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ORIGINAL ARTICLE

CA27.29 as a tumour marker for risk evaluation and therapy monitoring in primary breast cancer patients

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Abstract Several trials showed that tumour markers are associated with an impaired prognosis for breast cancer. Whether earlier treatment can improve the course of the disease remains controversial. The SUCCESS Trial compares FEC (500/100/500)-docetaxel (100) vs. FEC (500/100/500)-docetaxel/gemcitabine (75/2000) as well as 2 vs. 5 years of zoledronate in high-risk primary breast cancer patients. In 2669 patients, CA27.29 was measured before and after chemotherapy with the ST AIA-PACK CA27.29 reagent for the AIA-600II automated enzyme immunoassay (Tosoh Bioscience, Belgium). Values above 31 U/ml were considered positive. Of the patients, 7.6 % (n = 202, mean 19, range 3–410) and 19.1 % (n = 511, mean 21, range 3–331) had elevated marker levels before and after chemotherapy, respectively. Of the patients, 4.9 and 78 % showed elevated and low CA27.29, respectively, at both time points. After treatment, 35 % of the pre-therapy positive patients were negative, and 15 % of the initially negative patients became positive. The correlation between both time points was significant ($p < 0.0001$). No correlations among nodal status, grading, hormonal status, HER2 status and CA27.29 levels were found. However, tumour size ($p = 0.02$), older age ($p < 0.001$) and post-menopausal status ($p = 0.006$) were significantly associated with higher CA27.29 levels. Before treatment, the prevalence of elevated CA27.29 was equally distributed between both treatment arms, whereas after chemotherapy, 13.7 % of the patients in the FEC-doc arm showed an increased level vs. 25.4 % of the patients in the FEC-doc/gemcitabine arm ($p < 0.0001$). However, we could not show a significant association between the G-CSF application (yes vs. no) and CA27.29 status before/after chemotherapy ($p = 0.75$). These results indicate a close relationship between CA27.29 levels and tumour mass. Increased values after the completion of chemotherapy might be attributed to treatment effects and should be considered with caution.

Keywords Breast cancer · Tumour marker · CA27.29 · Chemotherapy · Treatment monitoring

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Introduction

When breast cancer is first diagnosed, minimal residuals of the disease have already disseminated to distant organs in certain patients. Although they appear to be metastasis-free at diagnosis, 20 % of the patients still die of breast cancer [1, 2]. Even in the low-risk subgroup of node-negative patients, about 10 % of the patients return with metastases or die within 10 years after diagnosis [3]. Our present diagnostic approaches lack the ability to clearly identify high-risk patients who might benefit from additional systemic treatment.

Several studies have been published identifying MUC-1 gene-derived glycoproteins, such asCA15–3 and CA27.29, as independent predictors at the primary diagnosis for disease outcomes in addition to classical prognostic markers such as tumour size and nodal status [4–9]. After primary therapy, these glycoproteins can predict disease recurrence approximately 3 to 6 months ahead of imaging diagnostics, including PET-CT scans [10–12]. However, according to the current tumour marker guidelines of the American Society of Clinical Oncology, CA15–3 and CA27.29 are not recommended for routine clinical use because there are no trials available that demonstrate a clear benefit regarding improved survival or diminished toxicity resulting from the timely detection of recurrence and early treatment initiation [13].

Because mucins are overexpressed in many adenocarcinomas and can shed into the blood stream, higher serum levels of CA27.29 may reflect an increased tumour burden associated with an increased risk of the spread of minimal residuals of the disease. In contrast to tumour tissue, serum is easily accessible at any time and is therefore the ideal source for a marker to select patients who are at risk of recurrence at the primary diagnosis and during follow-up and to monitor treatment efficacy. In this trial, we prospectively evaluated the role of the tumour marker CA27.29 before and after taxane-based adjuvant chemotherapy in a large number of primary breast cancer patients.

