Inhibin A Is Down-regulated During Chemotherapy in Patients with Breast Cancer

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Abstract. *Background: Inhibins are dimeric glycoproteins, composed of an alpha-subunit (INH-α) and one of two possible beta-subunits (βA or βB), with substantial roles in human reproduction and in endocrine-responsive tumours. Aims of this study were to determine the serological measurement of inhibin A (α-βA) in breast cancer patients during chemotherapy. Patients and Methods: A series of 30 breast cancer patients who underwent standardised chemotherapy were prospectively evaluated before chemotherapeutic treatment as well as four weeks after chemotherapy and two years after chemotherapy for the serological expression of inhibin A. For statistical analysis the Wilcoxon rank sum test was used for paired samples. Statistical significance was assumed at p<0.05. Results: The concentration of inhibin A showed a significant decrease between data obtained before chemotherapy and after chemotherapy (p<0.005) and two-year follow–up (p<0.001). Interestingly, there were no differences in inhibin A concentrations between the four-week and two-year follow-up (p=0.744). Discussion: Chemotherapy significantly decreases inhibin A concentration during chemotherapy. This might reflect a suppression of ovarian function, being also a marker for chemotherapy-induced amenorrhoea. Moreover, it has been suggested that inhibin A might be a tumour marker for breast cancer, and therefore a sudden increase in its concentration might be indicative of breast cancer recurrence.*

Inhibins and activins are secreted polypeptides, representing a subgroup of the TGF-β superfamily of growth and differentiation factors (1, 2). Inhibins are heterodimeric glycoproteins, composed of an alpha-subunit (α) and one of two possible beta-subunits, named βA or βB, in the formation of either inhibin A (α-βA) or B (α-βB), respectively (1, 2). Two additional β-subunits (named βC and βE) have been cloned, although their precise function and dimerisation potential remains yet unclear (1). Activins are, in contrast to inhibins, homodimers of the β-subunits linked by a disulphide bridge (1-3).

Inhibin subunits have been detected in endocrine tumours (4) and their differential expression in malignant tissue has suggested an important role in malignant cell transformation (4-6). Inhibin subunits have been observed in healthy breast epithelia as well as in benign and malignant breast tissue (7, 8). Moreover, activin A is synthesized in malignant mammary tissue as well as in the serum of postmenopausal women with breast cancer, suggesting it as a possible tumour marker in breast cancer patients, since average activin A levels have been observed to be higher in cancer patients (9).

It has been demonstrated that premenopausal breast cancer survivors have diminished ovarian reserve compared with a control group (10), as assessed by biochemical markers including inhibin B (10). Moreover, pre-chemotherapy inhibin B and anti-müllerian hormone (AMH) have been observed to be lower among women experiencing chemotherapy-associated amenorrhoea, suggesting a predictive value for the occurrence of amenorrhoea (11).

In order to investigate the potential role of inhibin A in breast cancer patients within the context of chemotherapy, serum samples of breast cancer patients obtained from the prospective, randomised German therapeutic SUCCESS trial receiving adjuvant chemotherapy were analysed for inhibin A.

Patients and Methods

Samples. This study used serum samples derived from 30 patients diagnosed with breast cancer from the prospective, randomised German therapeutic SUCCESS trial (12). All of the patients underwent surgery leading to R0 resection of the tumour and received adjuvant chemotherapy. For each patient, three serum samples at three different time-points were taken: before the beginning of chemotherapy, four weeks after termination of chemotherapy and two years after chemotherapy. Therefore, a total of 90 serum samples were tested for their inhibin concentration.

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The SUCCESS trial is a multicentric, prospective randomised trial comparing the effectiveness and compatibility of two different chemotherapy regimens in breast cancer patients, followed by two- or five-year bisphosphonate therapy (12). The aim of the trial is the comparison of recurrence-free survival after randomisation of patients who received three cycles of epirubicin–5-fluorouracil–cyclophosphamide (FEC) chemotherapy, followed by three cycles docetaxel (D) chemotherapy *versus* three cycles of FEC chemotherapy followed by three cycles gemcitabine–docetaxel (DG) chemotherapy.

