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Enzymes and Protein Markers

Comparison of the Diagnostic Accuracy of CA27.29 and CA15.3 in Primary Breast Cancer

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Background: **A new, fully automated method that measures the breast cancer-associated glycoprotein CA27.29 has become commercially available. The aim of the present study was to compare this CA27.29 assay with the assay that measures CA15.3 in primary breast cancer.**
Methods: **The study was performed retrospectively on preoperative serum samples collected from 275 patients with untreated primary breast cancer (154 node positive and 121 node negative). Eighty-three healthy control subjects were also evaluated. CA27.29 was measured using the fully automated Chiron Diagnostics immunochemiluminescent system (ACS:180 BR). CA15.3 was measured with a manual immunoradiometric method (Centocor CA15.3 RIA).**
Results: **In healthy subjects, CA15.3 was significantly higher than CA27.29 (P <0.0001). On the other hand, in breast cancer patients CA27.29 was higher than CA15.3 (P = 0.013). The mean value found in the control group plus 2 SD was chosen as the positive/negative cutoff point. The overall positivity rates were 34.9% for CA27.29 and 22.5% for CA15.3. The area under the ROC curve was greater (P <0.001) for CA27.29 (0.72) than for CA15.3 (0.61). Both markers showed a statistically significant, direct relationship, with pathological stage being higher in node-positive than in node-negative cases and in larger than in smaller tumors. Neither CA27.29 nor CA15.3 showed significant associations with age, menopausal status, or tumor receptor status.**
Conclusions: **CA27.29 discriminates primary breast cancer from healthy subjects better than CA15.3, especially in patients with limited disease. Prospective studies are necessary to confirm this conclusion.**

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Mucins are large and complex glycoproteins expressed by several epithelial tissues. Mucins have been grouped into seven distinct families (MUC1 through MUC7) according to their genetic and biomolecular characteristics *(1)*. MUC1 represents large transmembrane glycoproteins with molecular weights ranging from M_r 250 000 to 1 000 000 with extracellular domains formed of a highly O-linked glycosylated protein core consisting of a variable number of highly conserved 20-amino acid repeat units (tandem repeats) *(2)*. MUC1 mucins are expressed physiologically at the luminal surface of glandular epithelia. The expression of MUC1 mucin is up-regulated frequently, and the protein is released in higher amounts in the blood of patients with breast cancer *(2, 3)*. Several murine monoclonal antibodies reactive with the MUC1 gene product have been generated *(4)*. Some of them have been used to set up immunoassays for the detection of the MUC1 mucin in body fluids. CA15.3 *(5)* is the MUC1 marker studied most extensively in breast cancer. It detects the mucin by two monoclonal antibodies: 115D8 *(6)*, which recognizes a still unidentified epitope, and DF3 *(7)*, specific for an epitope site on the protein backbone *(8)*.

In 1996, the American Society of Clinical Oncology (ASCO) prepared and distributed Clinical Practice Guidelines for the Use of Tumor Markers in Breast and Colorectal Cancer *(9)*. The ASCO panel considered carcinoembryonic antigen and CA15.3 and recommended that these markers should not be used for screening, diagnosis, staging, or surveillance after primary treatment of breast cancer.

The restrictive ASCO recommendations reflect the low diagnostic sensitivity of the markers for limited tumor burden, precluding their use in the recognition of early disease. In 1997, the ASCO Panel updated these guidelines *(10)*. They considered an additional tumor marker, the mucin-associated antigen CA27.29, which had been evaluated in a well-designed study and approved by the Food and Drug Administration *(11)*. However, the ASCO panel did not recommend routine use of the marker because data on the possible impact of the marker on survival, quality of life, reduced toxicity, or reduced costs were not available.

The CA27.29 antigen is detected by the monoclonal antibody B27.29, specific for the protein core of the MUC1 product *(8, 12)*. Preliminary studies evaluated CA27.29 in comparison with either CA15.3 or other tumor markers *(13–15)*. Using a manual RIA method, Chan et al. *(11)* showed in a double-masked, prospective clinical trial that CA27.29 was effective for the early detection of recurrence in patients in follow-up after treatment of primary breast cancer. The diagnostic performance of CA27.29 found in the latter study seemed superior to those reported to date for CA15.3 in stage II patients *(16–20)*. Although both immunoassays detect the MUC1 product, differences are not unexpected. The MUC1 product may be glycosylated differently among patients or in the same patient in different phases of the disease *(3, 21)*. Accordingly, assay methods set up using monoclonal antibodies against different epitopes may react in different ways with the MUC1 product.

In 1995, a fully automated version of the CA27.29 assay (ACS180:BR; Chiron Diagnostics, East Walpole, MA) became commercially available *(22)*. Bon et al. *(23)* compared this method with CA15.3 measured with different assays in a large patient series with different clinical conditions, including 114 patients with primary breast cancer. The present study was performed to evaluate the fully automated assay for CA27.29 in a representative cohort of patients with primary breast cancer with the following aims: (*a*) to evaluate the diagnostic performances of the method; (*b*) to compare CA27.29 and CA15.3 in breast cancer and in a control group of apparently healthy women; and (*c*) to evaluate the relationships of both markers with the clinical and pathological characteristics of the patients.

