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SHORT REPORT

Relationship between prognostic factors of breast cancer and ^{99m}Tc-sestamibi uptake in patients who underwent scintimammography: Multivariate analysis of causes of false-negative results

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Summary The complementary role of sestamibi scintimammography (SSM) in patients with breast cancer (BC) is well established. The aim of this study was to establish whether a relationship exists between sestamibi uptake, evaluated as a tumour-to-background ratio (TBR), and the main prognostic factors of BC. SSM with the measurement of TBR was performed preoperatively in 102 women (median age 57 years, range 32–81 years) who underwent curative surgery for primary BC. Final pathology showed 4 (3.9%) with pT1a, 17 (16.7%) with pT1b, 44 (43.1%) with pT1c and 37 (36.3%) with pT2 breast carcinomas. The overall sensitivity of SSM was 80.4%. An ANOVA showed significant ($P<0.01$) differences between the TBR of patients with G1 vs. G3 tumours, and between the TBR of those with G2 vs. G3 breast carcinomas. Moreover, there was a difference ($P=0.021$) between the TBR of patients ($n=12$, 11.8%) with CEA serum levels >10 ng/ml (2.031 ± 0.420), and those with normal ($n=90$, 88.2%) CEA values (1.713 ± 0.446), whilst no difference ($P=$ NS) was found between patients ($n=27$, 26.5%) with CA 15-3 >30 U/ml (1.893 ± 0.401) and those with normal ($n=75$, 73.5%) CA 15-3 values (1.699 ± 0.462). There was a mild inverse correlation between TBR and both the oestrogen ($R=0.25$, $P=0.011$) and the progesterone receptor ($R=0.23$, $P=0.02$) rate. The logistic regression analysis

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showed that only size and CA 15-3 serum levels represent true independent parameters, but the function was able to predict only 11 out of 21 (52.4%) patients with false-negative SSM. TBR is independent of age and mainly correlates with the size of the tumour. There are no reliable preoperative prognostic factors that are really useful for improving SSM sensitivity in patients with small breast carcinomas.

Introduction

Breast cancer (BC) is a significant global public health problem and a progressive disease. Early diagnosis of BC decreases mortality and facilitates conservative therapy. Mammography (MG) is the most widely used diagnostic technique to detect BC, but is not always decisive in differentiating between benign and malignant lesions, especially in young women, patients with dense breast tissue, scars and implants, or in those who have undergone previous radiation therapy.[1–3] The reported rates of false-positive results with MG affect both specificity and positive predictive values (PPV). The subsequent unnecessary surgical procedures, usually performed under ultrasound or stereotactic guidance, result in a large number of non-malignant specimens, with several economic and psychological consequences.[4–7] ^{99m}Tc sestamibi scintimammography (SSM) is a reliable diagnostic tool in BC detection, especially in premenopausal women. It has a higher specificity compared with MG, and its complementary role in surgical planning is well established.[4,7–9] The aims of this study were to establish whether a relationship exists between sestamibi uptake, evaluated as a tumour-to-background ratio (TBR) and the main prognostic factors of BC, and to analyze the causes of the false-negative results of SSM.

Materials and methods

Study population and tumour staging

We retrospectively reviewed data regarding a series of 102 consecutive women (median age 57 years, range 32–81 years) who had undergone curative surgery for primary BC. There were 33 (32.4%) premenopausal and 69 (67.6%) menopausal patients. None of the patients had undergone preoperative chemotherapy, and none had evidence of multicentric BC at final pathology. According to the American Joint Committee on Cancer (AJCC), tumour size (pT) was defined as the maximum diameter measured by the pathologist, and lymph node involvement (pN1) was histologically confirmed.[11] The presence of distant metastases was excluded by routine laboratory tests, liver ultrasound, standard chest X-ray and bone scanning.

CEA and CA 15-3 levels were determined by automated test systems using a two-site enzyme-linked immunosorbent assay (ELISA, monoclonal antibody). A cut-off limit of 10 ng/ml (CEA) and 30 U/ml (CA 15-3) was set as recommended by the kit manufacturer and previously described.[12,13] Oestrogen (ER) and progesterone (PgR) receptors were assayed using a quantitative standard immunoenzymatic method, and the results were expressed as a percentage of positivity in the overall cell population. Immunostaining of Ki-67 antigen was performed using the monoclonal antibody MIB-1 using a microwave antigen retrieval technique, and the MIB-1 labelling index was expressed as a percentage.[13] Histological grade (G) was defined according to the Scarff–Bloom–Richardson classification.

According to the TNM classification, the tumours were classified as follows: pT1a = up to 5 mm in size, pT1b = 6–10 mm and pT1c = 11–20 mm. Table 1 reports the main clinical, biochemical and pathological parameters of the overall population.