Immunoassay (ELISA). For serological measurement of inhibin A, Ultrasensitive Inhibin A ELISA (DSL-10-18200 ACTIVE® Ultrasensitive Inhibin A ELISA, DSL, U.S.A) was used. The principle of the test is an enzymatically amplified 'two-step' sandwich type immunoassay. It involves two antibodies which absorb the antigen (inhibin) at two different sites. The primary antibody, anti-inhibin βA subunit antibody, was located in special microtitration wells. In the assay, standards, controls as well as serum samples were incubated in these coated microtitration wells. A protein buffer was also added. After several hours of incubation at room temperature the inhibin βA antibody was able to absorb any inhibin that was present. After washing with wash solution, the wells were treated with biotinylated anti-inhibin alpha subunit detection antibody, the secondary antibody, which also was able to bind inhibin during incubation. The immunoreaction was continued by subsequent addition of streptavidin labelled with enzyme horseradish peroxidase (HRP) to link the enzyme to the secondary antibody. After a third incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine with hydrogen peroxide. This substrate generated a change of colour which was catalysed by the already present HRP. An acidic stopping solution was added afterwards and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm.

The absorbance measured was directly proportional to the concentration of inhibin A present in the samples. A set of inhibin A standards was used to plot a standard curve of absorbance *versus* inhibin A concentration from which the inhibin A concentrations in the unknowns could be calculated.

Statistical analysis. The concentration of inhibin in the serum samples of patients receiving chemotherapy was measured at three time points: before the beginning of chemotherapy, four weeks after the last cycle of chemotherapy and two years after chemotherapy.

For statistical analysis, the statistics programme SPSS (SPSS version 15.0; SPSS Inc., Chicago, IL, USA) for Windows was used. The established data of inhibin concentration were compared by Friedman test and the Wilcoxon rank sum test for paired samples. Statistical significance was assumed at $p<0.05$.

Results

In chronological sequence, a significant difference between the three points of time was observed. The concentration of inhibin A showed a significant decrease between data obtained before chemotherapy and after chemotherapy ($p<0.005$) and between before chemotherapy and two-year follow-up ($p<0.001$). Interestingly, there were no differences between the four-week and two-year follow-up data ($p=0.744$) (Figure 1).

Discussion

The inhibin/activin-subunits have been detected in normal female reproductive tissue and endocrine tumours (4), including ovarian cancer (4, 13), endometrial tissue (14-19) and breast cancer (7, 20), suggesting possible roles in cancer proliferation and growth (4, 21).

This widespread expression of these subunits might reflect several autocrine and paracrine roles. In women with ovarian cancer, elevated serological levels of inhibin have been demonstrated, suggesting it as a useful tumour marker in this type of cancer (22). Additionally, similar observations and suggestions have been observed for activin A in breast cancer tissue (9). However, activin might not be a clear-cut marker of breast cancer recurrence, but it still constitutes a potential candidate for recurrence surveillance (20).

Recent investigations have focused in assessing ovarian reserve by using several surrogate markers, including inhibin B, in breast cancer survival (23). Meanwhile, it has been demonstrated that premenopausal breast cancer survivors have diminished ovarian reserve compared with a control group (10) as assessed by biochemical markers, including inhibin B (10). Moreover, pre-chemotherapy inhibin B and AMH have been observed to be lower among women experiencing chemotherapy-associated amenorrhea, suggesting a predictive value for the occurrence of amenorrhoea (11). However, the most evaluated serological glycoproteins of the TGF-β group are inhibin B and AMH. In this study it was demonstrated that inhibin A concentration can be detected in serum of breast cancer patients with a significant concentration decrease during the course of chemotherapy. An interesting fact is that there were no differences between the values at end of chemotherapy and the two-year follow-up. Since no patient in the analysed study group has presented with a breast cancer recurrence to date, no definite statement on the value of inhibin A as a marker for tumour recurrence can be made at the time of writing.

