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CA27.29: a valuable marker for breast cancer management. A confirmatory multicentric study on 603 cases

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Abstract

Recently, a fully automated method has become commercially available to measure the MUC-1-associated antigen CA27.29. The present investigation was performed in order to compare CA27.29 and CA15.3 in a wide series of patients affected with breast cancer. Overall, 603 cases with breast cancer and 194 healthy controls were investigated. Patients were enrolled in 4 institutions, while assays were performed in one laboratory. CA27.29 was measured by the ACS:180 BR assay (Bayer Diagnostics) and CA15.3 by the AxSYM (Abbott Laboratories). An excellent correlation was found between the results obtained by the two methods. The two markers showed comparable results in healthy controls, with higher levels in post-menopausal than in pre-menopausal subjects. The markers were significantly higher in primary breast cancer than in controls. The areas under the receiver operating characteristics (ROC) curves of the two tests were comparable, but CA27.29 showed better sensitivity in cases with low antigen concentrations (below the cut-off point). Accordingly, when comparing each test in different stage categories, significance levels of the differences were higher for CA27.29 than for CA15.3 for all T categories versus healthy controls, for pT1 versus pT2, for all N categories versus healthy controls and for node-negative versus N1-3 patients. From the results of the present study, that has been performed on samples taken at diagnosis and prior to any treatment from the widest series of patients with primary breast cancer reported so far, we can draw the following conclusions: CA27.29 provides comparable results to CA15.3; CA27.29 seems more sensitive than CA15.3 to limited variations of tumour extension; however, it cannot help clinicians in distinguishing stage I patients from stage II patients. However, from the point of view of clinical decision making, CA27.29 provides comparable results to CA15.3. CA27.29 is therefore suitable for routine use in the management of patients with breast cancer. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Breast cancer; CA27.29; CA15.3; MUC-1; Serum tumour markers; Mucin markers

1. Introduction

In the last decade, the position of the scientific community towards the use of tumour markers in breast cancer has been controversial. The recommendations first provided by the American Society for Clinical Oncology are quite restrictive, advising for the use of carcinoembryonic antigen (CEA) and CA15.3 or CA27.29 only for monitoring the therapy of advanced cancer patients with non-measurable lesions [1,2]. More recently, on the basis of published evidence, the European Working Group in Tumor Markers recommended the use of CEA and CA 15.3 in prognosis, early diagnosis of recurrence and follow-up of patients with breast cancer [3]. Molina and colleagues showed that CEA, CA 15.3 and c-erbB-2 are useful in the early detection of breast cancer recurrence during the follow-up [4,5]. Robertson et al. showed that changes in tumour markers excellently reflect International Union Against Cancer (UICC) status in the monitoring of therapy for metastatic breast cancer [6]. Shering et al. reported that high pre-operative serum CA15.3 levels

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are associated with a poorer outcome [7]. Nicolini et al. showed in a retrospective study that early treatment of patients on the basis of the increase of tumour marker levels during postoperative follow-up improved the survival length with respect to those patients treated when metastatic disease was evident [8]. In addition, the interest towards tumour markers has been recently renewed thanks to the meaningful changes in the management strategy of cancer patients. Tumour markers seem indeed to impact on several emerging issues. First of all, new therapeutic regimens are currently under evaluation, including both traditional chemotherapeutic agents and newly developed drugs addressed to biological targets. The clinical assessment of response or failure to treatment usually requires a relatively long observation time. Tumour markers may provide early indications, thus driving cost-effective changes in therapeutic strategies. Secondly, the assessment of the true endpoints of adjuvant therapeutic treatments in node-negative patients with intermediate risk may require a very long time. Therefore, surrogate endpoints are urgently needed to identify an early failure [9].

The use of tumour markers is now supported by a better knowledge of their physiology. Basic research has identified putative functions for mucin markers related to the *MUC-1* gene. Their biological actions have been extensively investigated [10], showing that they may elicit different effects favourable to tumour dissemination [11].

In this changing scenario, MUC-1-associated markers seem to play a pivotal role. Several assay kits have been developed to measure MUC-1-associated markers. Recently, a fully automated method (ACS:180 BR) has been set-up to measure CA27.29 [12]. Preliminary studies that compared CA27.29 and CA15.3 reported mixed results [12–21].