Patients and Methods

The study was performed retrospectively using serum samples stored in the serum bank of the Regional Center for the Study of Biological Markers of Malignancy of Venice. The inclusion criteria were as follows: (*a*) patients affected by primary breast cancer, stages I to III; (*b*) no radiotherapy, chemotherapy, or endocrine manipulations before the surgery; and (*c*) no clinical or laboratory evidence of benign diseases of the liver, pancreas, ovary, or kidney.

Patient staging was carried out according to the International Union Against Cancer criteria. Histological typing was done according to the WHO classification.

The estrogen and progesterone receptors were measured in high-speed cytosol, using a radioligand-binding assay set up according to the European Organization for Research and Treatment of Cancer standardization criteria *(24)*.

Serum was separated by centrifugation at room temperature and stored in multiple aliquots at −80 °C. Samples (n = 275) from patients with primary untreated breast cancer (median age, 65 years; range, 28–80 years) were randomly selected from sera stored from 1987 to 1993. Serum samples from 80 apparently healthy controls (median age, 50 years; range, 35–70 years) were also evaluated.

One hundred fifty-four patients were node negative, 121 were node positive; 150 cases were pT_1, 113 were T_2, and 11 were pT_3; 105 were in stage I, 148 were in stage II, and 22 were in stage III. Tumor receptor status was available in all cases.

ASSAY METHODS

The ACS:180 BR assay (Chiron Diagnostics) is a fully automated competitive chemiluminescent immunoassay. A mouse monoclonal antibody, raised against a peptide epitope in the tandem region of the MUC1 backbone and labeled with acridinium ester, is incubated for 7.5 min with both the patient sample and purified CA27.29 coupled covalently to paramagnetic particles (solid phase). Both the antigen in the sample and the solid-phase CA27.29 compete for binding to the labeled antibody. Therefore, an inverse relationship is found between the amount of antigen in the sample and the amount of relative light units detected by the system. The assay was performed according to the instructions of the manufacturer.

The CA15.3 RIA (Centocor Diagnostics) is a two-site, solid-phase, manual immunoradiometric assay. The method was performed according to the instructions of the manufacturer.

ACS:180 BR ASSAY PERFORMANCE

Within- and between-assay imprecision (CV) was assessed using both a commercial quality-control serum (tumor marker control, two levels, lot nos. 44031 and 44032; Bio-Rad) and three in-house serum pools prepared using human serum samples to cover a clinically relevant range (~10–100 kilounits/L).

Assay imprecision was <7% for samples with intermediate (30~40 kilounits/L; CV <7.0%) and high antigen concentrations (~115 kilounits/L; CV <5.0%), whereas imprecision tended to increase at very low antigen concentrations (10~12 kilounits/L; CV <15%).

STATISTICAL ANALYSIS

Data were evaluated using the Spearman correlation, the Kruskal–Wallis one-way ANOVA on ranks, the χ^2, and the Wilcoxon signed-rank tests. Bland–Altman plots were also used. ROC curves were generated plotting sensitivity vs 1 − specificity. The Astute package (DDU software) was used.

Results

HEALTHY SUBJECTS

For CA27.29 and CA15.3 in healthy subjects, the Spearman *r* was 0.774. The Bland–Altman plot (Fig. 1) suggested a tendency to lower CA27.29 values. This was confirmed by the paired statistics, which showed significant differences (P <0.0001), between CA27.29 and

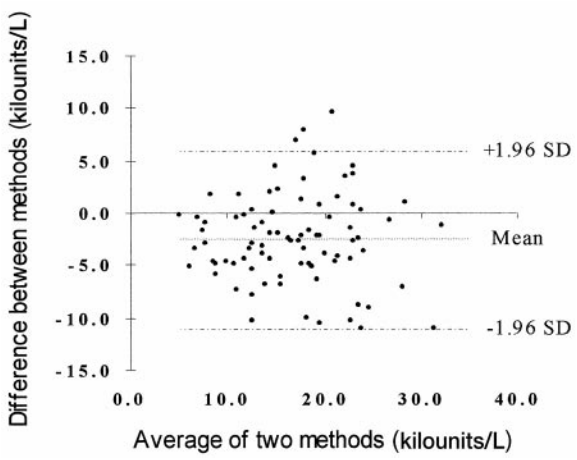

Fig. 1. Bland–Altman plot of differences between individual case values of CA27.29 and CA15.3 in healthy women.
Horizontal lines represent the mean differences and ± 1.96 SD.

