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Clinical Study

Clinical Usefulness of Cancer Markers in Primary Breast Cancer

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The aim of this study was to investigate the diagnostic power of CA 549, MSA and CA 15-3 in identifying breast cancer. The study included 232 patients of which 56 were healthy, 43 had benign breast cancer and 191 with other growths. The results were obtained using a specific immunoassay and using producers' cut offs. The following sensitivity and specificity of markers were found: CA 549 (sen.: 40%/spec.: 90%), MSA (sen.: 22%/spec.: 96%), and CA 15-3 (sen.: 33%/spec.: 86%). Ideal cut offs were defined with ROC curves. A significant correlation was found between CA 549, MSA, and CA 15-3. The combination of markers does not improve the clinical usefulness to identify only breast cancer. Serum tumor markers are abnormally elevated in patients with breast cancer. CA 549, MSA, CA 15-3 are useful clinical markers, good indicators of disease extent, and may have important prognostic value. This study demonstrates the role of the tumor markers in breast cancer.

1. Introduction

An ideal cancer marker for breast cancer would be clinically useful in many ways and, therefore, has been searched for decades [1–4].

CA 15-3 is regarded as the most suitable cancer marker and therefore became established in the clinical routine worldwide. However, its sensitivity is still unsatisfactory in the early stages of primary breast cancer [5–11].

During the last years, two new cancer markers, CA 549 and MSA (mammary serum antigen) were put on the market [12–19]. First analyses showed, in some cases, very promising sensitivity levels with a sufficient specificity.

For example CA 15-3, CA 549, and MSA belong to the molecular family of breast mucins. CA 549 is an antigen associated to breast cancer circulating in blood, which is defined by the monoclonal antibody BC4E 549 and which belongs to the polymorphic epithelium mucins (PEMs). CA 549 is part of the human milk fat globulin proteins (HMFGPs).

The mammary serum antigen (MSA) is defined by the antibody 3EL.2. MSA represents a macromolecular glycolic protein with a molecular weight over 300000.

The aim of our study was to check sensitivity and specificity levels of these new markers within our group of patients. For this purpose, we determined sensitivity and specificity levels for breast cancer in different stages using various cutoffs.

Furthermore, we compared our results obtained from breast cancer patients with control groups having other diseases also able to induce positive marker results.

2. Materials and Methods

In this study, a total of 522 serum samples were analysed. The samples were stored deep-frozen at a temperature of −18°C. Among all samples tested, 232 were obtained from breast cancer patients in different stages, 63 were primary breast cancer and 56 samples came from healthy women. The remaining samples were obtained from the following:

43 patients with benign breast diseases;
21 patients with benign lung diseases;
27 patients with benign heart diseases;
27 patients with benign kidney diseases;
20 patients with benign liver diseases;
22 patients with lung cancer;
19 patients with stomach cancer;

18 patients with colon carcinoma;
37 patients with ovarian cancer.
All patients were female gender.

2.1. Methods. Serum levels of CA 549 were determined using the Hybri-BREScan Kit from Hybritech GmbH, Cologne. This test kit is based on the sandwich principle. Two monoclonal antibodies were used. One of them, BC4E 549, is an IgG aimed at the human breast cancer cellular line T417. This antibody is linked to an enzyme and was developed by Hybritech Inc. (San Diego, CA, USA). The second antibody, BC4N 154, is used as a solid-phase antibody and therefore it is fixed to polystyren-globules. This antibody is an IgM deriving from mouse and it is aimed at the human milk fat globulin membrane.

During the test, 20 uL patient serum or standard or control substance is incubated with the antibody globules in an horizontal mixer for an hour.

Following a first washing, 200 uL of enzyme-linked antibody conjugate is added. After one hour of incubation and a second washing, a colour reaction is set off by adding 200 uL test substrate. The substrate conversion is detected colorimetrically at 405 nm and is directly proportional to the CA 549 concentration. We used the producer's cutoff of 12,6 U/mL.

With regard to the determination of the MSA serum levels, we used an inhibition ELISA containing the murine monoclonal antibody 3EL.2 [20].

The kit is commercially available at MEDAC company, Hambourg.