Patients and methods

Study design

The SUCCESS study is a prospectively randomized, open-label phase III trial to evaluate the role of gemcitabine in the adjuvant treatment of early breast cancer as well as the optimal duration of adjuvant zoledronate therapy. In a 2 × 2 factorial design, 3.754 node-positive or high-risk node-negative patients were randomized to receive FEC-doc (3 cycles of 5-FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w, followed by 3 cycles of docetaxel 100 mg/m^2 q3w) or FEC-doc/gemcitabine (3 cycles of 5-FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w, followed by 3 cycles of docetaxel 75 mg/m^2, gemcitabine 1.000 mg d1,8 q3w). In a second randomization, all patients were assigned to 2 vs. 5 years of zoledronate (4 mg x q3mx24m vs. q3mx24m followed by q6mx36m). Node-positive or node-negative patients with additional risk factors could be included. Risk factors included pT ≥ 2, histopathological grade 3, age ≤ 35 or negative hormone receptor status.

Blood samples for translational research were collected before the start of systemic treatment and after the completion of chemotherapy but before the start of endocrine treatment and after 2 and 5 years. Results based on quantification of the tumour marker CA27.29 before and after chemotherapy are shown in this study.

Patients

Patients were recruited at 251 German study sites. Blood samples before and after chemotherapy were available from 2669 early breast cancer patients. The study was approved by all of the involved ethical boards in Germany and complied with the Declaration of Helsinki guidelines. The tumour stage at primary diagnosis was classified according to the revised AJCC tumour-node-metastasis (TNM) classification [14]. Histopathological grading of the primary tumours was performed according to the Bloom-Richardson system [15]. The primary surgical treatment consisted of either breast conservation or modified radical mastectomy leading to a R_0 resection in all reported cases. Routine axillary dissection included lymph nodes at levels I and II, whereas nodes of level III were excised only in cases with macroscopic metastatic involvement of the lower levels. For the diagnosis of lymph node metastasis, single-embedded lymph nodes were screened up to three levels. In all patients who were treated with breast conservation, external beam radiation therapy was mandatory. Chest wall irradiation following mastectomy was performed in patients with more than three involved lymph nodes or T3 and T4 tumours.

All patients received either FEC-doc or FEC-doc/gemcitabine chemotherapy according to the randomization. Following chemotherapy, pre-menopausal hormone-receptor positive women received tamoxifen alone or in combination with goserelin for 2 years if they were younger than 40 years of age. Post-menopausal patients were treated with tamoxifen for 2 years followed by anastrozole for 3 years.

Methods

Serum preparation

Laboratory analysis was performed centrally at the Ludwig-Maximilians-University of Munich Women's Hospital. Approximately 10 ml of peripheral blood was drawn by peripheral vain puncture in standard serum tubes and centrifuged

(10 min, 2000*g*, room temperature) within 24 to 72 h following the collection time to remove clots. Serum was immediately transferred to an immunoreaction cup from the ST AIA-Pack 27.29 (Tosoh Bioscience, Belgium) series for further analysis.

CA27.29 quantification

CA27.29 serum concentration was measured using the AIA-600 II automated enzyme immunoassay system (Tosoh Bioscience, Belgium) according to the manufacturer's instructions. In brief, serum samples were combined with a diluent (1:20) and transferred to an immunoreaction cup from the ST AIA-Pack 27.29 series (Tosoh Bioscience, Belgium). CA27.29 was immobilized using magnetic beads conjugated to antibodies. Then enzyme-labelled antibodies attached to a different epitope were bound to the CA27.29 antigen to form a sandwich. Then, the samples were then incubated at 37 °C, followed by a washing step to remove any unbound antibody. The fluorogenic substrate 4-methylumbelliferyl phosphate (4-MUP) was added to the test cup, and the enzyme activity was measured based on the amount of fluorescence. Values above 31 U/ml were considered positive. In all positive samples, repeated determinations were performed, and the mean was used for analysis. Sixty-six healthy donors were analysed, and only one sample had a value above 31 U/ml. Clinical information was obtained directly from the electronic study documentation of the SUCCESS Trial. Data quality was ensured by electronic data management, including automated plausibility checks and regular monitoring visits to the study site by an independent clinical research organization (Alcedis GmbH, Giessen, Germany).