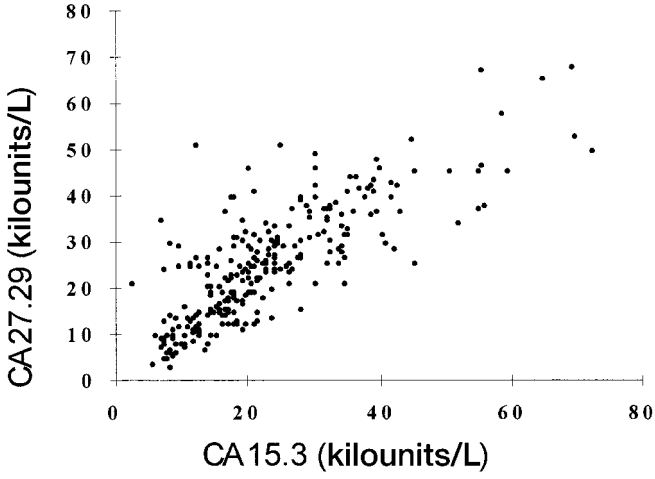

Fig. 2. Scatter plot of CA27.29 and CA15.3 in patients with primary breast cancer.

CA15.3 (data not shown). The values of CA27.29 and of CA15.3 as well as the mean + 2 SD and +3 SD cutoff points are summarized in Table 1. For further evaluation, we used the 2 SD cutoff because it corresponded to the CA15.3 value (31 kilounits/L) frequently used as cutoff point in clinical practice. The distribution of both CA27.29 and CA15.3 was not significantly different from gaussian. Therefore, the mean + 2 SD was used as the cutoff point to assess tumor marker positivity in patients with breast cancer. No association between the markers and either age or menopausal status was found in the control group. Therefore, the cutoff points did not require adjustments related to these variables.

In contrast to Bon et al. *(23)*, we found no discordant values in healthy subjects with very high CA27.29 values and low CA15.3.

PATIENTS WITH PRIMARY BREAST CANCER

For CA27.29 and CA15.3 in patients with breast cancer (Fig. 2), the Spearman *r* was 0.797. We found no cases with abnormal increases of CA27.29 with respect to CA15.3 in breast cancer patients. The scattergram showing the relative difference of CA27.29 in comparison with CA15.3 (Fig. 3) confirmed this agreement, but it suggests a tendency toward higher CA27.29 values in the low range. Both the values and the positivity rates of CA27.29 are significantly higher than those of CA15.3 (Table 2). The ROC curve showed that CA27.29 (area under the curve, 0.72; 95% confidence interval, 0.62–0.82) was superior to CA15.3 (area under the curve, 0.61; 95% confidence interval, 0.55–0.67) in the present patient series (Fig. 4). The sensitivity, specificity, and likelihood ratio are summarized in Table 3. Although CA27.29 performs better than CA15.3, it is worth noting that the sensitivity and specificity of both markers are relatively low. At a predetermined specificity of 90%, the sensitivity was only 51% for CA27.29 and dropped to 29% for CA15.3. We had to accept poor specificity (58% for CA27.29 and 40% for CA15.3) to reach a sensitivity of 70%.

The ROC curves indicate superior performance of CA27.29 in the low antigen dose range, i.e., better specificity in cases with sensitivity ≤70% (areas under the ROC curves, 0.721 for CA27.29 and 0.606 for CA15.3; $P < 0.0001$).

RELATIONSHIP WITH OTHER CLINICAL OR PATHOLOGICAL INDICATORS

Neither CA27.29 nor CA15.3 showed a significant association with age or menopausal status.

Table 1. CA27.29 and CA15.3 serum concentrations in healthy women.

			CA27.29, kilounits/L				CA15.3, kilounits/L			
Menopausal status	n	Value distribution	Mean	SD	Mean + 2 SD	Mean + 3 SD	Mean	SD	Mean + 2 SD	Mean + 3 SD
Overall	83	Gaussian	15.7	6.4	28.5	34.9	18.2	6.4	31.0	37.4
Premenopausal	44	Gaussian	15.4	6.4	28.2	34.6	17.6	6.8	31.2	38.0
Postmenopausal	39	Gaussian	15.9	6.3	28.5	34.8	18.8	6.0	30.8	36.8
			F-ratio, 0.14; $P = 0.704$[a]				*F*-ratio, 0.69; $P = 0.409$[a]			

[a] ANOVA for menopausal status.

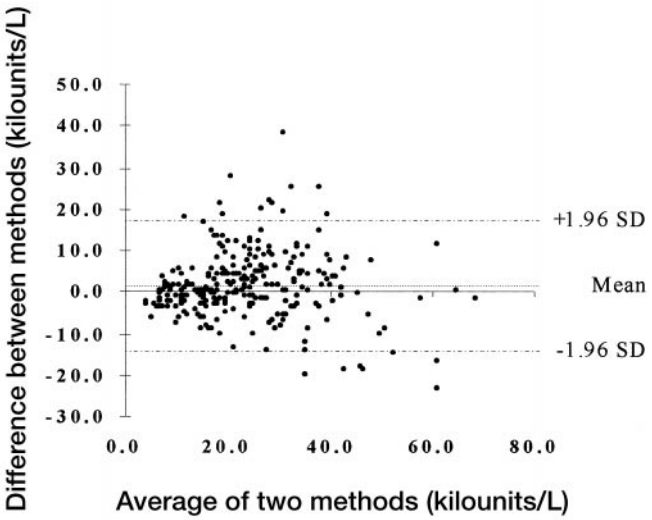

Fig. 3. Bland–Altman plot of differences between individual case values of CA27.29 and CA15.3 in patients with primary breast cancer. *Horizontal lines* represent the mean differences and ± 1.96 SD.

