Prevalence of CA 27.29 in Primary Breast Cancer Patients before the Start of Systemic Treatment

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Abstract. Background: Several trials show that tumor markers at primary diagnosis of cancer have prognostic relevance and can predict dissemination of the disease. While MUC-1 markers are frequently used to monitor treatment efficacy in metastatic breast cancer, their role at primary diagnosis or during follow-up remains unclear. This translational research project within the SUCCESS trial evaluates the role of the tumor marker CA 27.29 before and after adjuvant chemotherapy, as well as after two and then five years in patients with early breast cancer. Patients and Methods: The SUCCESS trial compared FEC (500/100/500)-docetaxel (100) vs. FEC (500/100/500)-docetaxel/gemcitabine (75/2000) and two vs. five years of zoledronate treatment in node-positive and high-risk node-negative patients with primary breast cancer. CA 27.29 was measured before chemotherapy in 2669 patients with the reagent ST AIA-PACK CA 27.29 for AIA-600II-Analyzer (Tosoh Bioscience, Belgium). Results of CA 27.29 above 31 U/ml were regarded as positive. Results: 7.6% of patients had elevated marker levels after the completion of primary surgical treatment but before initiation of chemotherapy (n=202, mean 19, range 3-410 U/ml). No correlation between nodal status (p=0.55), grading (p=0.85), hormonal status (p=0.21), HER2/neu status on the primary tumor (p=0.58) and CA 27.29 was shown. However, larger tumor size (p=0.02), lobular histology (p<0.0001), older age (p<0.001) and postmenopausal hormone status before the start of treatment (p=0.006) were significantly associated with higher CA 27.29 levels. Conclusion: These data indicate a close relationship between CA 27.29 and tumor mass persisting even several weeks after surgery, but also identify potential confounding factors that should be considered in interpreting tumor marker results. Further follow-up of the SUCCESS trial will clarify whether CA 27.29 measured after surgery but before the start of systemic treatment is prognostically relevant and whether it is a useful marker for treatment monitoring in the adjuvant setting.

Mucin MUC-1 is physiologically present at the luminal surface of glandular epithelia. Since this glycoprotein is up-regulated in many adenocarcinomas and released into the blood stream in higher amounts than in healthy individuals, it has been investigated extensively in breast cancer patients. Several studies identified MUC-1 markers such as Ca15-3 and CA 27.29 at primary diagnosis as being independent predictors for disease outcome, in addition to the traditional prognostic markers such as tumor size and nodal status (1-3). After primary therapy, the level of these markers can predict disease recurrence about six months earlier than available methods (4, 5). However, according to the current tumor marker guidelines of the American Society of Clinical Oncology, CA 15-3 and CA 27.29 are not recommended as prognostic markers for routine clinical use because there are...
Patients and Methods

Study design. The SUCCESS study is a German, multicenter prospectively randomized, open label, phase III trial, evaluating the role of gemcitabine in the treatment of primary breast cancer patients, as well as the optimal duration of adjuvant zoledronate treatment. In this 2x2 factorial design trial, 3754 node-positive or high risk node-negative patients were randomized to receive FEC-Doc (3 cycles of 5-fluorouracil (5-FU) 500 mg/m², epirubicin 100 mg/m², cyclophosphamide 500 mg/m², q3w) or FEC-Doc/gemcitabine (3 cycles of 5-FU 500 mg/m², epirubicin 100 mg/m², cyclophosphamide 500 mg/m², q3w, followed by 3 cycles of docetaxel 100 mg/m² q3w) or FEC-Doc/gemcitabine (3 cycles of 5-FU 500 mg/m², epirubicin 100 mg/m², cyclophosphamide 500 mg/m², q3w, followed by 3 cycles of docetaxel 75 mg/m², gemcitabine 1.000 mg q1.8 q3w). In a second randomization, all patients were assigned to two vs. five years of zoledronate (4 mg q3mx24m vs. q3mx24m followed by q6mx36m). Node-negative patients were included in case of pT≤2, histopathological grade 3, age ≤35 years or negative hormone receptor status.

As part of the study, blood samples were collected before the start of systemic treatment, after chemotherapy and after two and five years. Analyses of the tumor marker CA 27.29 before chemotherapy are presented.