Statistical analysis

To compare categorical variables, the $\chi 2$-test was used. A two-tailed *t* test was used to calculate the differences of the mean of independent samples that had continuous variables. *P* values less than 0.05 were considered significant in two-sided tests. No adjustment of the error probability for multiple tests was performed. The computer software Statistical Package for the Social Sciences 16.0 (SPSS Inc., Chicago, IL, USA) was used.

Results

Prevalence of CA27.29 positivity at primary diagnosis

The data from 2669 patients with histologically confirmed primary breast cancer were analysed (Table 1). The mean patient age was 53 years in the CA27.29 negative group and 57 years in the CA27.29 positive group. Overall, 59.7 % of the patients had large tumours (T2 to T4), 33.9 % were node-negative and 95.3 % had an unfavourable tumour grade (G2 or G3) (Table 1). Before the start of systemic treatment but after complete removal of the primary tumour, 7.6 % of the patients presented with elevated CA27.29 ($n = 202$, mean 19, range 3–410). CA27.29 levels were not associated with lymph node positivity ($p = 0.55$), histopathological grading ($p = 0.85$), hormone receptor status ($p = 0.21$) or HER2 status of the primary tumour ($p = 0.58$). Additionally, no correlation with the surgical treatment ($p = 0.08$ for breast surgery and $p = 0.31$ for axillary treatment) or systemic chemotherapy randomization ($p = 0.5$) was found. However, elevated levels of CA27.29 were more frequently seen in patients with larger tumours ($p = 0.02$) and in older patients ($p < 0.001$) and those with post-menopausal status before the start of treatment ($p = 0.006$).

Prevalence of CA 27.29 positivity after chemotherapy and correlation of both time points

The second sample was drawn between the end of chemotherapy and the start of endocrine and bisphosphonate treatment. Overall, 19.1 % of the patients returned with elevated tumour marker levels after chemotherapy ($n = 511$, mean 21, range 3–331). The distribution of the CA27.29 values at both time points is shown in Fig. 1. Whereas the vast majority of patients (77 and 72 %) presented with tumour marker levels between 10 and 30 U/ml before and after treatment, we observed an increased positivity rate after completion of chemotherapy.

Whereas 4.9 % of the patients showed elevated CA27.29 before and after therapy, 35 % of the pre-therapy positive patients were negative afterwards. Approximately, 78 % of the patients presented with low CA27.29 at both time points, whereas 15 % of the initially negative patients became positive after treatment. The correlation between both time points was significant ($p < 0.0001$) (Table 2).

Before treatment, the prevalence of elevated CA27.29 was equally distributed between both treatment arms, whereas after chemotherapy 13.7 % in the FEC-doc arm showed an increased level vs. 25.4 % in the FEC-doc/gemcitabine arm ($p < 0.0001$). We identified two potential reasons for this discrepancy between the treatment arms, i.e. G-CSF application during chemotherapy and irradiation treatment simultaneous to the time point of blood sampling.

In 691 patients, the information on G-CSF use was available at the time of analysis. G-CSF support was given significantly more often in the FEC-DG arm (FEC-DG: 57.8 %, FEC-D: 36.3 %, $p < 0.001$). However, we could not show a significant association between the G-CSF application during chemotherapy (yes vs. no) and CA27.29 status before/after chemotherapy ($p = 0.75$).