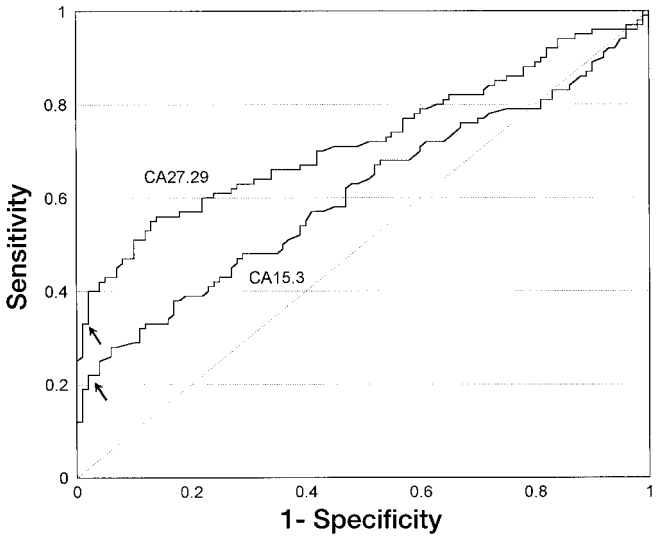

Fig. 4. ROC curves of CA27.29 and CA15.3.
Area under the curve ± SE: CA27.29, 0.721 ± 0.027; CA15.3, 0.606 ± 0.031 (P <0.0001). *Arrows* indicate selected cutoffs.

Both markers were associated with tumor burden, being significantly higher in late vs early stages (P <0.0001 for CA27.29; P = 0.0004 for CA15.3), in large vs small tumors (P = 0.007 for CA27.29; P = 0.019 for CA15.3), and in node-positive vs node-negative patients (P = 0.002 for CA27.29; P = 0.087 for CA15.3; Table 4). Accordingly, the serum concentrations of both markers were significantly correlated with both the number of positive lymph nodes (Spearman correlation for CA27.29, r = 0.242, P <0.0001; for CA15.3, r = 0.188, P = <0.005) and the tumor diameter (Spearman correlation for CA27.29, r = 0.216, P <0.0003; for CA15.3, r = 0.206, P = <0.0006).

Moreover, CA27.29 was higher than CA15.3 in patients with more limited tumor burden. The ratio of the CA27.29 positivity rate to the CA15.3 positivity rate was 1.9 in stage I, 1.6 in stage II, and 1.1 in stage III. Likewise, according to tumor size, it was 1.8 in pT_1, 1.5 in pT_2, and 1.0 in pT_3.

Neither CA27.29 nor CA15.3 was associated with the cytosol concentration of either the estrogen receptor or the progesterone receptor (data not shown).

Discussion

The real usefulness of tumor markers in the management of breast cancer has been questioned because of the low diagnostic sensitivity for early disease *(9, 10)*. The majority of published studies have shown that serum concentrations and the positivity rates of tumor markers increase with the extent of the disease. These results have been reported for carcinoembryonic antigen and for the mucin markers evaluated to date *(16, 20, 25–29)*. Basically, the markers showed low sensitivity and specificity in early stages of the disease and for the early detection of relapse.

Mucin markers have been investigated extensively to improve their diagnostic effectiveness. In particular, much effort has been aimed at basic knowledge of MUC1. The biology of monoclonal antibodies against MUC1 has been highlighted excellently in the International Society for Oncodevelopmental Biology and Medicine TD4 International Workshop, which represents a milestone in the characterization of MUC1 epitopes *(8)*. Different investigators have demonstrated that many antibodies recognize very simple, linear peptide motifs of few amino acids in the tandem repeat. Using the technique of minimum

Table 2. CA27.29 and CA15.3 in patients with primary breast cancer.

		CA27.29			CA15.3		
Menopausal status	n	Median, kilounits/L	Interquartile range, kilounits/L	Positive cases, %[a]	Median, kilounits/L	Interquartile range, kilounits/L	Positive cases, %[a]
Overall	275	24.3	14.5–32.0	34.9%	20.5	14.6–29.4	22.5
Premenopausal	57	23.7	14.2–32.0	31.6%	20.8	14.6–28.8	19.3
Postmenopausal	202	24.1	14.8–32.1	36.1%	20.5	14.3–29.4	22.9
			P = 0.788[b]	P = 0.524[c]		P = 0.513[b]	P = 0.559[c]

[a] Cutoff: mean + 2 SD of values found in healthy subjects.
[b] Kruskal-Wallis for menopausal status.
[c] χ^2 for menopausal status.

Table 3. Sensitivity, specificity, and positive likelihood ratios of CA27.29 and CA15.3.

