Preoperative transforming growth factor-beta 1 (TGF-beta 1) plasma levels in operable breast cancer patients

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Summary

Objectives: The aim of this project was to search for new risk prognostic markers in the early stage of breast cancer. We tested preoperative plasma transforming growth factor - beta 1 (TGF- beta 1) levels in patients with operable breast cancer. Correlation with traditional prognostic markers and with positivity/negativity sentinel lymph node was evaluated. *Materials and Methods:* Between 2003 and 2005, 36 patients with operable breast cancer (T1-2, N0-1, M0) with positive or negative sentinel lymph nodes were evaluated for their plasma TGF-beta 1. Twenty-seven healthy individuals (9 premenopausal and 18 postmenopausal) served as controls. Patients were evaluated for the traditional prognostic markers including tumor characteristics, positivity and negativity of sentinel lymph node, TNM, tumor grade, expression of tumor markers CA 15-3 and CEA, hormonal status (pre- or postmenopausal patients, estrogen and progesteron receptor expression), ERB and p53 expression. Predictive value of TGF-beta 1 level and correlation with either of the assessed parameters was tested by one way ANOVA analysis. *Results:* Measurements of preoperative plasma TGF-beta 1 levels in patients with operable breast cancer were significantly higher compared with healthy individuals (median 15293 and 3983 pg/ml $p < 0.0001$). TGF-beta 1 level in plasma of patients with a positive sentinel lymph node was significantly higher than in patients with negative sentinel lymph nodes (high vs low, median 18,9 and 14,5 ng/ml, respectively, $p = 0.05$). *Conclusion:* The determination of TGF-beta 1 status might help to identify a high-risk population early in tumor progression, for which a more appropriate therapy should be established. In the node-negative population, the up-regulation of TGF-beta 1 might constitute an early event that promotes further progression of breast tumors.

Key words: Operable breast cancer; Transforming growth factor-beta 1 (TGF- beta1); Preoperative assessment; Sentinel lymph node; Risk factor; Prognostic marker.

Introduction

Breast cancer is the most frequent cancer in females and its incidence in developed countries of the world is still increasing [1, 2]. Most patients in early stage of disease (with negative regional lymph node, without signs of primary tumor metastasis) can be effectively treated with surgery and local radiotherapy. Patients with positive axillary lymph nodes are indicated for adjuvant chemotherapy. Besides involvement of local lymph nodes, other parameters, such as size of the prime tumor, histology of the tumor, grade of tumor cells, and hormone receptor Her2/neu status help in choosing the appropriate therapy. Unfortunately, those criteria are not sufficient. Although lymph node status is one of the best prognostic factors in breast cancer, it is not sufficiently accurate to predict the clinical course of the disease. Indeed, 20-30% of node-negative breast cancer patients will experience disease recurrence and metastatic dissemination [3].

This is the reason new markers need to be searched for which could, as soon and as objective as possible, determine the exact situation of breast cancer patients and consequently adequate therapy could be planned and therapy response monitored. Transforming growth factor-beta 1 (TGF-beta 1) is thought to be implicated in breast cancer progression. TGF-beta is a pleiotropic growth factor, which affects many different cell functions such as proliferation and extracellular matrix synthesis. TGF-beta can stimulate tumor angiogenesis, alter the stromal environment and cause local and systemic immunosuppression, all of which contribute to tumor progression and metastatic dissemination [4-7].

This study was conducted to further analyze the role of TGF-beta 1 in breast cancer and to evaluate its significance as a prognostic marker in early stages of breast cancer. The aim was to compare preoperative plasma TGF-beta 1 levels in early breast cancer patients and to compare them with those of healthy individuals and with the patients with advanced stage of disease (positive sentinel lymph node).

The association between preoperative plasma level of TGF-beta 1 and traditional prognostic markers (lymph node status, TNM classification, tumor grade, hormonal status, expression of tumor markers CA 15-3, CEA) was studied for all patients by two way ANOVA analysis.