Considering that high sensitivity and specificity are a key point for a more effective clinical use of MUC-1 markers, we performed the present multicentric study in order to compare the diagnostic performances of the new fully automated ACS:180 BR assay kit with those of CA15.3 in a large patient series. The present study was mainly focused on patients with primary breast cancer, in whom low CA15.3 serum levels are expected. Therefore, excellent precision performances were required to investigate differences between the tests. Given the poor precision of manual immunoradiometric assays when measuring low concentrations of mucin markers [22], we planned to use a fully automated CA15.3 assay (AxSYM Abbott) in order to have analytical precision comparable to that of ACS: 180 BR.

2. Patients and methods

The study was performed retrospectively using serum samples stored in serum banks of four institutions. All tests were performed in only one laboratory (Venice). The assays for both methods were carried out on the same analyser by the same technician.

Five hundred and sixty-six samples from patients with breast cancer (median age 61 years, range 17–89 years) were enrolled in the study.

One hundred ninety-four patients were node-negative, 176 node-positive; 166 cases were pT1, 156 pT2, 24 pT3 and 29 pT4; 128 were in stage I, 173 in stage II, 149 in stage III and 116 in stage IV.

For stage I–III cases, patients' characteristics required for inclusion in the study were:

- no treatment (including surgery, radiotherapy, chemotherapy or endocrine manipulations) before the blood sample;
- no clinical or laboratory evidence of benign diseases of the liver, pancreas, ovary or kidney;

Patient staging was carried out according to the UICC criteria. Histological typing was done following the World Health Organization (WHO) classification.

Serum samples from 194 apparently healthy controls (median age 46 years, range 17–78 years) were also evaluated.

Serum was separated by centrifugation at room temperature and one frozen aliquot was sent on dry ice to the laboratory where the assays were performed. Serum samples were stored at −80°C for a variable time span, ranging from 1 month to 2 years.

2.1. Assay methods

CA27.29 was measured by the ACS:180 BR assay (Bayer Diagnostics, Tarrytown, NY, USA), a fully automated competitive chemiluminescent immunoassay performed according to the manufacturer's instructions. In the present investigation, we used the new version of the ACS:180 BR assay kit, designed to eliminate possible discrepancies due to anti-idiotypic antibodies.

CA15.3 was assayed by the AxSYM CA15.3 (Abbott Laboratories; Abbott Park, IL, USA), a fully automated microparticle enzyme immunoassay performed according to the manufacturer's instructions. The AxSYM CA15.3 assay showed an excellent correlation and provided superimposable results with the manual immunoradiometric CA15.3 assay by Centocor (not shown).

2.1.1. Assay performances

Within- and between-assay precision were assessed with two in-house serum pools prepared using human serum samples. ACS:180 BR assay imprecision was low for samples with high antigen levels (~130 kU/l, CV $<4.0\%$), whereas it tended to increase for samples with intermediate levels (30–40 kU/l, CV $<9.0\%$). AxSYM CA15.3 assay precision was good for both samples with

intermediate (~25 kU/l, CV <6.0%) and high levels (~100 kU/l, CV <7.0%).

2.2. Statistical analysis

Data were evaluated as both continuous variables and dichotomised according to positive/negative cut-off points, as described in detail below. Data were analysed using the Kruskal-Wallis one-way ANOVA on ranks test. The Spearman correlation, the Bland-Altman plot and the Mountain plot were used to compare the results of the two tests. Receiver operating characteristics (ROC) curves were generated plotting sensitivity versus 1-specificity. The Number Cruncher Statistical Systems (NCSS) (Kaysville, UT, USA) package was used. A P value of $\leqslant 0.05$ was considered to be significant.

3. Results

3.1. Comparison between the two methods

An excellent correlation was found between the values of CA27.29 and CA15.3 both in healthy controls ($n=194$, $r=0.892$, $P<0.0001$) and in patients with primary ($n=450$, $r=0.921$, $P<0.0001$) and advanced breast cancer ($n=116$, $r=0.979$, $P<0.0001$). CA27.29 tended to be higher than CA15.3 except in samples with low values (<20 U/ml), whereas CA27.29 seemed to detect lower antigen levels, as was confirmed by both the Altman-Bland plot and the Mountain plot (data not shown).

