Influence of a Dose-dense Adjuvant Chemotherapy on sVCAM-1/sICAM-1 Serum Levels in Breast Cancer Patients with 1-3 Positive Lymph Nodes

HOLM EGGEMAN1, FRANK STÖBLEN2, MARC THILL3, SUSANNE KORLACH1, PETER SCHMID4, DIANA LÜFTNER5, DIRK ELLING6, FLORIN-ANDREI TARAN1, SHERKO KÜMMEL7 and SOLVEIG LANDT8

1Department of Gynecology and Obstetrics, University Hospital Magdeburg, Magdeburg, Germany; 2Department of Radiology, and 7Breast Center, Hyssensstift Kliniken Essen-Mitte, Essen, Germany; 3Department of Gynecology and Obstetrics, University Hospital Schleswig-Holstein, Lübeck, Germany; 4Brighton and Sussex Medical School, Trafford Centre, University of Sussex, Brighton, UK; 5Department of Oncology and Hematology, Charité, University Hospital Berlin, Berlin, Germany; 6Department of Gynecology and Obstetrics, Oskar Ziethen Hospital, Berlin, Germany; 8Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Düsseldorf, Germany

Abstract. Background/Aim: The aim of the present study was to investigate the effects of conventional and dose-dense chemotherapy on serum levels of soluble adhesion molecules sICAM-1 and sVCAM-1 in node-positive patients with breast cancer. Patients and Methods: sICAM-1 and sVCAM-1 were measured in the blood serum of 147 patients with breast cancer and with 1 to 3 affected lymph nodes prior to and after conventional or dose-dense chemotherapy within a randomized phase III study (NOGGO trial). Results: The increase in sICAM-1 (p<0.0001) and sVCAM-1 (p<0.001) levels after chemotherapy was statistically significant within the entire sample and the dose-dense study arm. sVCAM-1 levels were not altered by conventional chemotherapy, but were markedly and significantly increased after the dose-dense regimen. Higher sICAM-1 concentrations were found in postmenopausal patients, and the difference was significant before, but not after treatment. There was no significant correlation with other prognostic criteria. Conclusion: Both sVCAM-1 and sICAM-1 levels changed significantly after adjuvant chemotherapy, the effect being more marked under the dose-dense regimen. The possible prognostic relevance of adhesion molecule concentration and the effect of different modes of chemotherapy remains to be determined.

Growth and dissemination of tumors are the result of a variety of processes, such as uncontrolled cell proliferation, loss of homophilic cell-to-cell contacts, migration of malignant cells into blood and lymphatic vessels, interaction with white blood cell (WBC) and platelet surface structures, and induction of neo-angiogenesis and their mutual interrelationships (1, 2).

Intercellular adhesion molecules play a key role in the facilitation of tumor invasion and dissemination. This group consists of selectins, integrins and cadherins, as well as intercellular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1). The latter are cell surface glycoproteins that belong to the immunoglobulin superfamily and whose expression and activity is enhanced in inflammation. Their biologic function is an interaction with integrins that results in the formation of cell connections which in turn promote the adhesion and diapedesis of WBCs (3-6). Via these mechanisms, ICAM-1/VCAM-1 appear to be involved in tumor cell adhesion to vascular endothelial cells, an important pathway of tumor dissemination and metastasis formation.

In the 1990s, soluble species of these adhesion molecules (sICAM-1/sVCAM-1) have been described (7-10), and an increase in their serum concentrations was found in patients with colorectal (11) and gastric cancer (12).

In breast cancer patients, increased serum concentrations of sICAM-1 were described, and their level seems to be related to poorer therapy response and prognosis (13, 14); however, these findings are not conclusive in the literature (9).

Correspondence to: Solveig Landt, Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Tel: +49 1622387535, e-mail: solveiglandt@yahoo.de

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Whereas the possible relevance of sICAM-1/sVCAM-1 as diagnostic and prognostic markers has been intensively studied, knowledge on the influence of chemotherapy on their expression is relatively scarce. In one of the few studies, Mills et al. found significantly increased sICAM-1 plasma levels after 4 cycles of an adjuvant, anthracycline-based chemotherapy compared to baseline in 26 breast cancer patients (15).