Those investigations should help to find markers which will allow us to precis the diagnosis in early stages of disease and to help adjust the agressivity of standard therapy.

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Materials and Methods

This study involved 36 patients diagnosed and treated in the Obstetrics and Gynecology Department of our institution, between early 2003 and late 2005. Patients were selected according to the following criteria: (1) primary unilateral breast tumor; (2) no evidence of metastatic disease or any other malignancy at the time of diagnosis; (3) cT1,T2, N0/N1 status according to UICC criteria; (4) surgery as the first treatment - operable breast cancer study entrance criteria:

– size of prime tumor ≤ 1 cm with negative lymph nodes (T1a,b, N0);

– size of prime tumor ≤ 1 cm with positive lymph nodes (T1a,b, N1);

– size of prime tumor > 1 cm and < 5 cm with negative lymph nodes (T1c,T2, N0);

– size of prime tumor > 1 cm and < 5 cm with positive lymph nodes (T1c, T2, N1).

The diagnosis of breast cancer was done by core-cut or open biopsy. All patients included in the research study were routinely examined prior to operation, including palpation, mammography and/or breast sonography. In patients who were breast cancer positive for tumor markers CEA and CA 15-3 (normal values till 32.4 for CA 15-3 and 2.5 for CEA), chest X-ray, liver sonography and skeleton scintigraphy were done. Data on age, primary tumor stage, TNM staging (according to the Union Internationale Contre le Cancer) [8-10] and imunohistochemistry (determination of hormone receptors, mitotic tumor index) were reviewed and recorded. Grade was established according to the Nottingham Grading System [11-12]. Estrogen and progesterone receptor positivity was tested by monoclonal antibody kits ER/PR (Immunotech Company, USA). Her2/neu was tested by Hercep Test (DAKO Company, UK), Her2/neu 3+ were considered positive.

Venous blood samples were collected before the surgery. Plasma samples were obtained by centrifugation and stored at -70°C until assayed. TGF-beta 1 plasma level was determined by modified ELISA (enzyme-linked immunoabsorbent assay) using monoclonal anti TGF-beta 1 antibodies from R&D Systems®.

In all patients detection for sentinel lymph nodes was performed. Most of the patients [26] underwent technetium-guided detection 18-20 hours after subareolar injection of technetium radiocoloid 99mTc Senti-scit, Radiob, H). Seven patients underwent blue dye perioperative-guided detection (Blue Patent© V, Guerbet) and in three cases detection was performed by combinations of both methods [13]. The sentinel lymph node was examined by frozen section, in case of a positive finding of malignant cells, axillary lymph node dissection (level I/II) was performed. All lymph nodes including SLN were routinely examined by hematoxilin-eosin in parafin embedded blocks.

Twenty-seven healthy individuals (32-74 years, median age of 52.9 premenopausal and 18 postmenopausal) served as controls.

Patients not included in the study: size of prime tumor > 5 cm, and patients with neoadjuvant therapy (hormonal, radio- or chemotherapy). The study was approved by the ethical board and patients signed informed consents regarding blood drawing and presentation of results.

Statistics

The association between preoperative plasma levels of TGF-beta 1 and traditional prognostic markers (lymph node status, TNM classification, tumor grade, hormonal status, expression of tumor markers CA 15-3, CEA, Ki 67) was studied for all patients by two-way ANOVA analysis. Statistical analysis was performed with the Mann-Whitney U-test. Data were presented as mean ± SEM or as percentage; a p-value of < 0.05 was considered to be statistically significant.

Results

Clinical and pathological characteristics (Table 1)

The patients were 38-78 years old at diagnosis, with a median age of 56 years. In total, 33.3% of patients were premenopausal. A total of 12 patients presented a tumour size less than 1 cm; 72.2% of patients were node-negative, 25.0% presented one to three axillary invaded nodes and 2.8% had more than three invaded nodes. Ductal carcinomas were diagnosed in 77.8% of patients and invasive lobular carcinomas in 22.2% of patients.