3.2. Healthy controls

The two markers showed comparable results in 194 healthy controls. (CA27.29: median 14.1 U/ml, interquartile 8.5–20.1 U/ml. CA15.3: median 14.1 U/ml, interquartile 10.1–17.8 U/ml). The concentrations of the two markers showed significant variations in relation to menopausal status, being higher in post-menopausal (CA27.29: median value 16.7 (95% CI: 14.1–18.2) U/ml, CA15.3 (95% CI: 13.9–16.7) median value 15.3 U/ml) than in pre-menopausal (CA27.29: median value: 11.7 (95% CI: 9.8–13.4) U/ml; CA15.3 median value 13.1 (95% CI: 11.0–14.4) U/ml; $P=0.0027$ for CA27.29, 0.012 for CA15.3). Both CA27.29 and CA15.3 tended to be higher in elderly patients. The trend was however significant only for CA27.29. The distribution of the values of both markers according to age (data not shown) suggests a step-like variation, with a fairly sharp increase over 50 years of age. For both markers, differences between cases <50 and $\geqslant$50 years showed differences comparable with those found with reference to menopausal status. Therefore, considering that menopausal information was not available in 10% of healthy subjects and 15% of cancer patients, we decided to use the age-based cut-off point. On the basis of these findings, we calculated two different cut-off points to be used in patients younger and older than and equal to 50 years of age respectively. Considering that the distribution of the markers was significantly different from Gaussian (tested by both Kolmogorov-Smirnoff and D'Agostino Skeweness/Kurtosis test) we preferred non-parametric threshold levels, choosing the 95th percentile value for both. Values were 29.3 U/ml (<50 years of age) and 34.9 U/ml ($\geqslant$50 years) for CA27.29; 23.0 U/ml (<50 years of age) and 25.8 U/ml ($\geqslant$50 years of age) for CA15.3. Positivity rates in cancer patients were calculated using the two different cut-offs in relation to the individual patient age.

3.3. Primary breast cancer patients (stages I, II, III)

First of all, we compared the values found in the patients' enrolled by the different centres participating in the study (total: 603 cases). No significant differences were found using both ANOVA ($f=1.21$ for CA27.29, 1.22 for CA15.3) and Kruskal–Wallis test ($\chi=4.85$ for CA27.29, 2.91 for CA15.3). Therefore, we decided to evaluate all cases as one group. Overall, tumour marker values were significantly higher in cancer patients (median values: CA27.29 22.8 (95% CI: 11.0–24.4) U/ml, CA15.3 18.3 (95% CI: 17.1–19.1) U/ml) than in healthy controls (CA27.29 14.1 (95% CI: 12.1–14.1) U/ml; CA15.3 14.1 (95% CI: 13.2–15.1) U/ml; $P=0.05$ for CA27.29; $P=0.05$ for CA15.3). The diagnostic effectiveness of the two markers was assessed by ROC analysis. The results are shown in Fig. 1. Although the area under the curve of the two markers did not differ significantly ($P=0.06$), CA27.29 showed higher sensitivity than CA15.3 in the concentration range below the cut-off points (i.e., at around 10 U/ml, for the same specificity value, sensitivity was 87% for CA27.29 and 78% for CA15.3). This difference between the two markers was more evident in patients younger than 50 years (Fig. 2), while in those $\geqslant$50 years the ROC curves of CA27.29 and CA15.3 were superimposable (data not shown).

The age profiles were significantly different in cancer patients and controls, with older cases more represented in the patient group than in the controls. However, in patients with breast cancer, the two markers showed an association with age comparable to that found in healthy controls, with both CA27.29 and CA15.3 values increasing with age.