The present study is, to our knowledge, the first attempt to identify a possible influence of dose-dense chemotherapy on soluble adhesion molecule expression in breast cancer patients. We assessed sICAM-1/sVCAM-1 serum concentrations before and after therapy as part of a randomized controlled trial (RCT) that compared two different adjuvant chemotherapy regimens (epirubicin/cyclophosphamide followed by paclitaxel vs. dose-dense application of epirubicin followed by paclitaxel) in 147 patients with node-positive breast cancer (NOGGO trial [Clinical trial gov: 170200]).

Patients and Methods

Patients. Median serum concentrations of sICAM-1 and sVCAM-1, respectively, were assessed in 147 patients with stage Ia or IIa breast cancer and with 1-3 affected lymph nodes who underwent adjuvant chemotherapy in a phase III clinical trial comparing dose-dense and conventional treatment regimens (NOGGO trial [Clinical trial gov: 170200]). Between 2000 and 2003, a total of 147 patients were enrolled. The patients were between 25 and 72 (median, 54) years of age. A total of 72 patients were randomly assigned to conventional chemotherapy and 75 patients to the dose-dense scheme.

All clinical and sociodemographical data was obtained from the NOGGO trial files. The baseline clinical characteristics are shown in Table I; treatment groups were well comparable, with the exception of a significantly larger proportion of locally advanced tumors (NOGGO trial [Clinical trial gov: 170200]).

Table I. Patient and tumor characteristics in the entire study group and in both study arms [A: 4* epirubicin (90 mg/m², d1, q21)/cyclophosphamide (600 mg/m², d1, q21) followed by 4* paclitaxel (175 mg/m², d1, q21); B: 4* epirubicin (120 mg/m², d1, q14) followed by 4* paclitaxel (175 mg/m², d1, q14)].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients</th>
<th>Arm A</th>
<th>Arm B</th>
</tr>
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<tbody>
<tr>
<td>No.*</td>
<td>147</td>
<td>72</td>
<td>75</td>
</tr>
<tr>
<td>Age years (median, range)</td>
<td>54 (25-73)</td>
<td>55 (25-73)</td>
<td>53 (31-72)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>n=145*</td>
<td>n=71*</td>
<td>n=74*</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>61 (42.1%)</td>
<td>29 (40.8%)</td>
<td>32 (43.2%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>84 (57.9%)</td>
<td>42 (59.1%)</td>
<td>43 (56.8%)</td>
</tr>
<tr>
<td>Tumor size</td>
<td>n=147</td>
<td>n=72</td>
<td>n=75</td>
</tr>
<tr>
<td>pT1</td>
<td>70 (47.6%)</td>
<td>41 (57.9%)</td>
<td>29 (38.7%)</td>
</tr>
<tr>
<td>pT2</td>
<td>73 (49.7%)</td>
<td>31 (43.0%)</td>
<td>42 (56.0%)</td>
</tr>
<tr>
<td>pT3</td>
<td>4 (2.7%)</td>
<td>-</td>
<td>4 (5.3%)</td>
</tr>
<tr>
<td>No. of lymph nodes</td>
<td>n=147</td>
<td>n=72</td>
<td>n=75</td>
</tr>
<tr>
<td>1</td>
<td>63 (42.9%)</td>
<td>29 (40.3%)</td>
<td>34 (45.3%)</td>
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<tr>
<td>2</td>
<td>50 (34.0%)</td>
<td>24 (33.3%)</td>
<td>26 (34.7%)</td>
</tr>
<tr>
<td>3</td>
<td>34 (23.1%)</td>
<td>19 (26.4%)</td>
<td>15 (20.0%)</td>
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</tr>
<tr>
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<td>11 (7.5%)</td>
<td>4 (5.6%)</td>
<td>7 (9.3%)</td>
</tr>
<tr>
<td>G2</td>
<td>83 (56.5%)</td>
<td>39 (54.2%)</td>
<td>44 (58.7%)</td>
</tr>
<tr>
<td>G3</td>
<td>53 (36.1%)</td>
<td>29 (40.3%)</td>
<td>24 (32.0%)</td>
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<tr>
<td>Hormone receptor status</td>
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<td>n=75</td>
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<td>117 (79.6%)</td>
<td>56 (77.8%)</td>
<td>61 (81.3%)</td>
</tr>
<tr>
<td>Negative</td>
<td>30 (20.4%)</td>
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<td>14 (18.7%)</td>
</tr>
<tr>
<td>c-Erb-2 status</td>
<td>n=147</td>
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<td>n=75</td>
</tr>
<tr>
<td>Negative</td>
<td>64 (43.5%)</td>
<td>31 (43.1%)</td>
<td>33 (44.0%)</td>
</tr>
<tr>
<td>Positive</td>
<td>83 (56.5%)</td>
<td>41 (56.9%)</td>
<td>42 (56.0%)</td>
</tr>
</tbody>
</table>

*Patients with valid data.