	Cutoff, kilounits/L		Specificity		Positive likelihood ratio[a]	
Preset sensitivity	CA27.29	CA15.3	CA27.29	CA15.3	CA27.29	CA15.3
0.99	4.2	6.1	0.01	0.01	1.00 (0.00–4.10)	1.00 (0.00–4.10)
0.95	7.2	7.7	0.13	0.04	1.09 (0.00–2.20)	0.99 (0.00–2.76)
0.90	9.3	9.0	0.19	0.08	1.11 (0.24–1.97)	0.98 (0.00–2.14)
0.80	13.1	12.8	0.37	0.19	1.27 (0.63–1.91)	0.99 (0.19–1.79)
0.70	16.6	15.9	0.58	0.40	1.67 (1.10–2.24)	1.17 (0.58–1.76)
	Cutoff, kilounits/L		Sensitivity		Positive likelihood ratio[a]	
Preset specificity	CA27.29	CA15.3	CA27.29	CA15.3	CA27.29	CA15.3
0.99	28.9	36.6	0.33	0.12	33.00 (31.02–34.98)	12.00 (10.00–14.00)
0.95	25.7	28.9	0.43	0.25	8.60 (7.56–9.64)	5.00 (3.82–6.18)
0.90	24.1	27.2	0.51	0.29	5.10 (4.32–5.87)	2.90 (2.14–3.66)
0.80	21.9	23.4	0.57	0.39	2.85 (2.24–3.46)	1.95 (1.35–2.55)
0.70	19.2	20.9	0.63	0.48	2.10 (1.53–2.67)	1.60 (1.05–2.15)

[a] 95% confidence interval in parentheses.

epitope mapping with overlapping synthetic peptide, they demonstrated that the monoclonal antibodies B27.29 (CA27.29 assay), DF3 (CA15.3 assay), b12 (MCA assay), and BC4E549 (CA549 assay) recognize virtually identical amino acid sequences *(8)*.

Several variables can affect the in vivo detection and quantification of MUC1-related markers. The variability of both the number of tandem repeats and the posttranscriptional glycosylation may affect epitopes accessibility *(3)* in individual patients. Moreover, the effect of glycosylation at sites flanking the epitope may either increase or decrease antibody recognition *(30)*. Considering that in cancer patients glycosylation enzymes may be altered, a marked heterogeneity of MUC1-derived molecules may be expected among different patients *(31)*. In addition, heterogeneity may also occur in an individual patient at different times during the course of the disease because of phenotypic variations, either spontaneous or related to the biological effects of treatments.

The in vivo detection of MUC1 products may be further complicated by the presence of circulating antimucin autoantibodies, occasionally found in cancer patients as a result of a host response to altered mucin biochemistry. These antibodies are capable of forming mucin-antibody mucin immunocomplexes. These complexes may variably affect MUC1 detection, depending on the assay format. The sensitivity of two-site immunometric assays (i.e., CA15.3) may be substantially lowered, whereas competitive assays (i.e., CA27.29) should be less affected *(11, 32)*.

The preceding causes of variability indicate the need for a thorough preliminary evaluation of any new assay for a MUC1 marker to test its actual clinical effectiveness before its routine use. A competitive manual RIA for CA27.29 has been preliminarily evaluated. Several published studies have demonstrated an excellent correlation between CA27.29 and CA15.3 and have reported almost comparable sensitivity and specificity values for the two markers *(13–15)*. In a recent paper, Chan et al. *(11)*, using the manual Truquant BR RIA, evaluated the effectiveness of CA27.29 for the early detection of breast cancer relapse. Their results showed that the marker had a relatively higher sensitivity compared with that of CA15.3 reported in the literature. They found a sensitivity of 57.7% for recurrence in a cohort of 166 breast cancer patients, represented by 133 cases (80.1%) in stage II at presenta-

Table 4. Relationship between both CA27.29 and CA15.3 and tumor extension.

		CA27.29			CA15.3		
Pathological stage	n	Median, kilounits/L	Interquartile range, kilounits/L	Positive cases, %[a]	Median, kilounits/L	Interquartile range, kilounits/L	Positive cases, %[a]
T_1	150	22.2	13.1–29.8	30.0	19.4	14.0–25.8	16.6
T_2	113	24.9	16.7–33.2	39.8	21.8	15.8–32.1	27.0
T_3	11	32.4	23.8–45.7	54.5	34.8	16.8–45.0	54.5
N_0	154	21.2	13.1–30.2	30.5	19.5	13.7–26.6	19.3
N^+	121	25.6	18.7–34.8	40.5	22.4	15.8–32.2	22.9
Stage I	105	20.2	11.9–29.5	28.6	19.4	12.9–24.5	15.2
Stage II	148	24.7	15.8–31.9	35.8	20.6	14.5–30.2	23.0
Stage III	22	33.7	25.7–40.9	59.1	31.6	22.3–42.1	54.5

[a] Cutoff: mean + 2 SD of values found in healthy subjects.