Scintimammography

Once the patients had given their informed consent, they underwent SSM with measurement of TBR 6–15 days prior to surgery. SSM was performed according to the procedure guidelines suggested by the Society of Nuclear Medicine.[14] Patients laid in the prone position, a single dose (750 MBq) of ^{99m}Tc-methoxyisobutylisonitrile (sestamibi) was injected intravenously into the arm contralateral to the side of the lesion, and the images were acquired within 5–10 min. A single detector gamma camera (Sopha Medical DSX) equipped with a parallel-hole low-energy high-resolution collimator, a 256×256 matrix and a 140 keV (10% window) was used, as previously described.[9,10] Planar (anterior, prone lateral and posterior oblique) projections were obtained in the first 11 (10.8%) patients, and a SPET technique

Table 1 Main clinical, biochemical and pathological parameters of the overall population (mean ± S.D).

Parameter		Parameter	
Age (years)	58.1 ± 13.9	N0	61 (59.8%)
Side (left vs. right)	46/56	N1	27 (26.5%)
Size (mm)	16.6 ± 6.6	Nx	14 (13.7%)
pT1a	4 (3.9%)	Number of positive nodes	4.5 ± 3.3
pT1b	17 (16.7%)	CEA (ng/ml)	5.6 ± 3.5
pT1c	44 (43.1%)	CA 15-3 (U/ml)	22.0 ± 12.6
pT2	37 (36.3%)	CEA > 10 (ng/ml)	10 (9.8%)
G1	34 (33.3%	CA 15-3 > 30 U/ml	25 (24.5%)
G2	50 (49.0%)	ER (%)	64.1 ± 27.5
G3	18 (17.7%)	PgR (%)	53.9 ± 29.3

Size = maximum diameter of the tumour measured by the pathologist, G = histological grading, ER = oestrogen receptor rate, PgR = progesterone receptor rate.

(128 × 128 matrix, 64 steps, 30 s/step) was available for 91 (89.2%) patients. The results of SSM were considered positive for BC when the uptake of the radiopharmaceutical, evaluated as TBR, was $\geqslant 1.4$, while a lower value was considered non-significant and SMM was classified as negative.[9] Sensitivity was defined as true-positives/true-positives+false-negatives.

Statistical analysis

The reported data are expressed as mean ± standard deviation (S.D.). Comparisons between different groups were performed using the two-tailed Student's *t*-test for means (i.e., biochemical parameters), and the chi-squared (χ^2) test. The Spearman's correlation (R) coefficient calculation was used to evaluate the linear relationship between age, biochemical parameters and size of the tumour vs. TBR. Analysis of variance (ANOVA) and post hoc (LSD test) analysis were also used. The identification of factors independently predicting true-positive results was obtained by using a logistic regression model. The parameters found to be significant related to the TBR in the univariate analysis were assessed for the multivariate analysis using the regression model, fitted using the method of maximum likelihood. The relative odds ratio (OR) was also calculated. A value of $P < 0.01$ was considered to be statistically significant.

Results

The overall sensitivity of SSM was 80.4%. No breast carcinomas of less than 7 mm were correctly detected by SSM. ANOVA showed significant ($P < 0.01$) differences between the TBR of patients with G1 (median 1.604) vs. G3 (median 2.261) tumours, and between the TBR of those with G2 (median 1.671) vs. G3 BC. There was a significant ($P = 0.021$) difference between the TBR of patients ($n = 12$, 11.8%) with CEA serum levels > 10 ng/ml (2.031 ± 0.420), and those with normal ($n = 90$, 88.2%) CEA values (1.713 ± 0.446), whilst no difference ($P = 0.56$) was found between patients ($n = 27$, 26.5%) with CA 15-3 > 30 U/ml (1.893 ± 0.401) and those with normal ($n = 75$, 73.5%) CA 15-3 values (1.699 ± 0.462).

There was no correlation between TBR and (1) the age of the patients ($R = 0.09$, $P = 0.39$), (2) their menopausal status ($P = 0.55$), and (3) the axillary node involvement ($P = 0.47$). We found a positive correlation between TBR and (1) the size of the tumour ($R = 0.32$, $P < 0.01$), (2) Ki-67 antigen expression ($R = 0.23$, $p = 0.02$), and (3) the CEA ($R = 0.30$, $P = 0.002$) and CA 15-3 ($R = 0.22$, $P = 0.03$) serum levels. There was a mild inverse correlation between TBR and both the oestrogen ($R = -0.25$, $P = 0.011$) and progesterone receptor ($R = -0.23$, $P = 0.02$) rate. Table 2 shows the results of the analysis of true-positive and false-negative SSM.

The post-hoc analysis (LSD test) showed that size, CEA, and CA 15-3 were significantly ($P < 0.01$) different between patients with false-negative SSM and those with true-positive results, while their ages did not differ ($P = 0.87$). The logistic regression analysis showed that only size and CA 15-3 serum levels represent true independent parameters, but the function was able to predict only 11 out of 21 (52.4%) patients with false-negative SSM. The resulting OR was 7.81 (95% CI: 2.6–23.0).

