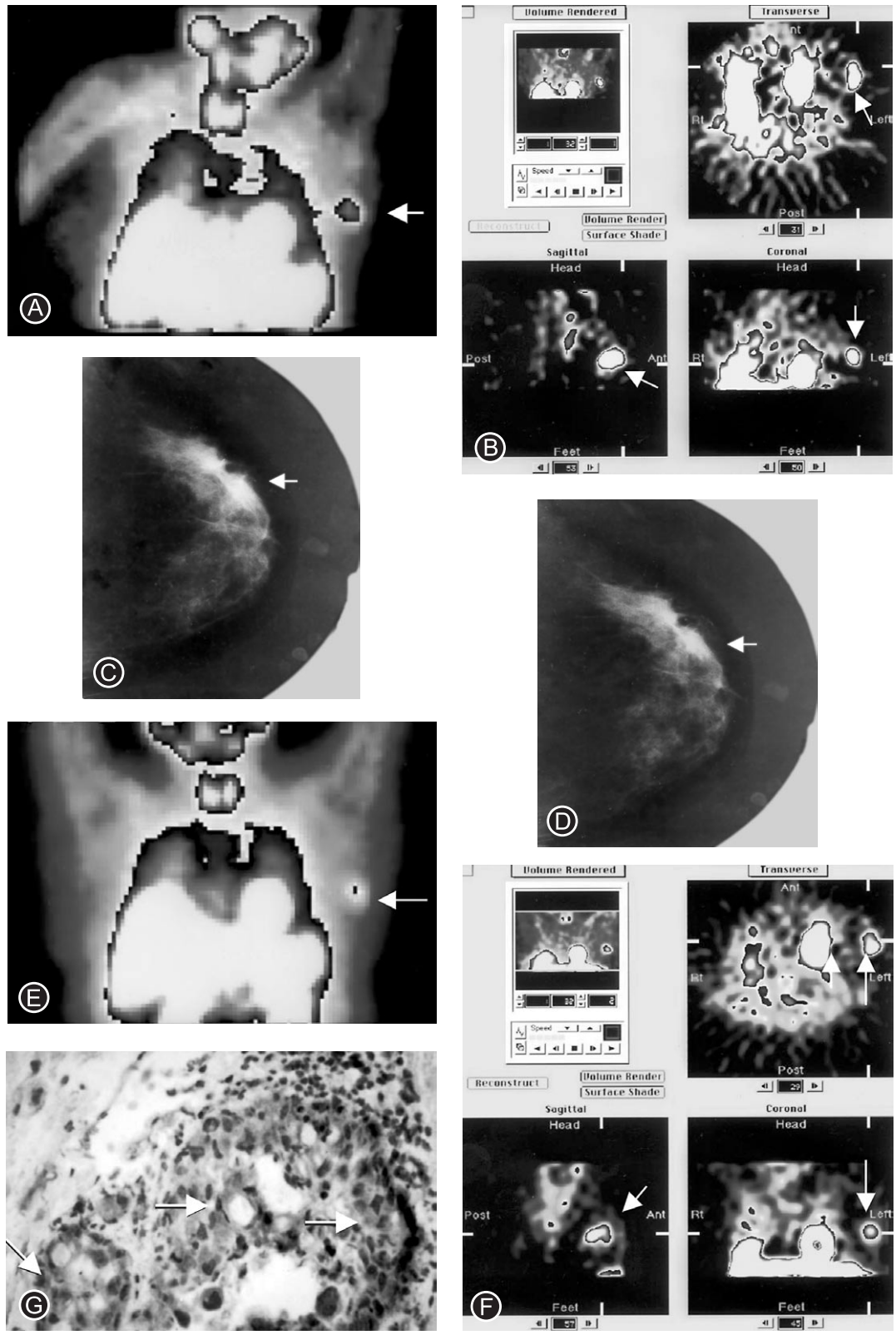

Figure 3. Baseline planar (A) and SPECT (B) ^{99m}Tc-MIBI scintigraphy of one of the patients before neoadjuvant chemotherapy (No.5 in Table1). Anterior planar and tomographic images demonstrated an intensive focal accumulation of the tracer in the region of the lateral segment of the left breast (A and B; arrows). Lateral mammogram (C) of the left breast showed two confluenting foci of cancer (arrow). There was no change on the repeat mammogram (D; arrow), planar (E; arrow) and SPECT (F; arrows) ^{99m}Tc-MIBI scintigraphy in the region of the tumour of the left breast after neoadjuvant chemotherapy, significant for the progression of the disease. Immunohistochemistry was very positive for P-gp overexpression (G; arrows).

Discussion

No significant correlation between P-gp overexpression and T/B Index obtained before and after neoadjuvant chemotherapy was found in our patients. Our results are similar to data published recently [10-12]. Nevertheless, it was seen that tracer uptake was reduced ≥ 0.20 in 54.5% (12/22 patients – in 2 cases with complete response, in 1 case with partial response and in 9 cases with minimal response). This suggests that ^{99m}Tc-MIBI scintigraphy may be used for response monitoring purposes. These results are similar to the data obtained in other studies [13-15].

Results from this analysis should be interpreted with caution because the number of patients in each chemotherapy group (FEC or CMF) is small and, in addition, patients in each group differed with regard to tumour stage, type of tumour lesion (ductal or lobular) and P-gp overexpression. Tracer distribution, uptake and retention generally depend on several factors such as neovascularisation and tumour metabolism [16-18]. The fact that non-MDR drugs (methotrexate, 5-fluorouracil, cyclophosphamide) are part of chemotherapy may complicate the analysis of studies correlating expression of P-gp and response to chemotherapy [13,14].

The data of the present study confirm that SPECT acquisition improves sensitivity of planar ^{99m}Tc-MIBI scintigraphy [18]. SPECT imaging correctly assessed multifocal disease in 3 patients and diagnosed additional tumour lesions [19]. In one patient planar imaging missed a primary breast cancer with deep location near the chest wall and poor vascularisation.

The limited use of 99mTc-MIBI scintigraphy for axillary lymph node staging may be improved by applying preoperative lymphatic mapping combined with gamma probe-guided biopsy of the sentinel node in breast cancer, especially in non-palpable nodes and in the presence of a small number of metastases. However, axillary lymph node dissection remains the method of choice for the detection of lymph node metastases in patients with breast cancer with a size more than 2 cm [19].

In the past decade a number of studies on the predictive role of ^{99m}Tc-MIBI scintigraphy in breast cancer and other malignancies have been published [12,13,15,20-24]. Different patterns of ^{99m}Tc-MIBI application were described but most of them suggest that the wash-out rate of this tracer related to P-gp expression rate [12,14,21,25].

It is possible to overcome MDR *in vivo* with different MDR modulators, but the dose of these modulators should be very high to reach the plasma levels needed for blocking the efflux pump. Side effects of these drugs are the main disadvantage that limits the use of MDR modulators in clinical practice [26]. It is very important to utilize non-cross resistant drugs in the chemotherapy of breast cancer patients with MDR and to avoid administration of MDR-inactivated anthracyclines [27].

Further multicentric clinical trials are needed to evaluate the efflux rate of ^{99m}Tc-MIBI as a predictive factor for P-gp transport activity in breast cancer patients before and after neoadjuvant chemotherapy.

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