3.4. Relationship to tumour burden

The levels of the two markers were evaluated in relation to tumour extension. As expected, both CA27.29 and CA15.3 were significantly higher in advanced than in loco-regional disease ($P<0.001$ for both). In order to

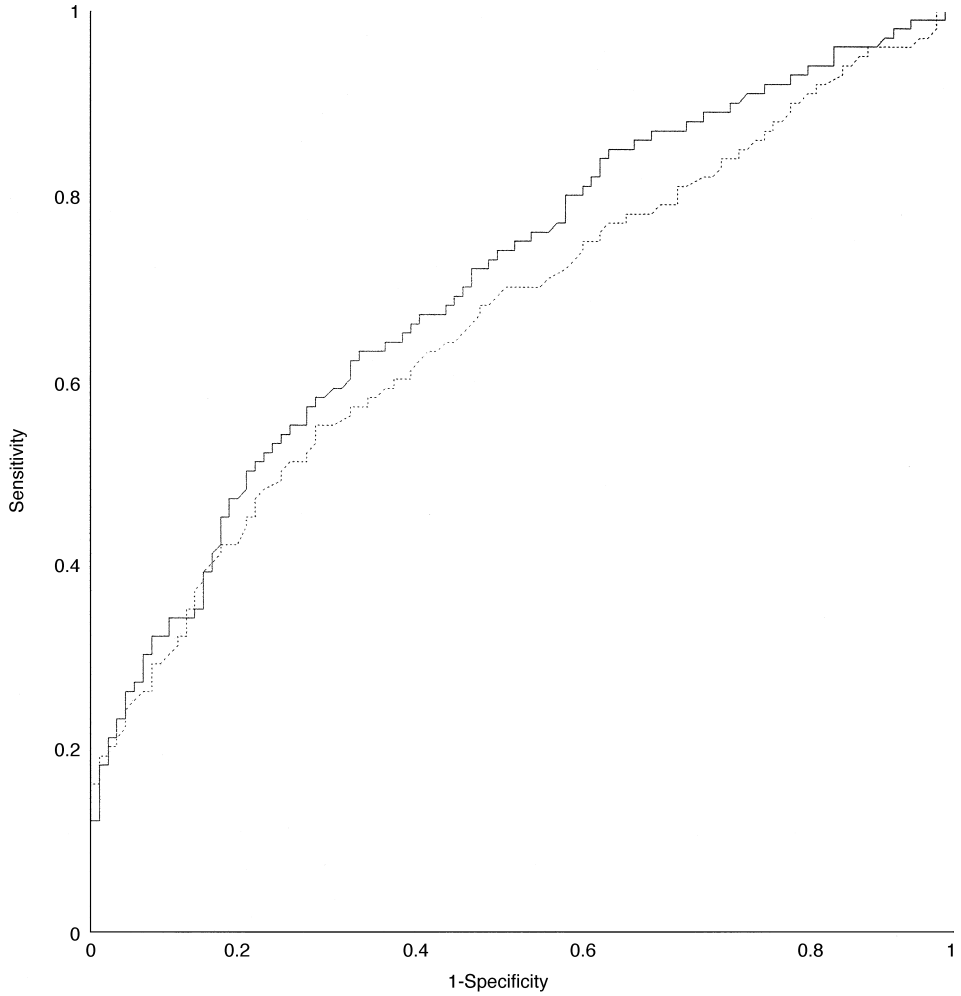

Fig. 1. Receiver operating characteristics (ROC) curves of CA27.29 (solid line) and CA15.3 (dotted line) (patients with primary breast cancer versus healthy controls).

evaluate if the markers were able to distinguish between different loco-regional stages, we compared their levels with reference to different T and N categories. Fig. 3 shows the relationship between the two markers and pT. PT3 and pT4 were considered together due to the small number of pT4 cases. Both markers tended to increase with tumour size. However, CA27.29 seemed to be more sensitive to small differences as is suggested by the statistical evaluation of differences (Table 1). Significance levels were higher for CA27.29 for all T categories versus healthy controls and when comparing pT1 to pT2. The relationship between the two markers and the number of positive lymph nodes grouped in the three categories (negative, 1–3 and more than 3 positive lymph nodes) showed essentially the same pattern (data not shown). Again, the levels of both markers tended to rise with an increasing number of positive lymph nodes. However, significance levels were higher for CA27.29 than for CA15.3 for all N categories versus healthy controls and for node-negative versus N1-3.