Serum concentrations of sICAM-1 and sVCAM-1 were determined with commercially available ELISA test kits (Quantikine®, R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The analysis is based on monoclonal antibodies showing no cross-reaction to other isoforms and thus specific for sICAM-1 and sVCAM-1, respectively. Duplicate measurements were performed, and the average of both results stored and employed for statistical analysis. Normal concentration ranges according to the manufacturer are 115-306 ng/ml for sICAM-1 and 395-714 ng/ml for sVCAM-1.

Statistical data evaluation. Data was stored in a Microsoft Excel™ spreadsheet and analyzed with the SPSS™ 15 program package (SPSS™ Inc., Chicago, IL). Normal distribution of values was not assumed, and therefore non-parametric methods were employed for analysis. Median and interquartile range (IQR) were used for description of concentrations. The changes in concentrations of adhesion molecules before and after therapy were assessed with the Wilcoxon test for paired differences, and differences between subgroups of the sample were analyzed with the Mann-Whitney U-test (for two subgroups) or the Kruskal-Wallis test (more than two subgroups). The χ² test was used for the comparison of frequency distributions, and Spearman’s rank correlation coefficient for linear regression.

For all tests, statistical significance was considered when the p value was <0.05.
Results

Previous to the therapy onset, median serum concentrations of sICAM and sVCAM-1 were mostly in the upper part of the normal range provided by the manufacturer, and about 30% of the patients had levels above the norm (sICAM-1 29.3%, sVCAM-1 28.6%).

Elevated sICAM-1 concentrations before treatment were significantly more prevalent in postmenopausal as compared to premenopausal patients, whereas sVCAM-1 status showed no such correlation (Figure 1). An elevated concentration before chemotherapy of either molecule was not related to nodal status, number of positive lymph nodes, tumor size, grading and c-erb-2 status. There was also no correlation with age, indicating the aforementioned relationship with menopausal status to be independent of other factors.

As a result of the chemotherapy, serum levels of both adhesion molecules increased significantly ($p<0.0001$; Table II). The increase of sICAM-1 (from 274 to 322 ng/ml; 17.5% of patients) was markedly more pronounced than that of sVCAM-1 (from 649 to 686 ng/ml; 5.7% of patients). Moreover, the change in sVCAM-1 concentrations occurred exclusively in patients undergoing dose-dense chemotherapy, whereas no appreciable difference was observed in the conventional treatment arm; conversely, sICAM-1 was affected by both chemotherapy modalities in equal measure.

The only prognostic factor that showed an appreciable difference was the menopause status: Postmenopausal women had significantly higher sICAM-1 levels before therapy, and the difference was maintained after treatment, albeit not significant. No such difference was observed for sVCAM-1. sICAM-1 concentrations showed no correlation with age before or after treatment.

The only other correlation with prognostic criteria concerned the c-erb-2 status: sICAM-1 concentrations were appreciably, albeit not significantly, lower in c-erb-2-positive patients, whereas sVCAM-1 showed no such relation.

Discussion

Prospective randomized controlled trials have demonstrated a clear benefit of dose-dense adjuvant chemotherapy for patients with node-positive high-risk breast cancer (16, 17), which may be further increased by the additional sequential application of taxanes (18). The present prospective randomized study investigated the possible influence of conventional and dose-dense chemotherapies on the serum concentrations of soluble adhesion molecules sICAM-1 and sVCAM-1, which have been linked to poor therapy response and prognosis in breast cancer patients (14, 19).

First of all, the results of our trial confirm previously published results with regard to increased sICAM-1/sVCAM-1 serum concentrations in patients with breast cancer (before chemotherapy) as compared to healthy women (9, 13). Moreover, we also demonstrated a substantial and significant increase of serum concentrations of sICAM-1/sVCAM-1 in the course of adjuvant chemotherapy. Whereas 62.7% of patients after dose-dense chemotherapy (arm B) had a ≥10% increase in sVCAM-1 concentrations, only 29.2% did so after conventional chemotherapy (arm A). In arm A, the sVCAM-1 increase, albeit appreciable, was not...
statistically significant. In contrast, sICAM-1 markedly and significantly increased in both treatment arms.