Table 1 Patients' characteristics at the time of primary diagnosis

	CA27.29 positive pts. (%)	CA27.29 negative pts. (%)	*p* value
Number of patients	202	2467	
Age	57 (28–74)	53 (21–76)	<0.001
Tumour size[a]			0.02
pT1	64 (33.3)	1012 (42.3)	
pT2	104 (54.2)	1228 (51.3)	
pT3	19 (9.9)	127 (5.3)	
pT4	5 (2.6)	25 (1.0)	
Lymph node metastases (LNM)			0.55
Absent (pN0)	65 (32.2)	839 (34.0)	
1–3 axillary LNM (pN1)	85 (42.1)	1146 (46.5)	
4–9 axillary LNM (pN2)	24 (11.9)	336 (13.6)	
≥10 axillary LNM (pN3)	28 (13.9)	140 (5.7)	
NX	0 (0.0)	6 (0.2)	
Grading (G)			0.85
G1	10 (5.0)	115 (4.7)	
G2–3	192 (95.0)	2352 (95.3)	
Hormone receptor status			0.21
Negative	50 (24.8)	714 (28.9)	
Positive	152 (75.2)	1753 (71.1)	
HER2 status[b]			0.58
Negative	144 (76.2)	1757 (74.4)	
Positive	45 (23.8)	605 (25.6)	
Histological type[c]			< 0.0001
Ductal	137 (71.4)	1975 (82.5)	
Lobular	39 (20.3)	262 (10.9)	
Other	16 (8.3)	157 (6.6)	
Menopausal status			0.006
Pre-menopausal	68 (33.7)	1077 (43.7)	
Post-menopausal	134 (66.3)	1390 (56.3)	
Primary operation[a]			
Breast conserving	126 (65.6)	1714 (71.6)	0.08
Mastectomy	66 (32.7)	679 (28.4)	
Sentinel lymph node only	37 (19.3)	520 (21.7)	0.31
Axillary dissection	155 (80.7)	1856 (77.6)	
No axillary staging	0 (0.0)	16 (0.7)	
Systemic therapy			0.50
FEC-doc	99 (49.0)	1270 (51.5)	
FEC-doc/gemcitabine	103 (51.0)	1197 (48.5)	

FEC-doc: 3 cycles of 5-FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w, followed by 3 cycles of docetaxel 100 mg/m^2 q3w. FEC-doc/gemcitabine: 3 cycles of 5-FU 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2 q3w, followed by 3 cycles of docetaxel 75 mg/m^2, gemcitabine 1.000 mg d1,8 q3w

[a] Tumour size and information on primary operation missing in 85 cases

[b] HER2 status missing in 118 cases

[c] Histological type missing in 83 cases

pts patients

Because blood sampling after chemotherapy could have been performed simultaneously with radiotherapy, we evaluated whether there was any correlation between CA27.29 levels and radiotherapy. However, elevated CA27.29 levels after chemotherapy were not observed more frequently in patients who received radiotherapy treatment ($p = 0.353$).