tion. They compared this sensitivity with the 10.6–29% sensitivity reported by others for CA15.3 in patients with stage II primary breast cancer *(12–19)*. The sensitivity of CA15.3 for the detection of recurrence is indeed higher in several published papers *(25–29, 33–37)* than the sensitivities cited by Chan et al. *(11)*. However, the increased concentrations of the markers in follow-up have been, in general, correlated with actual tumor burden at the time of relapse, whereas patients have not been evaluated with reference to the stage of their disease at presentation. The comparison between CA27.29 and CA15.3 performed by Chan et al. *(11)*, although innovative and interesting, is indirect and should be confirmed by more stringent studies because the authors did not compare CA27.29 and CA15.3 in the same patient series. Such a study has been published by Bon et al. *(23)*, who compared the ACS:180 CA27.29 assay with four different commercially available CA15.3 assays. They examined a large case series, including 114 patients with primary breast cancer. In general, they found a very good correlation between CA27.29 and the examined CA15.3 assays. In addition, the correlation with the Centocor manual CA15.3 RIA was excellent. However, although describing their results separately for the different clinical conditions, they compared the various methods by considering all the clinical cases together, i.e., benign breast disease, breast cancer (combined stages), and other malignancies. In the present study, we directly compared the two markers in a cohort of patients with primary breast cancer larger than that of Bon et al. (275 vs 114) to elucidate the relationship of the marker with other clinical and biological characteristics of the disease.

We previously compared CA15.3 with other MUC1 markers, namely MCA and CA549. In both instances, we found good correlation between CA15.3 and either MCA or CA549 *(38, 39)*. Although the results were not interchangeable in individual patients, the different methods provided comparable information in terms of sensitivity and specificity.

In the present patient series, CA27.29 was higher than CA15.3 in those patients with primary breast cancer, whereas it was lower than CA15.3 in healthy subjects. The opposite behavior of the two markers in healthy and in cancer subjects suggests that CA27.29 may be more cancer-specific. This led to a lower cutoff point for CA27.29 and, consequently, to superior sensitivity and specificity values.

Although we calculated both the mean + 2 SD and + 3 SD cutoff points, we used only the 2 SD cutoff for the method comparison because it corresponds to the CA15.3 value (31 kilounits/L) frequently used as a cutoff point in clinical practice. The choice of the 2 SD instead of the 3 SD cutoff did not affect the results of the comparison between the two markers because the cutoffs of both markers were calculated in the same control group using the same statistics and were then subsequently used to examine the same patient series.

CA27.29 and CA15.3 are directly associated with the extent of disease. These findings are in contrast with those of Bon et al. *(23)*, who could not find different CA27.29 or CA15.3 values in relation to the disease stage. This may have been because of the small number of cases from each clinical stage that they evaluated. Indeed, CA27.29 concentrations and positivity rates are higher than CA15.3 in patients with earlier stages of disease, whereas the two markers show comparable results in stage III cases. Although the latter data should be interpreted cautiously because of the relatively low number of stage III cases, they indicate an overall higher sensitivity of CA27.29 for early disease and corroborate the findings of Chan et al. *(11)*, who showed a high sensitivity for relapse and suggested that CA27.29 is more sensitive than CA15.3 in that setting. Of further note, and in agreement with a higher sensitivity for early disease, CA27.29 was significantly higher than CA15.3 in patients when marker concentrations were within the health-related reference intervals (data not shown).

As with all tumor markers, increases of a particular analyte are not absolute evidence of the presence of malignancy and should be interpreted within the overall context of patient management. Although in our study we did not see any evidence of abnormal increases of CA27.29, Bon et al. *(23)* did report that in 3 of 250 apparently healthy individuals, CA27.29 unexpectedly increased above the health-related cutoff. Of note, this increase persisted in only one individual after further analysis. It was subsequently shown that these anomalous increases could be attributed to the presence of a unique interfering factor in these samples that had the characteristics of an anti-idiotypic antibody to B27.29 (P. Maimonis, personal communication).

Chiron Diagnostics has developed a new version of the ACS:180 BR assay, designed to eliminate these discrepancies. A pretreatment step, involving the addition of NaOH to all samples before antibody incubation, was added to the assay. The NaOH presumably denatures this putative interfering antibody without affecting the CA27.29 concentrations. The new assay, made available only after the completion of our study, has been validated extensively, and it was determined that the pretreatment has no effect on samples not containing the interfering factor (data not shown). Therefore, because we observed no samples with falsely increased values, it is extremely unlikely that our results would change if we used the new version of the assay. In confirmation of our supposition, a study comparing the new version of the ACS:180 BR assay with CA15.3 was performed recently. Supporting our results, Aspeslet et al. *(40)* reported the superior performance of CA27.29 over CA15.3 in all stages of breast cancer.

The superior diagnostic performances of CA27.29 may be ascribed to the ability of the assay to detect a well-defined peptide epitope, whereas CA15.3 relies on the detection of two epitopes, one of which is still uncharacterized and could possibly be of carbohydrate nature.

Because most carbohydrate structures are not unique and occur on many different molecules, recognition of carbohydrate by CA15.3 could theoretically lead to higher interindividual variability *(41)*. However, in a previous study CA15.3 showed higher sensitivity values than CA549 *(39)*, which is assayed through a monoclonal antibody specific for a peptide epitope almost identical to that recognized by B27.29 *(8)*. Therefore, the superior performance of CA27.29 may be attributed not only to the epitope specificity or the antibody, but may be related to assay architecture as well. Although CA27.29 is superior to CA15.3, both assays still present limited sensitivity and specificity values. These findings should therefore prompt researchers to work toward newer assay formats with high sensitivity for the detection of early disease.