Table 1
CA27.29 versus CA15.3 — relationship with tumour size. Kruskal–Wallis multiple comparison Z-value test

	CA 27.29			CA 15.3		
	pT1	pT2	pT3-4	pT1	pT2	pT3-4
Healthy controls	3.77	6.07	8.06	2.59	4.65	7.51
pT1		2.28	5.39		2.03	5.65
pT2			3.75			4.18

Median significantly different if Z-value > 1.96.

ROC analyses were performed comparing the levels of the two markers in patients subdivided into different stage categories and in healthy subjects. The areas under the concentration curves (AUCs) of CA27.29 were in all instances wider than those of CA15.3 (summarised in Table 2). Interestingly, the difference between the AUCs of the markers and the 0.5 area was more evident in stage I versus healthy controls than in the other comparisons (Table 2). AUCs of CA27.29 were wider than those of CA15.3 also when comparing the two markers in stage I versus stage II, in stage II versus stage III and

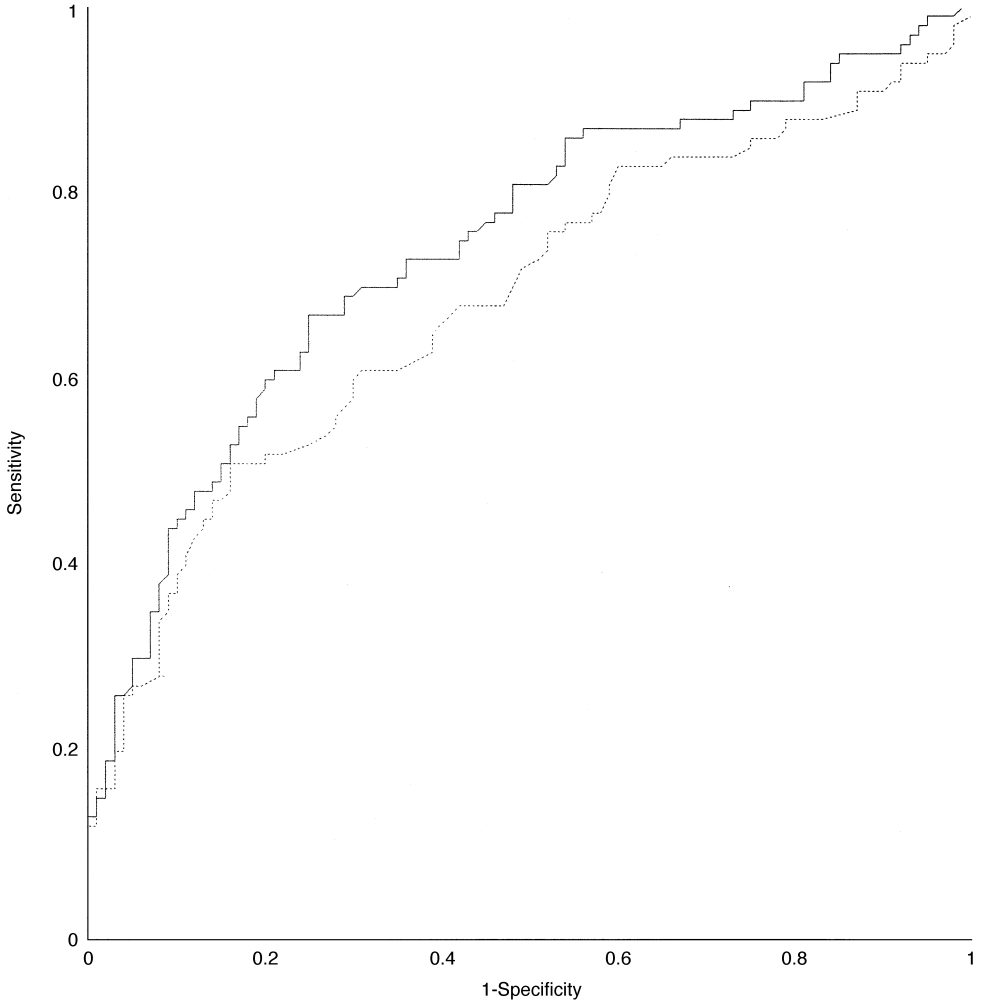

Fig. 2. ROC curves of CA27.29 (solid line) and CA15.3 (dotted line) (patients with primary breast cancer < than 50 years versus healthy controls).

