

## Hormone Receptor Rate, MIB-1 Score and Serum Tumour Markers CEA and CA 15-3 Relationship in Elderly Women with pT1-2 Breast Cancer

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**Abstract.** *The aim of this study was to analyse whether preoperative serum levels of CEA and CA 15-3 correlate with oestrogen (ER) and progesterone (PR) receptor rate and MIB-1 score in elderly (>65 years) women with breast cancer (BC). Data from a series of 349 women (median age 61 years, range 26-89) with pT1-2 BC who underwent curative surgery were reviewed. Patients were divided into two groups: Group A, 237 (60.2%) women <65 years, and Group B, 157 (39.8%) women >64 years. Size of the tumour, ER, PR, CEA and CA 15-3 preoperative serum levels were higher in older patients, while the MIB-1 rate was lower. In both groups, a significant ( $p<0.05$ ) inverse correlation between ER and MIB-1 was found, while there was a relationship between MIB-1 and both PG and CA 15-3 only in younger patients. These data suggest that only MIB-1 index should be considered an effective parameter for assessing tumour proliferation.*

Breast cancer (BC) is the second leading cause of cancer-related deaths, and more than 194,000 new cases of BC were reported in the USA in 2009 (1, 2). Despite advances in its diagnosis and therapy, BC remains one of the main causes of death in women over the age 50 (3, 4). Patients with BC may potentially develop disease progression and will thus need an effective lifelong follow-up (5). BC is common in elderly (>64 years) women, but the relationship between

serum tumour markers, proliferation markers, oestrogen receptor (ER) and progesterone (PR) receptor rate is unclear. The aim of this study was to analyse whether preoperative serum levels of tumour markers carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3) correlate with well-established prognostic variables, such as ER, PR and mindbomb homolog 1 (MIB-1), a monoclonal antibody found in patients with BC, according to age.

### Patients and Methods

A consecutive series of 349 women (median age 61 years, range 26-89 years) with pT1-2 BC who underwent curative surgery was evaluated. Entry criteria for this retrospective study included: (i) no history of previous cancer, (ii) no evidence of multicentric BC or distant metastases and (iii) no multifocal BC at final pathology. According to the American Joint Committee on Cancer tumour size was defined as the maximum diameter measured by the pathologist, while the presence of distant metastases was excluded by standard chest x-ray, liver ultrasound and whole-body bone scintigraphy (6). Informed consent was obtained from all participants who were alive at the time of the retrospective study, in accordance with institutional review board approval.

Patients were divided into two groups: Group A, 237 (60.2%) women <65 years (median age 53 years, range 26-64 years); and Group B, 157 (39.8%) women >64 years (median age 74 years, range 65-89 years). The following data were recorded: age of the patients (years), tumour size (mm), CEA and CA 15-3 baseline serum levels, ER and PR rate and MIB-1 labelling index.

CEA and CA 15-3 serum levels were determined by automated testing using a two-site enzyme-linked immunosorbent assay (ADVIA Centaur® XP Immunoassay System, Siemens, München, Germany). The cut-off limit of 10 ng/ml (CEA) and 30 U/ml (CA 15-3) was used, as previously described (3, 7). Both ER and PR were assayed using a quantitative standard immunoenzymatic method and results were expressed as percentage of positivity in the overall cell population. The immunostaining was obtained for both ER and PR in samples from all patients. The immunohistochemical assay (IHA) was performed on 4 µm sections cut from the blocks; retrieving the antigen; blocking the endogenous peroxidase and non-specific proteins; binding with primary mouse monoclonal antibody

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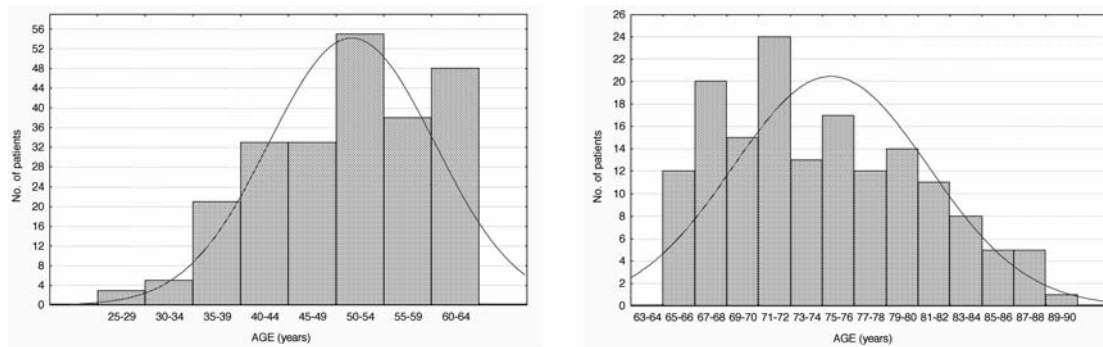


Figure 1. Different age distribution in Groups A (left) and B (right).

against ER and PR; and linking with biotinylated rabbit antibody against mouse immunoglobulin G (8, 9). The specimens were stained manually. Immunostaining of the 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 (5). Histological grade was defined according to the modified Bloom-Richardson classification (10). Monoclonal antibody MIB-1 and polyclonal Ki-67 appeared to be the best antibody reagents available for measuring proliferation fraction in conventionally processed tissues in diagnostic immunopathology. Multiple studies have been performed which utilised the immune reactivity of MIB-1 to determine the growth fraction of malignant tumours (11).

The reported data are expressed as mean $\pm$ standard deviation (SD). Differences between means were tested by Student's *t*-test. The Pearson's correlation coefficient (R) calculation was also used to evaluate the linear relationship between pairs of variables. The differences were considered significant at a *p*-value <0.05.