The effects and action mechanisms of inhibin in cancer cells are not yet completely elucidated. However, the most important function is the tumour suppressor activity of the α-subunit, first identified after functional deletion of the inhibin-α gene in mice (24, 25). Whether the inhibin-βA and -βB subunits have similar tumour-suppressive properties as observed for the inhibin-α subunit remains controversial (4), although recently obtained evidence suggests that activin A, the homodimer of the βA subunit, can inhibit cancer cell proliferation in various experimental models *in vitro* and *in vivo* (26, 27). Whether these assumptions are also true for breast cancer cells is not yet known. Activin A can inhibit breast cancer cell proliferation of cell lines *in vitro* (28-31). Moreover, activin resistance

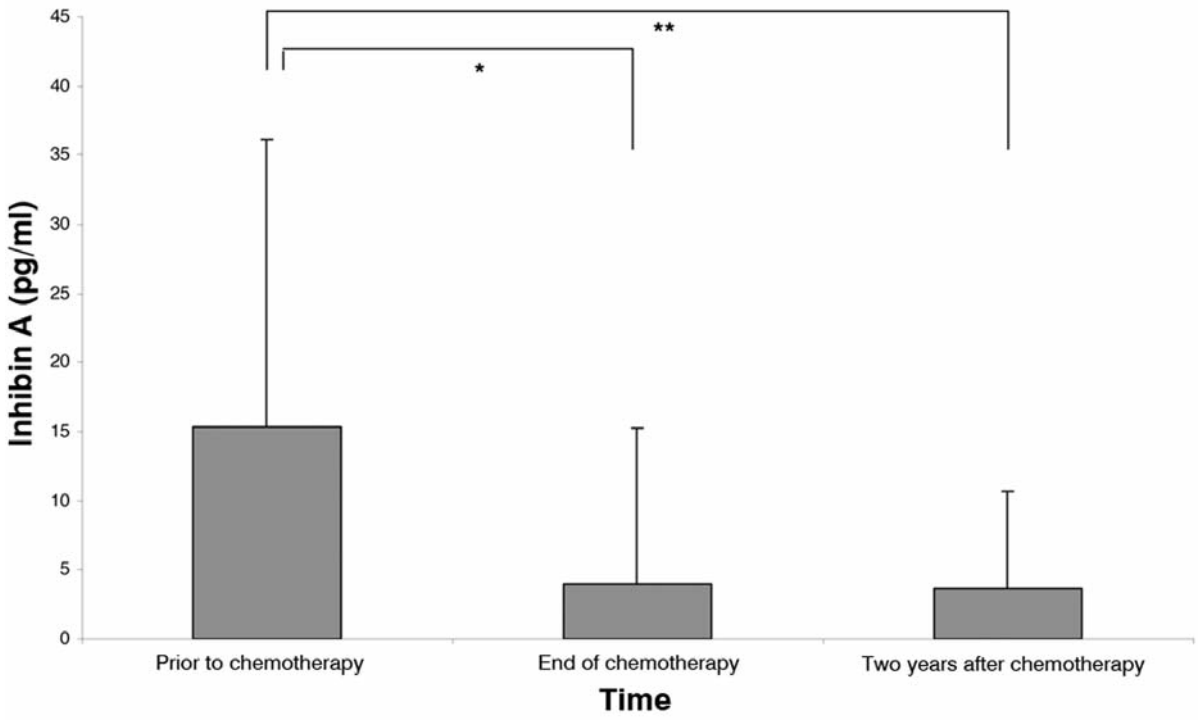

Figure 1. *Serological concentrations of inhibin A in patients with mammary carcinomas. The concentration of inhibin A showed a significant decrease between data obtained before and after chemotherapy (*p<0.005), and at two-year follow-up (**p<0.001).*

in oestrogen receptor-negative breast tumour cell lines might be involved in breast cancer carcinogenesis and in increased malignancy compared with oestrogen receptor-positive cells (28).

In conclusion, chemotherapy significantly decreased the inhibin A concentration in this study. This might reflect a suppression of ovarian function, being also a marker for chemotherapy-induced amenorrhoea. Whether inhibin A also constitutes a prognostic serological tumour marker in breast cancer patients is still under investigation.

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