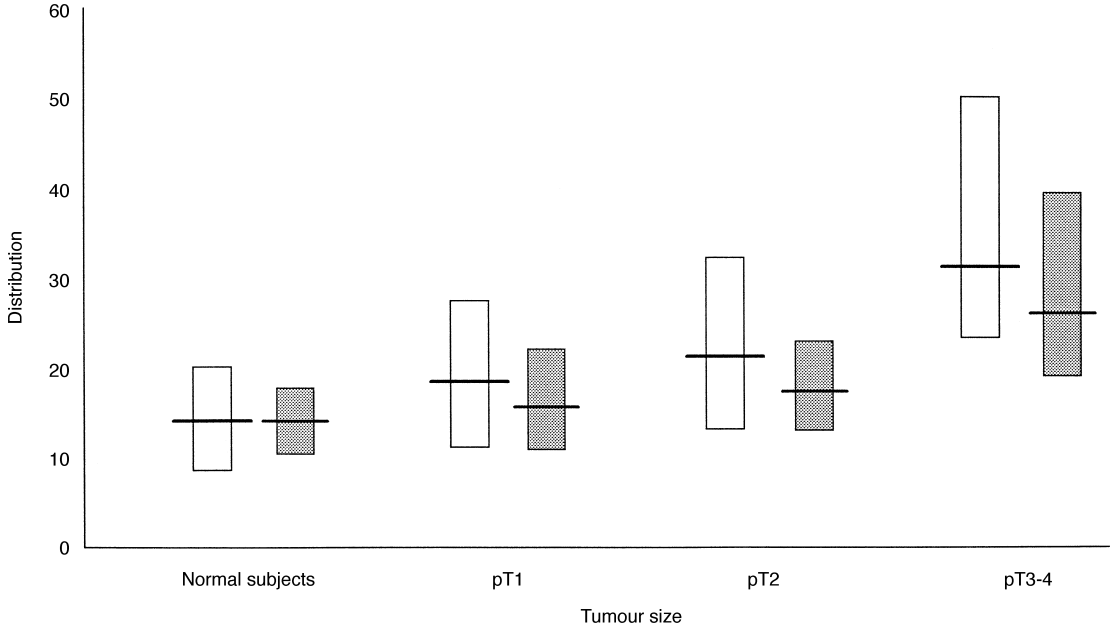

Fig. 3. Distribution of CA27.29 (open boxes) and CA15.3 (grey boxes) according to tumour size. Each box represents the interquartile range; the line represents the median.

Table 2
ROC analysis of CA27.29 and CA15.3 according to the pathological stage

		AUC (95% CI)		CA27.29 versus CA15.3		CA27.29 versus 0.50 area		CA15.3 versus 0.50 area	
		CA27.29	CA15.3	Z	P	Z	P	Z	P
Stage I versus healthy controls	All cases	0.6042 (0.540–0.668)	0.5649 (0.499–0.630)	1.19	0.1970	3.20	0.0007	1.93	0.0265
	Cases < 50 years	0.5865 (0.449–0.723)	0.5544 (0.499–0.723)	0.451	0.6527	1.24	0.1082	0.75	0.2259
Stage II versus healthy controls	All cases	0.6676 (0.649–0.686)	0.6245 (0.606–0.643)	1.50	0.1336	5.96	< 0.0001	4.23	< 0.0001
	Cases < 50 years	0.7307 (0.643–0.818)	0.6324 (0.534–0.731)	2.07	0.0384	5.19	< 0.0001	2.63	0.0042
Stage III versus healthy controls	All cases	0.8406 (0.799–0.882)	0.8018 (0.754–0.849)	1.70	0.091	15.88	< 0.0001	12.44	< 0.0001
	Cases < 50 years	0.8752 (0.807–0.944)	0.8509 (0.772–0.930)	0.645	0.5220	10.75	< 0.0001	8.68	< 0.0001

95% CI, 95% confidence interval; AUC, area under curve; ROC, receiver operating characteristics.

in stage I versus stage III (data not shown). However, differences were less evident than when each stage was compared with healthy controls. The higher sensitivity of CA27.29 than CA15.3 for the different stages was generally more evident in patients younger than 50 years than in the overall group. However, the limited number of patients available after the latter stratification hampers a reliable statistical evaluation of these findings.