Patients. Serum samples from 2669 primary breast cancer patients recruited from 251 German centers were used. The study was approved by relevant ethical boards involved in Germany. The tumor stage at primary diagnosis was classified according to the revised AJCC tumor-node-metastasis (TNM) classification (9). Histopathological grading of the primary tumors was performed according to the Bloom-Richardson system (10). The primary surgical treatment consisted of either breast conservation or modified radical mastectomy, leading to R0 resection in all reported cases. Sentinel lymph node excision alone was performed in all cases with no histopathological evidence of tumor in the sentinel nodes. Routine axillary dissection in patients with metastases in the sentinel nodes included lymph nodes of levels I and II, while those of level III were excised only in cases with microscopic 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 treated with breast conservation, external beam radiation therapy was administered. Chest wall irradiation following mastectomy was performed in patients with more than 3 involved lymph nodes or T3 and T4 tumors.

All patients received either FEC-Doc or FEC-Doc/gemcitabine chemotherapy according to their randomization. Following chemotherapy, premenopausal patients with hormone receptor positive disease were treated with tamoxifen for five years. Additionally patients below 40 years of age or that had premenopausal blood status after chemotherapy, were treated with two years of gosereline. Postmenopausal patients were switched from tamoxifen to anastrozole after two years.

Methods. Laboratory analysis was performed centrally at the Ludwig Maximilians-University of Munich Women’s Hospital. CA 27.29 concentrations were measured by the ST AIA-Pack 27.29 reagent using the AIA-600II Analyzer (Tosoh Bioscience, Tessenderlo, Belgium) according to the manufacturer instructions. The ST AIA-Pack 27.29 assay is an automated monoclonal fluorometric assay directed against the MUC-1 antigen. Samples were shipped at room temperature and either analyzed immediately on their arrival in the laboratory or stored at –20°C until analysis at a later date. Serum (150 μl) was diluted by 1:20 and the monoclonal antibody bound to magnetic pearls was then added. The pearls were washed and incubated at 37°C with the fluorogenic substrate 4MUP. Assay results above 31 U/ml were regarded as positive. In all positive samples, two repeat assays were performed and the mean was used for analysis. Sixty-six controls without malignant disease were analyzed, from whom one sample was found to be above 31 U/ml (1%). Clinical information was obtained directly from the electronic study documentation of the SUCCESS trial.

Statistical analysis. The χ²-test was used to compare categorical variables. The two-tailed t-test was used to calculate the differences of the mean of the independent samples that had continuous variables. P-values of less than 0.05 were considered significant in two-sided tests. No adjustment for the error probability for multiple testing was performed. The Statistical Package for the Social Sciences 16.0 (SPSS Inc., Chicago, IL, USA) was used throughout.

Results

Prevalence of CA 27.29 positivity at primary diagnosis. The serum samples were acquired after primary surgical treatment leading to R0 resection and before the start of chemotherapy. At that time point, 7.6% of patients presented with elevated marker levels (n=202). The mean CA 27.29 value was 19 U/ml, with a range from 3 U/ml to 410 U/ml. CA 27.29 levels categorized by percentiles are depicted in Figure 1.

The median age of all patients was 53 years (range 21 to 76 years). The majority of patients had small tumors (41.6% T1, 51.5% T2, 5.7% T3, 1.2% T4) and 34.3% were node-
negative (45.5% N1, 14.2% N2, 6.1% N3). Overall, 4.7% presented with G1 tumors, 71.4% of patients were hormone-receptor positive and 25.5% Her2/neu positive on the primary tumor.

Correlation of CA 27.29 at primary diagnosis with classical prognostic markers. Table I summarizes the patient characteristics at the time of primary diagnosis in relation to CA 27.29 positivity. Elevated CA 27.29 levels did not correlate with the majority of tumor characteristics at the time of primary diagnosis, such as nodal status (p=0.55), histopathological grading (p=0.85), hormone receptor status (p=0.21) or Her2/neu status on the primary tumor (p=0.58), nor with the surgical treatment (p=0.08 for breast surgery and p=0.31 for axillary treatment) or systemic chemotherapy randomization (p=0.5). However, larger tumor size (p=0.02) and lobular histological tumor type (<0.0001), as well as older age (p<0.001) and postmenopausal status before the start of treatment (p=0.006) were significantly associated with higher CA 27.29 levels.