Table 2 Differences between patients with true-positive and false-negative SSM (*χ^2 test).

Parameters	True positives (TBR⩾1.4)	False negatives (TBR<1.4)	*P* value
No. of the patients	81 (79.4%)	21 (20.6%)	—
Age (years)	58.0±14.2	58.3±12.9	0.93
Side (left vs. right)	34/47	12/9	0.78*
Size (mm)	18.1±6.0	12.0±4.8	<0.01
pT1a	0	4 (100%)	—
pT1b	10 (58.8%)	7 (41.2%)	0.76*
pT1c	34 (77.3%)	10 (22.7%)	0.003*
pT2	37 (100%)	0	—
G1	23 (67.6%)	11 (32.4%)	0.08*
G2	41 (82.0%)	9 (18%)	0.002*
G3	17 (94.4%)	1 (5.6%)	0.001*
N0	47 (77.0%)	14 (23%)	0.005*
N1	23 (85.2%)	4 (14.8%)	0.002*
Number of positive nodes	4.6±3.3	4.0±3.5	0.46
CEA (ng/ml)	6.0±3.7	4.0±2.5	0.02
CA 15-3 (U/ml)	24.0±12.9	14.1±7.0	0.001
CEA>10 ng/ml	10 (12.3%)	0	—
CA 15-3 >30 U/ml	24 (96%)	1 (4%)	0.001*
ER (%)	61.7±30.0	73.7±6.8	0.07
PgR (%)	51.5±30.9	63.5±19.1	0.09
MIB-1 (%)	23.9±17.3	16.7±15.0	0.08

Size = maximum diameter of the tumour measured by the pathologist, G = histological grading, ER = oestrogen receptor rate, PgR = progesterone receptor rate, MIB-1 = Ki-67 antigen positivity rate.

Discussion

It is well established that tumour size, nuclear grading, steroid receptor status, and several biological parameters of tumour proliferation may relate to BC behaviour. Other variables, such as serum tumour markers, have low sensitivity, especially in early stage BC, and their usefulness is still under discussion.[12,13,15–17]

The main modalities for detecting BC are MG and ultrasonography, and both these tests utilize anatomical approaches. SSM, however, utilizes a fundamentally different approach to detect BC, since it is a functional imaging modality. Unfortunately, MG is not useful for predicting the prognosis of patients with BC, and, moreover, has a low PPV and a false-negative rate of more than 20% in young and pre-menopausal women.[1–3,6,10] The detectability of SSM is well established and its complementary role, in conjunction with MG, as a diagnostic tool in patients with BC has already been confirmed.[9,10,18] The sensitivity of SSM for detecting BC is lower than that of MG, ranging between 80% and 85% in large series.[18,19] It is even lower when applied in a population with non-palpable or <1.0-cm breast tumours, and it is not a reliable method for the latter.[20] However, a recent meta-analysis showed that SSM has a sensitivity of 88%, a specificity of 87% and an accuracy of 88% in patients with palpable BC.[8] Moreover, the sensitivity can be partially improved by using a high-resolution breast-specific gamma camera. Rhodes et al.[21] obtained an overall sensitivity of 92% in patients with breast lesions smaller than 2 cm on a mammogram, and a sensitivity of 86% for the detection of lesions 1 cm or smaller in diameter.

The size of the tumour plays a major role in the accuracy of SSM, and no breast carcinomas smaller than 5 mm have ever been directly visualized using the imaging techniques currently available.[19,22] Cwikla et al.[23] reported a correlation between TBR and both age of the patients and size of the tumour. Tofani et al.[22] reported that SSM has a better sensitivity in premenopausal women, while specificity was higher in postmenopausal ones. We did not find a significant correlation between age and SSM accuracy, but we did find a relationship between BC aggressiveness and the expression of Ki-67 (MIB-1 rate), which correlated with TBR in the univariate analysis but was unconfirmed in the regression model. Similarly, ER and PgR status were found to be negatively correlated to TBR. These findings confirm previous studies suggesting that cell proliferation and tumour growth rate do not represent an important regulating factor of sestamibi uptake, which is strongly correlated to the

number of metabolically active neoplastic cells.[23,24] Moreover, we found a relationship between TBR and both CEA and CA 15-3 serum levels, together with size of the tumour, in the univariate analysis. However, the logistic regression showed that CEA was not a true independent parameter. In our study the size of the tumour was the main parameter affecting SSM results, but the combination of size and CA 15-3 was able to predict only 55% of patients with false-negative SSM.

In conclusion, TBR is independent of age and mainly correlates with the size of the tumour. There are no reliable preoperative prognostic factors that are really useful for improving SSM sensitivity in patients with small breast carcinomas.

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