The latter result is in accordance with the aforementioned study by Mills et al. (15) who showed a significant increase of sICAM-1 in 26 patients after 4 cycles of a conventional, anthracycline-based adjuvant chemotherapy. Moreover, Mills and co-workers demonstrated an initial decrease of sICAM-1 levels in the first week after course 1, possibly as a consequence of direct immunosuppressive anthracycline effects. We were unable to verify this effect in the present study because no serum sampling was scheduled after the first treatment course. Since all our patients had nodal positive disease, we were also unable to confirm the finding of Mills et al. in regard to their findings of higher sICAM-1 concentrations in nodal-positive as opposed to nodal negative disease. However, a consistent relationship of sICAM-1 concentrations with the number of affected nodes failed to be present in our sample.

The fact that cyclophosphamide was only administered in the conventional arm was a consequence of several prospective trials that demonstrated the lack of an additional benefit achieved by cyclophosphamide as compared to anthracycline/taxane alone (e.g. the PACS 04 trial (20)); moreover, it rather emphasizes the more pronounced effect of the dose-dense regimen which was basically more effective according to the present study and previously published clinical results (e.g. 16, 17).

With regard to the conventional prognostic factors, the only significant interrelation was found between sICAM-1 and menopausal status: Before and after treatment,
postmenopausal patients had about 9% and 6.5% higher concentrations, respectively, the former difference being statistically significant and the latter only just failing to meet this criterion \( p=0.058 \). There was no correlation with age or any other of the prognostic factors, despite a similar correlation between sICAM-1 and c-erb-2 status that was, however, not significant.

All in all, we were unable to confirm the findings of O’Hanlon et al. (13), who demonstrated higher concentrations of both molecules, especially of sVCAM-1, in connection with a poorer spectrum of prognostic criteria, and our respective findings are contradictory.

The exact correlation of adhesion molecule concentrations and breast cancer prognosis, as well as the underlying mechanisms involved, remain elusive, and so does the diagnostic significance of elevated sICAM-1/sVCAM-1 concentrations. Some studies point towards elevation of sICAM-1 as a reflection of endothelial or other tissue activities, and others assume there to be a reaction to inflammatory processes (21,22).

A therapy-associated increase would be well conceivable and is indeed strongly suggested by the results of the present trial. The underlying mechanism might be an increase in circulating interleukin or tumor necrosis factor concentrations that have been described previously after chemotherapy (23, 24); these inflammatory mediators could in turn have stimulated sICAM-1/sVCAM-1 release (14, 25, 26). However, Mills et al. (15) determined IL-6 levels parallel to their sICAM-1 measurements and found no such relationship.

An association of sICAM-1/sVCAM-1 elevation with the tumor tissue as such is unlikely, since the presence of tumor cells in the body is negligible in the adjuvant situation on which the present trial is based; it should be pointed out, however, that blood samples for the adhesion molecule assessment were obtained after – and not before – surgery.

Ultimately, the clinical significance of elevated sICAM-1/sVCAM-1 concentrations in breast cancer patients and as a consequence of chemotherapy remains unclear. It remains to be seen if the NOGGO trial will confirm the finding of Zhang et al. (14) of a poorer therapy response and a shorter survival in patients with elevated sICAM-1 levels. The appropriate results of the trial, when available, will give guidelines for further research for which a baseline assessment has been provided herein.

**Conclusion**

The present prospective randomized trial provides the very first insight into sICAM-1/sVCAM-1 response to dose-dense chemotherapy and its correlation to conventional prognostic factors. In accordance with previous observations under conventional chemotherapy (15), serum sICAM-1 levels increased significantly and markedly in both treatment arms, whereas an sVCAM-1 response was only seen after dose-dense treatment. Apart from a higher sICAM-1 concentration in postmenopausal women, there was no appreciable relationship of either molecule with conventional prognostic factors. Follow-up examination of the patients of the NOGGO trial will reveal if the poorer survival in patients with elevated sICAM-1 concentrations reported by Zhang et al. (14) can be confirmed.

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**References**


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