4. Discussion

Several MUC-1-associated markers have been developed and used in clinical practice [13,23,24]. The most widely evaluated is CA15.3, which recognises the antigen through two monoclonal antibodies, DF3 and 115D8. Different monoclonal antibodies have been developed [25] and used to set up other commercially available assay kits: MCA (monoclonal antibody B12), CA549 (monoclonal antibody BC4E549) and CA27.29 (monoclonal antibody B27.29). Comparative studies between CA15.3 and both MCA and CA549 failed to show evidence of advantages in these latter markers in comparison with CA15.3 [26,27]. Basic knowledge [28–32] supports a re-evaluation of MUC-1 markers in order to identify the most suitable one for clinical use. MUC-1 glycoprotein is indeed markedly heterogeneous, exhibiting a wide range of molecular weights and showing a remarkably different number and assembly of carbohydrate molecules [10,28]. In addition, different MUC-1 sub-populations may be present in serum samples from different patients or from the same patients in different phases of the disease [33–35]. Reddish and coworkers showed that the epitope sequence on the MUC-1 core-protein recognised by B27.29 and DF3 antibodies is essentially the same [36]. However, the binding of DF3 is reduced by neuraminidase treatment of MUC-1 antigen, thus suggesting that immunoreactivity is influenced by the presence of sialic acid. On the contrary, B27.29 binding seems not to be affected by glycosylation, suggesting that the antibody has a peptide restricted specificity [36]. The possible presence of MUC-1 antibodies in the sample may be a further cause of variability in the results obtained with different methods. Those auto-antibodies, which may be generated by a response of the host to cancer related changes of the MUC-1 glycoprotein, may form immunocomplexes with circulating mucins. The possible interference of these complexes with MUC-1 assay systems may vary depending upon the assay format. Namely, it should be minimised by using competitive assays (i.e. CA27.29) while it may significantly reduce assay sensitivity in two-sites immunoradiometric assays (i.e. CA15.3).

From these findings, it appears that the two markers could provide different results in individual patients in spite of the fact that their values are essentially well correlated. Preliminary findings by Correale and colleagues [14] and by Aspeslet and colleagues [16] showed that the sensitivity of CA27.29 for an early detection of relapse was higher than that of CA15.3. Chan and colleagues [17], using the Truquant BR manual assay, reported that CA27.29 has a sensitivity for recurrence of 57.7% during the follow-up of a cohort of patients including 80% of stage II cases. This sensitivity figure is higher than that reported for CA15.3 in the post-operative follow-up of stage II breast cancer patients, which ranges from 10 to 29%. In a previous investigation performed on 275 patients with primary breast cancer and 83 healthy controls enrolled in one institution [21], CA27.29, measured by the ACS:180 BR assay, showed a sensitivity and specificity higher than CA15.3 measured by the Centocor manual immunoradiometric assay. The two markers showed an opposite pattern in cancers and controls, with CA27.29 higher than CA15.3 in cancer and lower in controls. This led to a lower cut-off point for CA27.29 than for CA15.3 and, ultimately, to higher diagnostic performances for the former [21]. In the present multicentric investigation, we could not

confirm the cut-off values previously found. Using a different control series, enrolled by three of the centres participating in the study, we found a cut-off point that is still lower than the majority of those previously reported in the literature [14–20] but higher than that found in our previous study [21]. It is important to note that the distribution of both CA27.29 and CA15.3 in healthy controls is also different in the present study (i.e. not Gaussian) than in our previous investigation (Gaussian). These differences may be related to a variety of reasons, including the number of evaluable cases and the characteristics of the subjects. In addition, the use of two different assays both for CA27.29, (ACS:180 BR assay with — in the present study — and without — in the previous study — pre-treatment to avoid interferences) and for CA15.3, may have contributed to these discrepancies. The use of a different assay kit for CA15.3 deserves some further comments. In the former study we used the manual Centocor CA15.3 IRMA version to have a reference with the historical standard method for this marker. However, imprecision in the low antigen dose range was higher with the manual CA15.3 kit than with the fully automated ACS:180 BR that we used for CA27.29. This probably led to a poorer diagnostic accuracy of CA15.3 for initial stage patients, in which low antigen levels are expected. In the present study, CA15.3 was determined with the AxSYM fully automated method, in order to have precision performances comparable to those of ACS:180 BR. Therefore, the comparison of the two markers in patients with low antigen levels should not be affected by imprecision bias in the present study.