The primary treatment was segmentectomy (69.4%) or modified radical mastectomy (30.6%) with axillary dissection. Twenty-six patients underwent technetium-guided detection of SLN, seven patients underwent blue dye perioperative guided detection, and in three cases detection was performed by combinations of both methods. The patients did not have any other therapy at the time of study entrance.

Table 1. — *Patient characteristics.*

Feature	Category	No. of patients	Percentage
Total population		36	
Age (years)	≤ 50	8	22.2
	> 50	28	77.8
Hormonal status	Premenopausal	12	33.3
	Postmenopausal	24	66.7
Tumor size	T1	7	19.4
	T2	29	80.6
Histology	Ductal	32	88.9
	Lobular	4	11.1
Grade	I	11	30.6
	II	17	47.2
	III	8	22.2
Receptor status	ER- PR-	5	13.9
	ER- PR+	0	0
	ER+ PR-	9	25
	ER+ PR+	22	61.1
Breast surgery	Segmentectomy	25	69.4
	Mastectomy	11	30.6
SLN status	Negative	26	72.2
	Positive	10	27.8

Biological characteristics of the breast cancer patients

A wide inter-patient variability in the levels of biological factors in breast cancer samples could be observed.

Thirty-one out of 36 patients (86.1%) showed ER positivity, 75.0% (27/36) were PR positive, three out of 35 were Her2/neu positive (8.3%). Three patients showed triple negativity for all three markers. Fifteen out of 34 patients (44.1%) showed p53 positivity, eight of those 15 showed p53 positivity less than 10%. Only three patients showed CA 15-3 positivity and two for CEA.

From the total of 36 eligible patients, 26 (72.2%) showed negativity of the sentinel lymph node, whereas

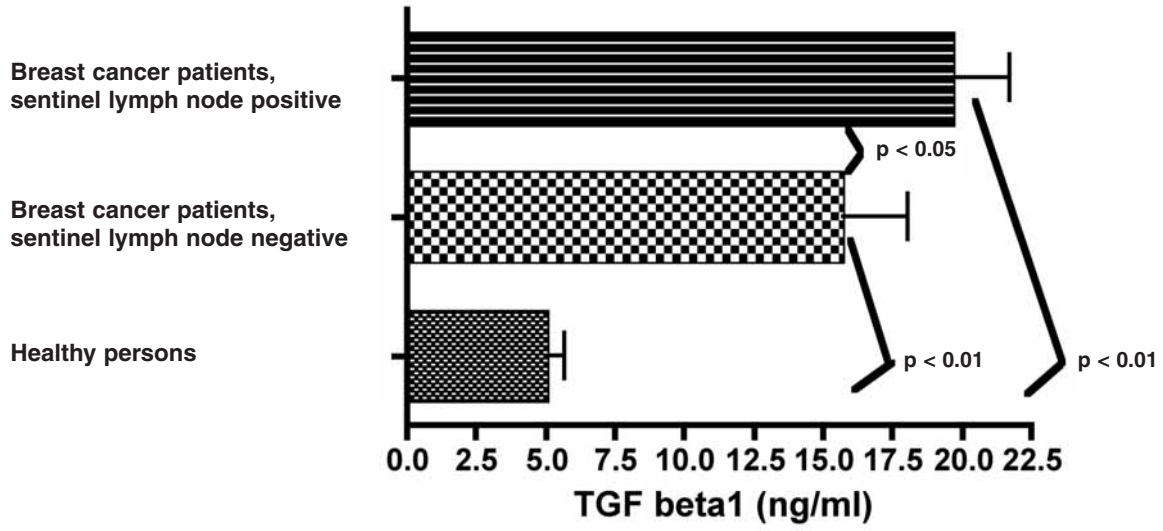

Figure 1. — Comparison: TGF beta 1 in plasma of healthy persons and operable breast cancer patients with negative or positive sentinel lymph nodes.

ten were sentinel lymph node positive. There was no correlation between above mentioned markers expresion and sentinel lymph node positivity.