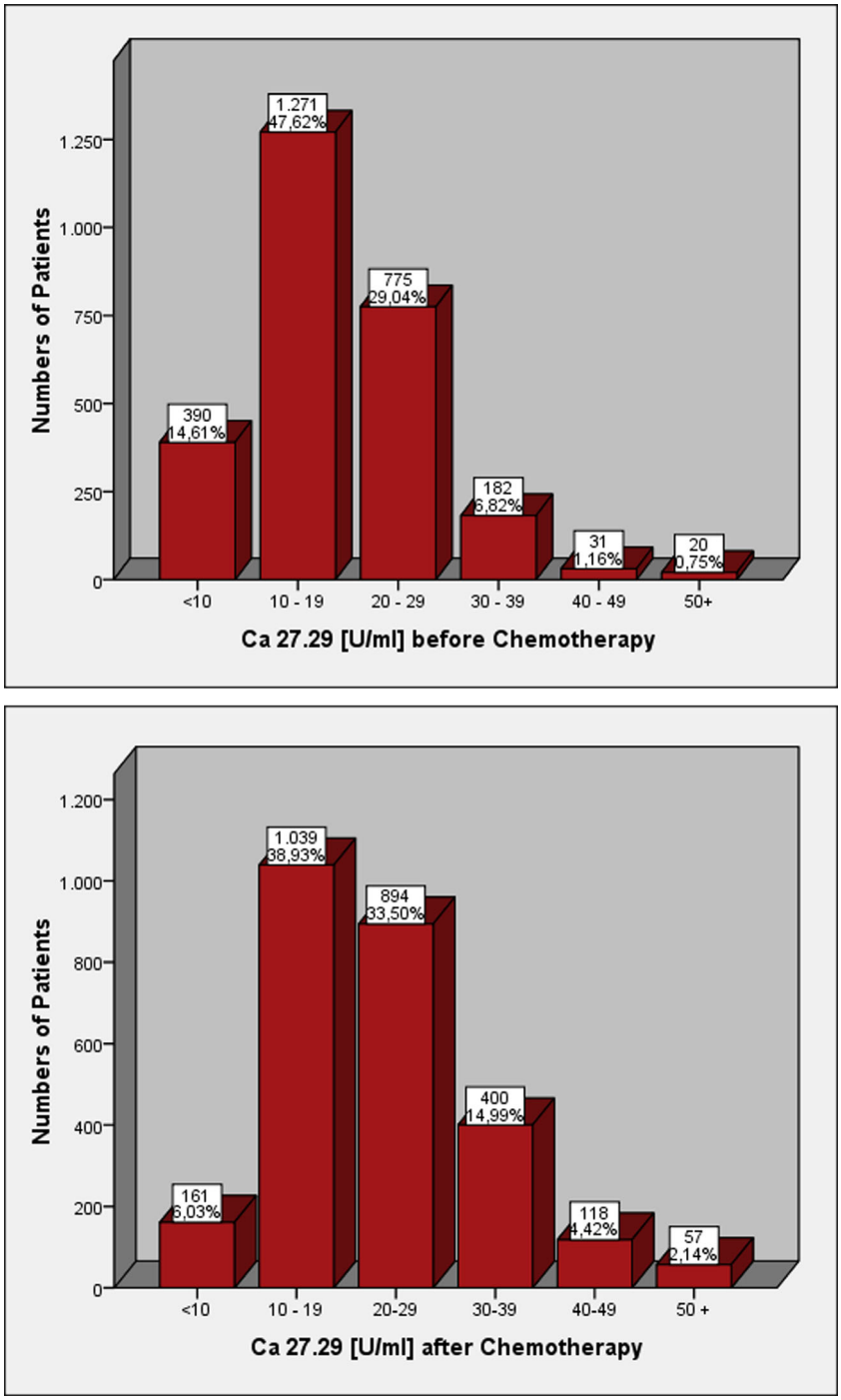

Fig. 1 CA27.29 values before and after chemotherapy

Discussion

We prospectively analysed the role of the MUC-1 marker CA27.29 in a large group of 2669 primary breast cancer patients before and after adjuvant taxane-based chemotherapy. CA27.29 is a well-standardized marker for the detection of the MUC-1 antigen. Mucins are complex membrane-associated glycoproteins that interact with the cytoskeleton and are frequently upregulated and shed to the blood stream in adenocarcinomas. MUC-1 was reported to be expressed in more than 90 % of breast cancer tissues, and high MUC-1 expression is associated with lower tumour grade, positive oestrogen receptor status and improved patient survival [16]. Its frequent overexpression in breast cancer tissue and well-characterized biological role in tumours makes MUC-1 one of the most promising targets for the development of tumour vaccines.

Table 2 CA27.29 values before and after chemotherapy

			After chemotherapy	
			Negative	Positive
			2158 pts.	511 pts.
			80.9 %	19.1 %
Before chemotherapy	Negative	2467 pts.	2087 pts.	380 pts.
		92.4 %	78.2 %	14.2 %
	Positive	202 pts.	71 pts.	131 pts.
		7.6 %	2.7 %	4.9 %

In daily clinical practise, circulating MUC-1 is used together with CEA to monitor treatment efficacy in metastatic breast cancer patients [17]. The prognostic and predictive role of this tumour marker in early disease is much more controversial. Because a clear benefit with respect to improved survival derived from tumour marker surveillance in early breast cancer has not been shown yet, the current guidelines do not recommend using tumour markers as routine care [13].