## Results

The main pathological and biochemical data are reported in Table I. As expected, a significant (*p*<0.05) relationship between (i) size of the tumour and CA 15-3 (*R*=0.21 and 0.25), (ii) ER and PR (*R*=0.74 and 0.70) and (iii) an inverse correlation between MIB-1 and ER (*R*=-0.38 and -0.41) was found in the two groups. Moreover, there was a correlation (*p*<0.05) between MIB-1 and both PR (*R*=35) and CA 15-3 (*R*=21) only in younger patients (Figures 1 and 2), and between CEA and CA 15-3 (*R*=0.71) only in Group B.

## Discussion

Despite many advances, the management of BC remains difficult. Several different prognostic parameters have been used by clinicians in the decision-making management of patients with BC, particularly in those without axillary node involvement (12). TNM staging, pathological grade and hormone receptor status are well known to be related to the prognosis of BC (5). However, prognostic factors are given

Table I. Main pathological and biochemical data, and *p*-value between groups.

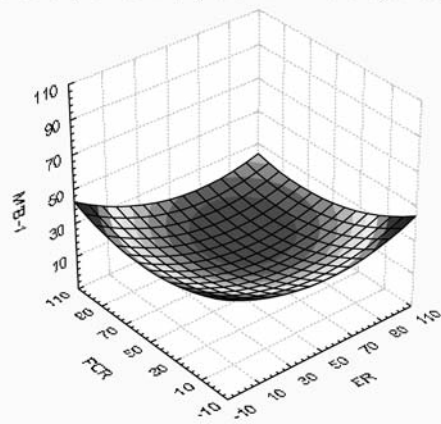
	Overall population	Group A	Group B	<i>p</i> -Value
No. of patients	393	236 (60.1%)	157 (39.9%)	-
Age (years)	60.8 $\pm$ 13.8	51.6 $\pm$ 8.7	74.5 $\pm$ 6.1	<0.001
Size (mm)	21.0 $\pm$ 13.8	19.9 $\pm$ 13.6	22.7 $\pm$ 14.1	0.050
ER (%)	58.5 $\pm$ 34.4	54.7 $\pm$ 35.9	64.8 $\pm$ 31.0	0.004
PgR (%)	51.0 $\pm$ 34.9	47.4 $\pm$ 34.9	57.0 $\pm$ 34.2	0.007
MIB-1 (%)	18.5 $\pm$ 18.4	20.3 $\pm$ 19.4	15.8 $\pm$ 16.3	0.017
CEA (ng/ml)	3.6 $\pm$ 9.8	2.7 $\pm$ 8.5	4.8 $\pm$ 11.6	0.039
CA 15-3 (U/ml)	21.4 $\pm$ 20.7	19.0 $\pm$ 14.3	24.9 $\pm$ 27.4	0.006

Group A: <65 years; Group B >64 years.

for women with BC irrespective of their relation to menopause and up to one third of patients are premenopausal, representing a high-risk group (13). CEA and CA 15-3 are the most common serum markers used for postoperative monitoring of BC, but unfortunately, due to their low sensitivity and specificity, they have no value for early detection of primary BC (14). According to the American Society of Clinical Oncology guidelines, CEA and CA 15-3 are not recommended for screening, diagnosis, staging and follow-up, while a rising marker level, in the absence of readily detectable disease, may suggest treatment failure (3, 15).

In several series, pre-operative CA 15-3 levels correlate with tumour size and nodal status and high pre-operative CA 15-3 serum levels were found to be associated with a poor outcome either in the overall group of patients or only in the subgroup of node-positive patients (16). In a retrospective study, CA15-3 was found to be elevated (defined as >51 U/ml) in 0% of Stage 1, 7.9% of Stage 2, 36.7% of Stage 3 and 68.6% of Stage 4 cases and patients with normal CA 15-3 at presentation had a 85% five-year overall survival rate, compared to 38% of patients with high levels (17). More recently, it has been shown

Relationship between ER, PgR and MIB-1 in women aged &lt;65 years



Relationship between ER, PgR and MIB-1 in women aged &gt; 65 years

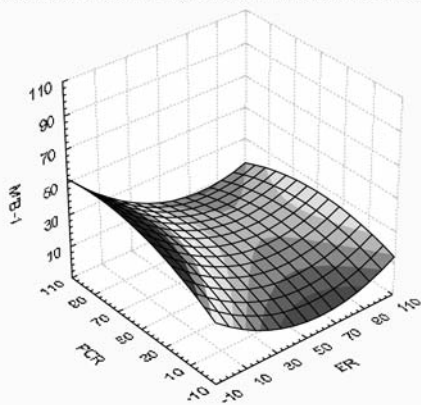


Figure 2. Relationship between estrogen receptor (ER), progesterone receptor (PGR) and MIB-1 in Group A (top) and Group B (bottom).

that an initial concentration of CA 15-3 > 40 kU/l is an independent factor associated with poor prognosis, suggesting its determination in the initial routine staging (18). The data from the current study confirm that CEA and CA 15-3 correlate exclusively with the size of the tumour. There is a general agreement that the proliferative activity of normal breast tissue declines after the menopause. However, BC maintains the ability to synthesize and metabolize oestrogens, and higher levels of aromatase activity have been found in BC from women older than 80 years, compared to younger ones (19). The current data are promising since tumour proliferation is the strongest prognostic factor in BC.

## Conclusion

These data suggest that preoperative serum CEA and CA 15-3 measurement are not useful in the therapeutic decision-making in patients with BC. However, MIB-1 index, showing

a significant inverse relationship with ER independent of age, should be considered an effective parameter for assessing tumour proliferation, especially in younger patients.

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