From the findings of the present study we can draw the following conclusions: (*a*) the fully automated immunochemiluminescent ACS:180 BR CA27.29 assay presents diagnostic performances superior to those of CA15.3 in patients with primary breast cancer; and (*b*) the higher sensitivity found in cases with limited disease confirms previous findings *(11)* and prompts the need for wide, prospective follow-up studies aimed at an early detection of the relapse.

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References

1. Gum JR. Mucin genes and the proteins they encode: structure, diversity and regulation. Am J Respir Cell Mol Biol 1992;7:557–64.
2. Gender S, Spicer AP, Lalani E-N, Duhig T, Peat N, Burchell J, et al. Structure and biology of a carcinoma associated mucin, MUC-1. Am Rev Respir Dis 1991;S42–7.
3. Graves R, Hilgers J, Fritsche H, Hayes D, Robertson JFR. MUC-1 mucin assays for monitoring therapy in metastatic breast cancer. Breast 1998;7:181–6.
4. Taylor-Papadimitriou J. Report on the First International Workshop on carcinoma-associated mucins. Int J Cancer 1991;49:1–5.
5. Tobias R, Rothwell C, Wagner J, Green A, Liu YSW. Development and evaluation of radioimmunoassay for the detection of a monoclonal antibody defined breast tumor associated antigen 115D8/DF3 [Abstract]. Clin Chem 1985;31:986.
6. Hilkens J, Buij F, Hilgers J, Hageman P, Calafat J, Sonnenberg A, van der Valk MA. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer 1984;34:197–206.
7. Kufe D, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. Hybridoma 1984;3:223–31.
8. Price MR, Rye PD, Petrakou E, Murray A, Brady K, Imai S, et al. Summary report on the ISOBM TD-4 workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. San Diego, Calif., November 17–23, 1996 [Review]. Tumor Biol 1998;19(Suppl 1):1–20.
9. American Society of Clinical Oncology. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1996;14:2843–77.
10. American Society of Clinical Oncology. 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. J Clin Oncol 1998:16:793–5.
11. Chan DW, Beveridge RA, Muss H, Fritsche HA, Hortobagyi G, Theriault R, et al. Use of Truquant BR radioimmunoassay for early detection of breast cancer recurrence in patients with stage II and stage III disease. J Clin Oncol 1997;15:2322–8.
12. Reddish MA, Helbrecht N, Almeida AF, Madiyalakan R, Suresh MR, Longenecker BM. Epitope mapping of Mab B27.29 within the peptide core of the malignant breast carcinoma-associated mucin antigen coded for by the human *MUC1* gene. J Tumor Marker Oncol 1992;7:19–27.
13. Correale M, Abbate I, Gargano G, Catino A, Dragone CD, Musci MD, et al. Analytical and clinical evaluation of a new tumor marker in breast cancer: CA27.29. Int J Biol Markers 1992;7:43–6.
14. Rodriguez de Paterna L, Arnaiz J, Estenoz J, Ortuno B, Lanzos E. Study of serum tumor markers CEA, CA15.3 and CA27.29 as diagnostic parameters in patients with breast carcinoma. Int J Biol Markers 1995;10:24–9.
15. Jensen JL, MacLean GD, Suresh MR, Almeida A, Jette D, Lloyd S, et al. Possible utility of serum determinations of CA125 and CA27.29 in breast cancer management. Int J Biol Markers 1991;6:1–6.
16. Hayes DF, Zurawski VR, Kufe D. Comparison of circulating CA15.3 and carcinoembryonic antigen levels in patients with breast cancer. J Clin Oncol 1986;4:1542–50.
17. Devine PL, Duroux MA, Quin RJ, McGuickin MA, Joy GJ, Ward BG, Pollard CW. CA15-3, CASA, MSA, and TPS as diagnostic serum markers in breast cancer. Breast Cancer Res Treat 1995;34:245–51.
18. Eastbrook A, Chen J, Oster M, Salinen R, Yemul S, Leon J. Clinical value of BCA 225: a new breast cancer serum marker. J Tumor Marker Oncol 1994;9:111–8.
19. Gion M, Mione R, Nascimben O, Valsecchi M, Gatti C, Leon A, Bruscagnin G. The tumor associated antigen CA15.3 in primary breast cancer. Evaluation of 667 cases. Br J Cancer 1991;63:809–13.
20. O'Hanlon DM, Kerin MJ, Kent P, Maher D, Grimes H, Given HF, et al. An evaluation of preoperative CA15.3 measurement in primary breast carcinoma. Br J Cancer 1995;71:1288–91.
21. Peterson JA, Ceriani RL. Breast mucin and associated antigens in diagnosis and therapy. In: Ceriani RL, ed. Antigen and antibody molecular engineering in breast cancer diagnosis. New York: Plenum, 1994:2–3.
22. Roth HJ, Zahn I, Maimonis P. Performance characteristics of the ACS-BR: a comparison with three other assays that also measure CA15.3. Clin Lab 1995;41:1031–5.
23. Bon GG, von Mensdorff-Pouilly S, Kenemans P, van Kamp GJ, Verstraeten RA, Hilgers J, et al. Clinical and technical evaluation of ACS-BR serum assay of *MUC1* gene-derived glycoprotein in breast cancer, and comparison with CA15.3 assays. Clin Chem 1997;43:585–93.
24. European Organization for Research and Treatment of Cancer. Revision of the standards for the assessment of hormone receptors in human breast cancer. Report of the Second E.O.R.T.C. Workshop held on 16–17 March 1979, in the Netherlands Cancer Institute. Eur J Cancer 1980;16:1513–20.
25. Dixon AR, Price MR, Hand CV, Sibley PE, Selby C, Blamey RW. Epithelial mucin core antigen (EMCA) in assessing therapeutic response in advanced breast cancer. A comparison with CA15.3. Br J Cancer 1993;68:947–9.
26. Safi F, Kohler I, Rottinger E, Suhr P, Beger HG. Comparison of