Conflicting results have been reported on the comparison between the diagnostic performances of the two methods. Some authors reported that CA15.3 is superior to CA27.29 [15,18], others reported no differences in the performances of the two tests [14,19,20], some others reported that CA27.29 was more effective than CA15.3 [16,17,21]. Overall sensitivity is quite different in various studies, certainly due, at least in part, to different characteristics such as the stage distribution in the patient series evaluated in the different studies. However, differences are also remarkable when observing positivity rates subdivided according to stage parameters. The lowest sensitivity figures have been reported by Bon and colleagues [18]. They compared the Biomira assay for CA27.29 with three different CA15.3 assay kits (the manual IRMA Centocor and two fully automated methods, the ELISA-Boehringer Mannheim-ES300- and the Abbott IMx). Surprisingly, they found lower CA27.29 positivity rates in stage III than in stage I patients. In addition, their data concerning CA15.3 was also quite mixed. Positivity rates of the Centocor CA15.3 did not show meaningful variations related to stage, while CA15.3 values obtained with both Boehringer Mannheim ES300 and Abbott IMx showed higher values in stage I than in stage II patients [18].

It should be emphasised that the results of studies concerning the determination of MUC-1-associated markers should be considered cautiously, taking into account the complexity of the MUC-1 related glycoproteins. As a matter of fact, in spite of the general agreement among results obtained with different assays, comparability of results of individual patients may be biased by a number of factors. Some of them are probably assay-related (assay architecture, antibodies, buffers, etc), while others may depend on the patient (clinical conditions, age, sample type, sample collection and storage, etc). The biological characteristics of the MUC-1 markers may be also affected by patient's age. Higher levels of both CA27.29 and CA15.3 were found in older than in younger patients. Similar findings have been previously reported in healthy females by Lynch et al, which showed higher levels of both markers in elderly patients using the same age threshold we used (50 years) [19].

The present study has been performed on samples taken at diagnosis and prior to any treatment from the widest series of patients with primary breast cancer reported so far. All samples have been assayed in one laboratory to reduce analytical bias. Statistical analysis showed an excellent agreement among results of clinical samples collected in different institutions, thus confirming a good homogeneity of both patient selection and sample handling. As expected, a strict correlation was found between the two markers in all the examined subgroups (normal subjects, patients in different stages). CA27.29 seems more sensitive to limited variations of tumour stage. Although it cannot help clinicians in distinguishing stage I from stage II, we believe, in agreement with Chan and colleagues, that this high sensitivity for minor disease variations may support a possible advantage of CA27.29 over CA15.3 testing during follow-up for the early detection of a relapse.

In 1996, Hayes and colleagues proposed a Tumour Marker Utility Grading System suited to evaluate the clinical impact of tumour markers in a standardised manner [37]. They clearly distinguished clinical outcome from the biological process and the end-points. The former depends on several variables not related to the marker, i.e. the effectiveness of available therapies. The latter is indeed characteristic of the biomarker and is the basis for its clinical use. According to Hayes and colleagues "...for a marker to be of value in a clinical setting, it must reflect a biological process with which it is putatively associated". This correlation with the biological process and end-points is the basis for considering the marker as a valuable tool if or when effective therapies for the disease are available.

In this changing scenario of breast cancer management, which ranges from 'molecular bullets' to chemoprevention trials, a marker reliably associated with variations in tumour extension may be of valuable clinical impact.

CA27.29 fits this need, since it correlates with the variation of tumour extension as well as, if not better than CA15.3. From the findings of the present study we can therefore confirm that CA27.29 may enter the diagnostic armamentarium of tumour markers that are effective for breast cancer.

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