For three hours, 25 uL serum sample incubate with the excess amount of 725 uL of the thinned monoclonal antibody 3E1.2. This monoclonal antibody is specifically aimed at MSA and binds to MSA contained in the serum sample. The antibody fraction which does not bind to MSA during the first reaction, is again incubated for 16–20 hours. In this step, the antibody binds to MSA linked to a microtiter strip. In the following step, 3E1.2 binds to a mouse IgM-antibody containing peroxidase. The result of this reaction is the following complex: a microtiter strip-bound MSA/ 3E1.2 peroxidase marked anti-mouse IgM. The amount of this complex is directly proportional to the colour reaction caused by the peroxidase reaction of the ABTS (2,2 azinobis(ethylbenzthiazoline-6-sulfonic acid), and it is inversely proportional to the concentration of MSA contained in the serum sample.

By the means of a standard curve comprising six standards, every extinction can be assigned to a certain MSA serum level.

In accordance with producer's recommendations, we used a cutoff 55 U/mL. For the determination of CA 15-3 serum levels we used the ElektroChemiLumineszenzImmunoAssay "ECLIA" that is used together with ELECSYS 2010 available at Fa. ROCHE. In order to illustrate the conne ction between sensitivity and specificity we established ROC curves. By the means of ROC curves, sensitivity can be presented graphically in a two-dimensional way in dependence on specificity. That means, that the sensitivity-specificity pairs of variates are put down in a diagram in relation to increasing cut-off levels. In these curves, the statistically ideal cutoff is represented as the point, which exceeds the first bisector of an angle the most. This point represents the biggest difference between healthy and ill.

Table 1: Sensitivity and specificity of tumormarkers in breast cancer with cutoffs recommended by the producer.

	CA 549	MSA	CA 15-3
Sensitivity ($n = 232$)	40%	22%	33%
Specificity ($n = 56$)	90%	96%	86%
Cutoff	12,6 U/mL	55 U/mL	25 U/mL

Table 2: Sensitivity of tumor markers in breast cancer at a standardized specificity of 95%.

	CA 549	MSA	CA 15-3
Sensitivity ($n = 232$)	25%	23%	19%
Specificity ($n = 56$)	95%	95%	95%
Cutoff	15,2 U/mL	48,2 U/mL	35,8 U/mL

3. Results

In the following, first the results obtained from all breast carcinoma patients analysed are represented. Apart from the producer's cutoffs, the ideal threshold values we obtained are shown. It is presented to what extent the markers correlate with each other and whether the combination of two markers improves the clinical usefulness. Finally, the population of the primary breast cancer patients is investigated and the most important disease groups able to induce positive marker results are presented.

Using the producer's cutoff, CA 549 was the most sensitive marker with 40% showing a specificity still acceptable of 90% (cutoff: 12,6 U/mL). In comparison, the sensitivity as well as the specificity of CA 15-3 were about 5% lower (sen.: 33%, spec.: 86%, cutoff: 25 U/mL).

With 22%, MSA's sensitivity was remarkably lower compared to the sensitivities of CA 549 and CA 15-3. In contrast, MSA's specificity was high with a value of 95% (cutoff: 55 U/mL) (Table 1).

In order to realise a more reliable comparison between the markers, sensitivity was measured and compared at a standardized specificity of 95%.

As a result, CA 549's sensitivity (25%) was similar to the one of MSA (23%) and slightly higher than the sensitivity of CA 15-3 (19%) (Table 2).

At the same time, it is possible to check whether the cutoffs recommended by the producer lead to an ideal separation between healthy women and women suffering from breast cancer in our study too. For CA 15-3, we obtained the best distinction between healthy an ill at a cut-off 25 U/mL. In this case, sensitivity is 33% and specificity 86%. With regard to MSA, the statistically ideal cutoff in our patient population lies at a value of 50 U/mL whilst sensitivity is 30% and specificity 89%. When it comes to CA 549, its ideal threshold value is 9 U/mL. In this case sensitivity is 59% whilst specificity is relatively low with 82%.

Table 3: Sensitivity of tumor markers in primary breast cancer in stages T1– T4.

	CA 549	MSA	CA 15-3
Sensitivity ($n = 63$)	33%	18%	35%
Sen. T1-Stage ($n = 26$)	35%	8%	15%
Sen. T2-Stage ($n = 27$)	33%	22%	48%
Sen. T3-Stage ($n = 4$)	25%	25%	0%
Sen. T4-Stage ($n = 6$)	33%	33%	83%
Specificity ($n = 56$)	90%	96%	89%
Cutoff	12,6 U/mL	55 U/mL	25 U/mL

In order to check whether one of these cancer markers is particularly suitable for early diagnosis, we analysed the patient population with primary breast cancer in dependence on the different stages. With reference to the overall population with primary breast cancer, CA 15-3 achieved best sensitivity (35%) when using the cutoff recommended by the producer. Sensitivity for stage T1 was 8% and stage T2 22% (Table 3).