CA15.3 and CEA in diagnosis and monitoring of breast cancer. Int J Biol Markers 1989;4:207–14.

27. Kallioneimi OP, Oksa H, Aaran RK, Hietanen T, Lehtinen M, Koivula T, et al. Serum CA15.3 assay in the diagnosis and follow-up of breast cancer. Br J Cancer 1988;58:213–5.

28. Miserez AR, Günes I, Müller-Brand J, Walther E, Fridrich R, Macke H, et al. Clinical value of a mucin-like carcinoma-associated antigen in monitoring breast cancer patients in comparison with CA15.3. Eur J Cancer 1991;27:126–31.

29. Dnistrian AM, Schwartz MK, Greenberg EJ, Smith CA, Schwartz DC. Evaluation of CA M26, CA M29, CA15.3 and CEA as circulating tumor markers in breast cancer patients. Tumor Biol 1991; 12:82–90.

30. Schol DJ, Meulenbroek MFA, Snijdewint FGM, von Mensdorff-Pouilly S, Verstraeten RA, Murakami F, et al. 'Epitope fingerprinting' using overlapping 20-mer peptides of the MUC-1 tandem repeat sequence. Tumor Biol 1998;19:35–45.

31. Spencer DIR, Price MR, Tendler SJB, De Matteis CI, Stadie T, Hanisch FG. Effect of glycosylation of a synthetic MUC1 mucin-core-related peptide on recognition by anti-mucin antibodies. Cancer Lett 1996;100:11–5.

32. Gourevitch MM, Mensdorff-Pouilly S, Litvinov SV, Kenemans P, van Kamp GJ, Verstraeten RA, Hilgers J. Polymorphic epithelial mucin (MUC-1) containing circulating immune complexes in carcinoma patients. Br J Cancer 1995;72:934–8.

33. Molina R, Filella X, Mengual P, Prats M, Zanon G, Daniels M, Ballesta AM. MCA in patients with breast cancer: correlation with CEA and CA15.3. Int J Biol Markers 1990;5:14–21.

34. Crippa F, Bombardieri E, Seregni E, Castellani MR, Gasparini M, Maffioli L, et al. Single determination of CA15.3 and bone scintigraphy in the diagnosis of skeletal metastases of breast cancer. J Nucl Biol Med 1992;36:52–5.

35. Colomer R, Ruibal A, Genollà J, Rubio D, Del Campo JM, Bodi R, Salvador L. Circulating CA15-3 levels in the postsurgical follow-up of breast cancer patients in non-malignant diseases. Breast Cancer Res Treat 1989;13:123–33.

36. Soletormos G, Nielsen D, Schioler V, Skovsgaard T, Winkel P, Mouridsen HT, Dombernowsky P. A novel method for monitoring high-risk breast cancer with tumor markers: CA15.3 compared to CEA and TPA. Ann Oncol 1993;4:861–9.

37. Molina R, Zanon G, Filella X, Moreno F, Jo J, Daniels M, et al. Use of serial carcinoembryonic antigen and CA15.3 assays in detecting relapses in breast cancer patients. Breast Cancer Res Treat 1995;36:41–8.

38. Bombardieri E, Gion M, Mione R, Dittadi R, Bruscagnin G, Buraggi G. A mucinous-like carcinoma-associated antigen (MCA) in the tissue and blood of patients with primary breast cancer. Cancer 1989;63:490–5.

39. Gion M, Plebani M, Mione R, Penzo C, Meo S, Burlina A. Serum CA549 in primary breast cancer: comparison with CA15.3 and MCA. Br J Cancer 1994;69:721–5.

40. Aspeslet L, Ballok J, Mansbach L, Maimonis PJ. A technical and clinical evaluation of 3 commercial MUC-1 breast cancer marker assays [Abstract]. Tumor Biol 1998;19(Suppl 2):13.

41. Laine RA. A calculation of all possible oligosaccharide isomers, both branched and linear yields $1.05 \times 10(12)$ structures for a reducing hexasaccharide: the Isomer Barrier to development of single-method saccharide sequencing or synthesis systems. Glycobiology 1994;4:759–67.