Serum and cytosolic levels of CA 549 in breast cancer patients

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Summary: CA 549 is a new mucinous circulating tumor marker recognized by two monoclonal antibodies (BC4E549 and BC4N154) recently proposed for breast cancer. In this report we compared the levels of CA 549 and CA 15.3, the best known biomarker for breast cancer nowadays, in 68 sieric and 59 cytosolic samples. Serum samples came from 59 breast patients (24 with primary disease = M−, 18 with systemic disease = M+, 17 with no evidence of disease after surgery = NED) and 9 women with benign breast disease = BBD.

The cytosols were prepared from primary breast carcinomas according to the method used for hormonal receptors. At first we evaluated the analytical performance of the immunoradiometric assay for CA 549 (Hybri-BREScan, Hybritech) and its applicability to the cytosolic determination.

Using a cut-off value of 12 U/mL for CA 549 and 28 U/mL for CA 15.3 serum levels, we obtained the following percentages of positivities: M− = 21%; M+ = 83%; NED = 0%; BBD = 22% for CA 549 and M− = 33%; M+ = 89%; NED = 18%; BBD = 22% for CA 15.3.

CA 549 gave information concordant with CA 15.3 in a high percentage of cases both in sera and in cytosols, but the clinical relevance of cytosolic determination remains to be investigated. Since serum CA 549 showed an adequate sensitivity in M+ patients only, it may be proposed in the follow-up to confirm CA 15.3 abnormal values or as an alternative to it.

Key words: Tumor markers; Breast cancer; Cytosol.

INTRODUCTION

Several tumor markers have been evaluated as clinically useful in the management of women with breast carcinoma, but only a few have proved of value (Neville, 1986).

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Nowadays, out of the "classic" carcinoembryonic antigen (CEA) (Beard, 1986) and tissue polypeptide antigen (TPA) (Gion, 1990), a large series of new cancer-associated substances detected by monoclonal antibodies (MAbs) have been proposed, such as CA 15.3 (Hilkens, 1986), MCA (Bombardieri, 1989), BCM (Ballesta, 1990) and also CA 549 (Bray, 1987).

Biochemical characterization of the molecules carrying the epitopes recognized by these MAbs, revealed that they are glycoproteins belonging the heterogeneous family of mucines, called polymorphic epi-

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thelial mucins (PEM) (Ceriani, 1982; Bon, 1990).

Mucins are a group of highly glycosylated, high molecular weight glycoproteins synthesized and secreted by various epithelial tissues with protective functions (Freizi, 1984).

In fact they constitute a biological barrier to protect epithelia against osmotic and pH gradients, physical injuries such as bacterial or viral infections (Bombardieri, 1990).

The neoplastic transformation leads to large changes in the structure of the tissue and in the cell function; mucines are released into the extracellular fluids and get into the bloodstream instead of on the surface of the secretory epithelia. For these reasons they become a suitable signal for cancer detection and monitoring (Price, 1988).

In this study we report our experience with CA 549 test in sera and cytosols from breast cancer patients (pts) in comparison with CA 15.3, which may today be regarded as the first choice marker for these neoplasms (Barak, 1990).

CA 549 is identified by means of two MAbs (BC4E549 and BC4N154) directed against distinct epitopes.

The first MAb was prepared against a human breast-tumor cell line (T417) and the other was developed against human milk fat-globule membranes (Bray, 1987).

MATERIALS AND METHODS

CA 549 serum levels were determined by a solid phase, two site immunoradiometric assay (Hybri-BREScan Hybritech Inc. USA). In the test procedure, samples containing CA 549 are reacted with a polystyrene bead (solid phase) coated with the BC4N154 and, sequentially, with the BC4E549 MAb labeled with radioactive iodine (^{125}I). Following the formation of the solid phase/CA 549/labeled antibody sandwich, the bead is washed to remove unbound labeled antibody. The radioactivity bound to the solid phase is measured in a gamma counter and worked out by the computer.

In the present report, we tested 42 apparently healthy subjects (K) to calculate our cut-off value, 9 women with benign breast disease (BBD) and 59 breast cancer pts, grouped in: 24 with primary disease (M—), 18 with systemic disease (M+) and 17 with no evidence of disease after surgery (NED). After a preliminary evaluation of its applicability, the same IRMA Kit was also used with no significant differences to test 39 cytosols prepared for steroid receptor determination (Gion, 1985) from primary breast cancer.

Cytosol protein concentration was determined by the Coomassie brillant blue method (Bradford, 1976) and we reset it to 1 mg/mL before the assay. CA 549 cytosol concentrations were expressed as U/mg of cytosol protein (c.p.).

All sera and cytosols from breast cancer pts were also assayed for CA 15.3 levels using a sandwich enzyme-immunoassay (EIA CA 15.3, CIS France).

RESULTS

At first, we evaluated the analytical performance of the CA 549 kit both in serum and cytosol with good overall results.

Table 1 summarizes and Figure 1 represents data of CA 549 serum levels in the study groups. According to a cut-off value of 12 U/mL for CA 549 and 28 U/mL for Ca 15.3, Table 2 shows the positivities of both markers in the sera of each group of pts.