In order to obtain a more reliable comparability, we used a uniform specificity of 95% when analyzing the specificity of the three cancer markers in our patient population with primary breast cancer. In this case, the total sensitivity of MSA was best (18%), however, in stage T1 sensitivity only was 8% and in stage T2 22%. Total sensitivity of CA 549 was 11% (T1 = 8%, T2 = 19%). Total sensitivity of CA 15-3 was 14% (T1 = 4%, T2 = 22%).

In order to find out, whether using only one marker out of the group of polymorphic epithelium mucins in the clinical routine is sufficient, we investigated the expressiveness of every single marker.

As a result, the correlation coefficient between MSA and CA 549 was 0,73. We found the same result for MSA and CA 15-3. CA 15-3 and CA 549 correlated best showing a correlation coefficient of 0,89.

Therefore, we obtained a quite satisfactory correlation for the biological systems between the members of this molecular family, CA 15-3, MSA, and CA 549. In order to find out to what extent the simultaneous use of different cancer markers in view of the quite high correlation coefficiencies is suitable, we combined the markers. The combination of two markers resulted in an improved sensitivity. When combining CA 15-3 and CA 549, sensitivity improved from 40% to 49% at a specificity of 77%. Sensitivity could be raised from 40% to 53% when MSA, CA 15-3, and CA 549 were combined whilst specificity was 73%. The combination of the markers CA 549 and MSA showed a sensitivity of 44% (spec.: 82%) and MSA and CA 15-3 together also achieved a sensitivity of 44% (spec.: 79%). All these results were obtained using the producer's cutoff (Table 4).

First, the rate of false positive marker results within the healthy control group is detected for the cancer markers CA 549 and MSA. These are compared to the results obtained from control groups suffering from other diseases, that are also able to induce positive marker results. Within the healthy control group, 11% were positive for CA 549. In contrast, CA 549 was highly elevated in patients with hepatitis (62%), with ovarian cancer (39%) as well as renal diseases (48%).

Table 4: The combination of tumor markers with the sensitivity and specificity.

Tumor marker combination	Sensitivity	Specificity
CA 549 + MSA	44%	82%
CA 549 + CA 15-3	49%	77%
MSA + CA 15-3	44%	79%
MSA + CA 15-3 + CA 549	53%	73

With regard to MSA, results for the healthy control population were even better. In this case, only 4% were MSA positive, whilst MSA was elevated in 38% in patients with hepatitis, 52% in patients with renal disease, 35% in patients with ovarian carcinoma, with lung carcinoma in 32%, 29% in patients with other lung diseases, and 26% in patients with heart diseases.

4. Discussion

One of the main requests for an ideal cancer marker is its suitability for preclinical screening. Therefore, a prerequisite would be a high sensitivity as well as specificity with respect to the detection of primary breast cancer. Using a specificity level of 95%, results for the sensitivity of CA 15-3 for breast cancer in different stages varied from 5% to 60%, depending on the work group. In our study, we were not able to confirm the extraordinary positive results obtained in some cases. As far as the sensitivity of the marker CA 549 is concerned, the data available also differs enormously. In different stages of breast cancer, between 12% and 77% are tested positive for CA 549.

According to our study, CA 549 showed the best sensitivity among all markers, but its specificity was low. With reference to MSA's sensitivity, literature shows results between 34% and 79% (spec.: 95%). In our study, its sensitivity was noticeably lower. According to our results, the usefulness of the cancer markers available is still not satisfactory with respect to primary breast cancer. In particular, this is the case in the early stages of this disease.

In the literature, CA 15-3's sensitivity ranges from 3% to 25% for T1 breast cancer. Our results for T1 breast cancer also lie within this scope. With regard to MSA's sensitivity, data from various authors fluctuate between 8% and 50%. Our own results confirm these rather reserved information. Sensitivity for CA 549 for T1 breast cancer stages varied from 3% to15% in the literature. With a sensitivity of 35% for this patient group, our result was quite positive. However, this result is put into perspective by the fact of an unsatisfactory specificity and the small group of patients analysed.

Recapitulatory, we support the opinion of Stenmans and Haikkinens that there is no cancer marker available so far, that shows a sufficient sensitivity in the early diagnosis of breast cancer. This fact is not changed noticeably when adding the new markers CA 549 and MSA.

A possibility to improve the clinical usefulness might be the combination of noncorrelating cancer markers. However,

CA 549, MSA, and CA 15-3 belong to the molecular family of breast mucins. The results of several work groups confirmed a good correlation. Our results corroborated this [21–23].

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