In the SUCCESS Trial, 8 % of the patients presented with elevated CA27.29 levels prior to the start of adjuvant systemic treatment. Compared to the published literature, our positivity rate at primary diagnosis was below the reported positivity rate of 9 to 75 % for stages I to IV disease [7, 8, 13, 18]. This discrepancy might be caused by different time points for blood sampling. Blood samples were acquired with the primary tumour in situ for many other studies, whereas blood sampling in our study was performed after excision of the primary tumour. The absence of the tumour mass, however, should result in an immediate drop of shed MUC-1 antigen in the circulation compared to pre-operative samples.

The results before and after chemotherapy had a highly significant correlation ($p < 0.0001$). However, 4 weeks after the completion of chemotherapy, the positivity rate increased to 19 %. Paradoxical transient tumour marker increase after the initiation of chemotherapy in advanced disease has been described before and is probably attributable to therapy-mediated apoptosis and necrosis of tumour cells [19, 20]. Usually, this chemotherapy-associated peak resolves within 60 days with patients returning to pre-treatment values. Therefore, an increase in the CA27.29 values following adjuvant chemotherapy might be caused by granulocyte colony-stimulating factors (G-CSF) that are triggered by chemotherapy. As described by others, the elevated CA27.29 values could reflect an increase in peripheral blood neutrophil numbers and induced neutrophil cytoplasmic MUC-1 expression [21].

In the SUCCESS Trial, G-CSF was applied as a secondary prophylaxis in cases with preceding haematotoxicity. An increased rate of grades 3 and 4 haematological toxicities was observed in the FEC-DG arm. As a consequence, G-CSF support was given significantly more often in this patient group (FEC-DG: 57.8 %, FEC-D: 36.3 %, $p < 0.001$). Therefore, this more frequent G-CSF application might have been the reason for the imbalance in CA27.29 values between the chemotherapy treatment arms, with a higher rate of CA27.29 positivity in the FEC-DG arm (13.7 vs. 25.4 %). In our patient cohort, we could not show a statistically significant association between increased tumour marker levels and G-CSF application during chemotherapy ($p = 0.75$). However, the information on the G-CSF application during chemotherapy was available for only 691 patients (21 %) at the time of this analysis. Therefore, this information should be interpreted with caution. Despite different cellular localization and glycosylation, MUC-1 is also expressed in normal breast tissue [22, 23]. Therefore, irradiation of the breast might influence the CA27.29 serum level and could be a relevant factor to explain the post-chemotherapy increased CA27.29 values. However, we could not find any association between radiotherapy and elevated tumour marker levels.

In addition to assay- or treatment-related factors, such as G-CSF, patient characteristics can influence the CA27.29 levels. In our trial, increased CA27.29 levels were observed in older patients ($p < 0.001$) and post-menopausal patients ($p = 0.006$). Similar findings have been reported by other researchers [8, 24, 25] and were confirmed in healthy women [26]. This observation could be explained by the diminished sialylation caused by ageing and unmasking MUC-1 antigenic sites recognized by the assay. Regarding primary tumour characteristics, we found no significant correlation with nodal status ($p = 0.55$), grading ($p = 0.85$), hormonal status ($p = 0.21$) and HER2 status ($p = 0.58$). As reported by others, [8, 25, 27] elevated tumour markers were associated with increased tumour size ($p = 0.02$) and reflected the higher tumour burden. However, in contrast to other findings, we could not prove an association between CA27.29 and lymph node positivity in our patient group.

Some recent publications demonstrated a prognostic relevance for MUC-1 tumour markers in early, newly diagnosed breast cancer cases independent of other established prognostic factors [7, 8]. During follow-up, an increase in marker concentration indicates a rising tumour mass in the organism and can predict disease recurrence an average of 5 to 6 months before other tests, including PET-CT scans [9, 12, 28]. Whereas monitoring in metastatic disease benefits from the timely evaluation of tumour burden as a measure for treatment efficacy, the role of tumour markers during recurrence-free follow-up is still matter of discussion because no improvement in overall survival by implementing tumour marker analysis has been demonstrated. Our data confirmed that tumour markers are influenced by tumour burden, whereas the role of treatment-associated factors is still unclear. This outcome should be considered when interpreting the tumour marker results.

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Conflicts of interest None

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