Discussion

In this trial, we prospectively analyzed the role of the MUC-1 marker CA 27.29 in a large group of 2669 primary breast cancer patients before adjuvant taxane-based chemotherapy. CA 27.29 is an established, standardized assay for the detection of the MUC-1 antigen. Mucins are complex membrane-associated glycoproteins that interact with the cytoskeleton and are frequently up-regulated and are shed into the blood stream in patients with adenocarcinomas (11). MUC-1 is reported to be expressed in about 90% of breast tumors (12-15). Several trials have compared different MUC-1 assays and found comparable sensitivity and specificity for CA 27.29 and Ca 15-3 (11,16-18). Some authors even assume a higher sensitivity for CA 27.29 in cases with low antigen concentrations or limited variations in tumor extension (18, 19). Albeit the difference between the two antibodies was small, it may support a possible advantage when the test is used during follow-up for early detection of disease recurrence.

In our trial, we found 8% of patients with CA 27.29 levels above the cut-off level when blood was drawn after primary surgery with R0 resection of the tumor but before the start of systemic chemotherapy.

![Figure 1. CA 27.29 values after surgical treatment but before the start of systemic chemotherapy.](image)

Table I. Patient characteristics at the time of primary diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>CA 27.29 positive pts (%)</th>
<th>CA 27.29 negative pts (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>202</td>
<td>2467</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 (28-74)</td>
<td>53 (21-76)</td>
<td>0.01</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>64 (33.3)</td>
<td>1012 (42.3)</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>104 (54.2)</td>
<td>1228 (51.3)</td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>19 (9.9)</td>
<td>127 (5.3)</td>
<td></td>
</tr>
<tr>
<td>pT4</td>
<td>5 (2.6)</td>
<td>25 (1.0)</td>
<td></td>
</tr>
<tr>
<td>LNM</td>
<td></td>
<td></td>
<td>0.55</td>
</tr>
<tr>
<td>Absent (pN0)</td>
<td>65 (32.2)</td>
<td>839 (34.0)</td>
<td></td>
</tr>
<tr>
<td>1-3 axillary LNM (pN1)</td>
<td>85 (42.1)</td>
<td>1146 (46.5)</td>
<td></td>
</tr>
<tr>
<td>4-9 axillary LNM (pN2)</td>
<td>24 (11.9)</td>
<td>336 (13.6)</td>
<td></td>
</tr>
<tr>
<td>≥10 axillary LNM (pN3)</td>
<td>28 (13.9)</td>
<td>140 (5.7)</td>
<td></td>
</tr>
<tr>
<td>NX</td>
<td>0 (0.0)</td>
<td>6 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>G1</td>
<td>10 (5.0)</td>
<td>115 (4.7)</td>
<td></td>
</tr>
<tr>
<td>G2-3</td>
<td>192 (95.0)</td>
<td>2352 (95.3)</td>
<td></td>
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<tr>
<td>Hormone receptor status</td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Negative</td>
<td>50 (24.8)</td>
<td>714 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>152 (75.2)</td>
<td>1753 (71.1)</td>
<td></td>
</tr>
<tr>
<td>Her2/neu status b</td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>Negative</td>
<td>144 (76.2)</td>
<td>1757 (74.4)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>45 (23.8)</td>
<td>605 (25.6)</td>
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<tr>
<td>Histological type c</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
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<tr>
<td>Ductal</td>
<td>137 (71.4)</td>
<td>1975 (82.5)</td>
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</tr>
<tr>
<td>Lobular</td>
<td>39 (20.3)</td>
<td>262 (10.9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>16 (8.3)</td>
<td>157 (6.6)</td>
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</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Premenopausal</td>
<td>68 (33.7)</td>
<td>1077 (43.7)</td>
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<tr>
<td>Postmenopausal</td>
<td>134 (66.3)</td>
<td>1390 (56.3)</td>
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<tr>
<td>Primary operation a</td>
<td></td>
<td></td>
<td>0.08</td>
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<tr>
<td>Breast-conserving</td>
<td>126 (65.6)</td>
<td>1714 (71.6)</td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>66 (32.7)</td>
<td>679 (28.4)</td>
<td></td>
</tr>
<tr>
<td>SLNE</td>
<td>37 (19.3)</td>
<td>520 (21.7)</td>
<td>0.31</td>
</tr>
<tr>
<td>Axillary dissection</td>
<td>155 (80.7)</td>
<td>1856 (77.6)</td>
<td></td>
</tr>
<tr>
<td>No axillary staging</td>
<td>0 (0.0)</td>
<td>16 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Systemic therapy</td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>FEC-Doc</td>
<td>99 (49.0)</td>
<td>1270 (51.5)</td>
<td></td>
</tr>
<tr>
<td>FEC-Doc/gemcitabine</td>
<td>103 (51.0)</td>
<td>1197 (48.5)</td>
<td></td>
</tr>
</tbody>
</table>