TGF-beta 1 levels (Table 2, Figure 1)

TGF-beta 1 level in plasma of cancer patients was significantly higher compared with healthy individuals (median 15293 and 3983 pg/ml $p < 0.0001$). TGF-beta 1 level in the plasma of patients with positive sentinel lymph nodes was significantly higher than that in patients with negative sentinel lymph nodes (high vs low, median 14.5 and 18.9 ng/ml, respectively, $p = 0.05$). There was no difference between pre- and postmenopausal patients.

Table 2. — *TGF-beta 1 levels in selected groups of patients.*

Category	Range	Mean (CI)*	Q25	Q50	Q75
SLN positive	13.45-33.71	19.75	13.81	18.97	24.09
SLN negative	1.124-47.67	15.72	5.992	14.5	21.02
Healthy controls	1.095-12.41	5.086	2.46	3.938	6.958

*95% confidence interval, CI.

Discussion

High levels of TGF-beta has recently been a discussed topic in correlation to patient therapy response, stage of disease and poor prognosis. Recent evidence continues to support a central role for TGF-beta in tumor maintenance and progression [14, 15].

There is evidence that TGF-beta acts as a suppressor of tumor initiation but also as a promoter of tumor progression, when the antiproliferative effect of the TGF-beta signalizing pathway has been overridden by other oncogenic mutations [16, 17]. In addition, there is increasing evidence that after malignant cells lose their sensitivity to TGF-beta 1 - mediated growth inhibition, autocrine TGF-beta signalling may promote tumorigenesis [18]. TGF-beta was shown to be produced by tumor cells and mainly by macrophages and T-regulatory cells of cancer patients [19].

TGF-beta is a pleiotropic cytokine with powerful immunosuppressive functions. Recent investigations have highlighted the role of TGF-beta in suppression of T-cell mediated anti-tumor immunity as well as cytotoxicity of NK immune cells and dendritic cells (DC) [20-22].

Immune response of cancer patients is often insufficient, supporting immunosupression and tumor growth [23-28]. Transforming growth factor-beta inhibits the antigen-presenting functions and antitumor activity of dendritic cell vaccines [29]. Targeting tumor-associated macrophages and T-regulatory cells producing TGF-beta seems to be a novel strategy against breast cancer.

Whereas TGF-beta 1 seems to be confirmed as a poor prognostic marker in a number of human tumors such as ovarian [30], colorectal, gastric [31], and prostatic [32] cancers, glioma [33] and in metastatic breast cancer [34]. The impact of TGF-beta 1 on the progression of breast cancer remains uncertain. Desruisseau *et al*. [3] described increased TGF-beta l protein level in breast cancer tissue samples and correlated with a shorter disease-free survival. This suggests that secreting higher levels of TGF-beta 1 may provide an advantage to tumor cells. The hormonal influence on activation of the TGF-beta system adds an additional layer of complexity.

Ivanovic *et al.* [34] showed that elevated plasma TGF-beta 1 levels correlate with decreased survival of metastatic breast cancer patients. In our study, we focused on patients with early stages of disease.

We proved for the first time that TGF-beta 1 levels are already elevated in early stages of the disease in patients with operable breast cancer. Moreover, TGF-beta 1 in plasma of patients with positive sentinel lymph nodes was significantly higher compared to patients with negative sentinel nodes. The fact that a high level of TGF-beta 1 was observed in a node-negative population strongly

suggests that TGF-beta 1 interferes at early stages of tumor progression, probably by making cell environment favorable for metastatic spread. Thus, in the node-negative population, the upregulation of the TGF-beta 1 group might constitute an early event that promotes further progression of breast tumors.

Whereas numerous predictive factors have been characterized thus far, early prognostic markers that interfere in the beginning of tumor progression are scarce. High TGF-beta 1 levels observed in node-negative breast cancer patients suggest that the determination of TGF-beta 1 status might help to identify a high-risk population early in tumor progression, for which a more appropriate therapy should be established. In this context, it appears fundamental to confirm the prognostic value of TGF-beta 1 in a large cohort of node-negative patients.

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