In our experience CA 549 gave information concordant (positive or negative) with CA 15.3 in 59/68 cases (87%), whereas Ca 15.3 alone was elevated in 8 cases and CA 549 in 1. As reported in Table 3, also cytosols had in the most part of cases a similar behaviour for both tumor

Table 1. — *Observed values of CA 549 (in U/mL) in the study groups.*

	No.	Mean	SD	Median	Range
K	42	6.9	2.7	6.5	2.2-13.7
BBD	9	8.6	3.4	7.7	4.7-14.1
M—	24	10.1	5.8	8.6	4.1-27.2
NED	17	5.9	1.8	6.2	2.7- 8.8
M+	18	80	94	61	3.5- 385

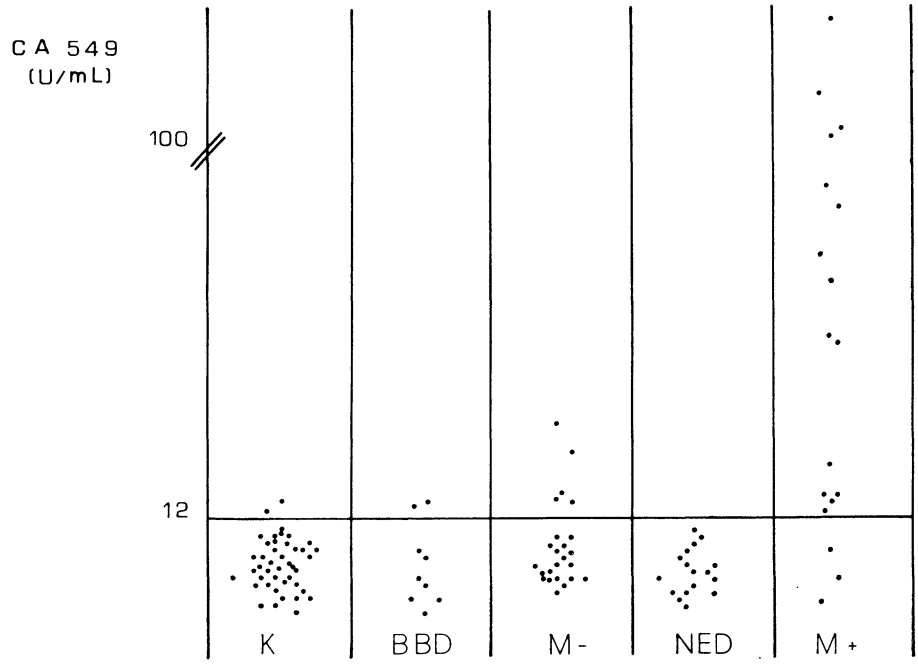

Fig. 1. — Distribution of CA 549 serum levels in study groups.

Table 2. — *Positivity rates of both tumor markers in the study groups.*

	No.	CA 1549 (%)	CA 15.3 (%)
BBD	9	2 (22)	2 (22)
M−	24	5 (21)	8 (33)
NED	17	0 (—)	3 (18)
M+	18	15 (83)	16 (89)

markers. In this table to quantify the cytosolic distribution we arbitrarily used as cut-off: 12 U/mg c.p. for CA 549 and 28 U/mg c.p. for CA 15.3, respectively.

Finally, the mean and median values obtained in our cytosol samples are demonstrated in Table 4.

DISCUSSION

To date, the main use of the measurement of tumor markers in women with breast cancer remains in the monitoring of the disease to detect early relapse (Walkers, 1984).

In fact, many of them show an elevated sensitivity in metastatic disease, but they

Table 3. — *Distribution of CA 549 and CA 15.3 cytosolic levels (in U/mg c.p.).*

	Lower than Cut off	1-4 times Cut off	4-6 times Cut off	>6 times Cut off
CA 549	15	12	8	4
CA 15.3	16	13	3	7

Cut off was arbitrarily set at: 12 U/mg c.p. for CA 549 and 28 U/mg c.p. for CA 15.3.

Table 4. — *Observed cytosolic values (in U/mg c.p.) of CA 549 and CA 15.3.*

	Mean	SD	Median	Range
CA 549	32	29.2	19	1-108
CA 15.3	109	166.7	38	4-707

lack clinical utility in early stages of breast cancer, when positivity rates are low (Pons-Anicet, 1987).

According to previous investigations (Beveridge, 1988, Demers, 1988; Cooper, 1990), our data demonstrated the same behaviour for CA 549 serum levels, with an adequate sensitivity in M^+ pts only. Furthermore, CA 549 gave information concordant with CA 15.3 in high percentage of cases: this clinical correspondence could be explained by a similar biological significance of these two markers (Leonard, 1988).

Therefore, CA 549 may be proposed in the follow-up of breast cancer to confirm CA 15.3 abnormal values or in alternative to it. Finally, we also showed the possibility of using the same radioimmunoassay for bot herum and cytosolic measurements, even if the pratical relevance of this cytosolic determination remains to be investigated.

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