aTumor size and information on primary operation missing in 85 cases; bHer2/neu status missing in 118 cases; cHistological type missing in 83 cases. LNM: Lymph node metastasis classification.
adjuvant systemic treatment. As multiple assays are used for
tumor marker detection, and cut-offs are not clearly defined, it
is difficult to compare positivity rates across different trials.
Overall, our positivity rate of 8% at primary diagnosis is below
the 9% to 75% reported for stage I to IV disease (20-23).
However, in contrast to most other trials, blood sampling in our
study was performed after excision of the primary tumor,
which should result in a drop of shedded MUC-1 antigen in the
circulation compared to preoperative samples.

The interpretation of tumor marker assays is complicated
by several factors influencing MUC-1 antigen expression. In
addition to assay- or treatment-related factors such as G-CSF
application, patient characteristics can also have an influence
on MUC-1 antigen expression. In our trial, increased
CA 27.29 levels were observed in older patients (p<0.001)
and patients with postmenopausal hormonal status before the
start of treatment (p=0.006). Similar findings were reported by
others (16, 24) and were confirmed in healthy women
(25). This observation can be explained by a diminished
sialylation caused by aging and unmasking of MUC-1
antigenic sites recognized by the assay.

We also evaluated the association of CA 27.29 at primary
diagnosis with other established prognostic factors and
primary surgical treatment, and found no significant
correlation with nodal status (p=0.55), grading (p=0.85),
hormonal status (p=0.21) or HER2/neu status on the primary
(p=0.58). As shown in Table I and reported by others
(18, 24, 26, 27), elevated CA 27.29 was associated with
increased tumor size (p=0.02). Reflecting the higher tumor
burden in the CA 27.29-positive group, these patients were
treated by mastectomy more frequently than were CA 27.29
-negative patients (33% vs. 28%, p=0.08), but this difference
did not reach statistical significance. However, we were unable
to prove an association of CA 27.29 with lymph node
positivity in our patient group.

Another finding in our data set is the association of CA 27.29
positivity with lobular histological tumor type. While we
found 11% of lobular carcinomas in the CA 27.29-
negative group, this rate increased to 20% in the CA 27.29-
positive group (p<0.0001). A high MUC-1 expression in up
to 90% of tumors has been reported both for ductal and
lobular breast carcinoma tissue, whereas mucinous
carcinomas were less MUC-1 reactive (12-14). However,
high MUC-1 tissue expression does not always coincide with
elevated MUC-1 serum levels (15, 28). As different
glycoforms of MUC-1 are expressed on cancer cells, serum
tests and immunohistochemistry might detect different
glycoforms. Consequently, the screening for different
glycoforms could be a reasonable approach. To our
knowledge, a correlation between elevated CA 27.29 and
lobular histological type has not been reported before.

These data indicate a close relationship between CA 27.29
and the tumor mass persisting even several weeks after primary
surgery. Furthermore, we identified tumor and patient
characteristics that should be considered in tumor marker
interpretation. Longer follow-up within the SUCCESS trial will
provide information on the prognostic relevance of CA 27.29
before the start of systemic treatment and will demonstrate the
role of this tumor marker in adjuvant chemotherapy and
endocrine, as well as bisphosphonate, treatment monitoring.

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