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Elevated plasma levels of TGF-beta1 in patients with locally advanced breast cancer related to other clinical stages

KEYWORDS: Breast Neoplasms; Transforming Growth Factor beta; Neoplasm Staging; Tumor Markers, Biological

ABSTRACT

Background: *The application of plasma tumor markers is mainly during the follow-up of cancer patients and especially in monitoring of advanced disease. These biomarkers do not require surgical intervention and provide relatively simple monitoring at any time during the disease course. TGF-beta1 is a pluripotent cytokine, with diverse effects in normal physiology and a role in both normal mammary gland development and progression of breast cancer. In early stages of breast carcinomas TGF-beta1 acts as tumor suppressor, while in later stages, when tumor cells become resistant to growth inhibition by TGF-beta1, it acts as tumor promoter. For that reason, the aim of this study was to assess the stage-related TGF-beta1 elevation in circulation of breast cancer patients, during disease progression.*

Methods: *We analyzed 52 breast cancer patients of different stages (I/II, III, IV) and 36 healthy donors. TGF-beta1 levels were determined by enzyme-linked immunosorbent assay (ELISA, R&D).*

Results: *Although there was no increase in plasma TGF-beta 1 in stage I/II patients (n =10, median value = 0.89 ng/ml), statistically significant elevation of plasma TGF-beta1 was found in locally advanced breast cancer (stage III, n = 9, median value = 2.30 ng/ml) and also in metastatic breast cancer (stage IV, n = 33, median value = 2.46 ng/ml) in relation to healthy donors and stage I/II.*

Conclusion: *This elevation of plasma TGF-beta1 in locally advanced breast cancer is probably the result of increased tumor mass and tumor-stromal interactions in this stage, as well as a possible cause of greater metastatic potential of tumor cells which lead to metastatic breast cancer. Prognostic role of TGF-beta1 is not fully understood, but from these results we could say that it could be a marker for monitoring patients disease course, as well as for understating the biology of breast cancer.*

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INTRODUCTION

Many studies are carried out in an effort to evaluate a significance of possible prognostic markers in breast cancer. Prognostic factors are associated with either growth rate or metastatic potential of the tumor (1). However, the results of published prognostic factor studies are often inconsistent or even contradictory, thus limiting their value and potential application, and there is still uncertainty about the importance of most prognostic factors.

TGF-beta1 is a multifunctional cytokine, whose increased or decreased production has been linked to numerous diseases, including many types of cancer (2). TGF-beta1 overexpression in breast tumors has been associated with more malignant phenotype (invasiveness and metastasis) (3), but its prognostic role still remains controversial (4,5) for many reasons. In this study, we tried to determine is there any change in circulating levels of TGF-beta1 in breast cancer patients and to relate those changes with different stages of disease.

PATIENTS AND METHODS

The present study included 52 breast cancer patients and 36 healthy women donors of similar age. For determination of plasma TGF-beta1 we used Quantikine ELISA kit (R&D Systems Inc. Minneapolis, MN, USA). Apyrase, Phenylmethylsulphonil Fluoride (PMSF) and Ethylendiaminetetraacetic acid (EDTA) was obtained from the Sigma Chemica Co (St. Louis, MO, USA). Aprotinin was purchased from Behring (Marburg, Germany).

For the plasma preparation, venous blood (4 ml) was collected into a test tube containing a final concentration of 0.25% EDTA, 1 mM PMSF, 250 Kallikrein Inaktivator Enhetier KIE/ml aprotinin, and 2.5 units/ml apyrase. The sample was mixed, centrifuged (20 min, 800 g, +4 C) and supernatant recentrifuged (10 min, 9 400 g, +4) to quantitatively remove residual platelets as possible source of nontumoral TGF-beta1. The recentrifuged supernatant was aliquoted (150 (l / aliquot) and stored frozen at -100 C. TGF-beta1 was analyzed by the receptor-based Quantikine ELISA kit, according to the manufacturers instruction. The absorbance at 450 nm was detected by the microplate ELISA Reader El 311 (Behring, Marburg, Germany). Concentrations were calculated from the constructed linear curve. The sensitivity of the kit is 20 pg/ml TGF-beta1. Validation of our protocol (for preparation of platelet-poor/protease-inhibited human plasma) was checked on *in vitro* degranulation of platelets by determination of Platelet Factor 4 (PF4) in the same samples, as we described in our previous work (6).

The Mann-Whitney test was used to assess the existence of significant statistical differences between the patient subgroups.

RESULTS

Table 1 shows clinicopathological features of breast cancer patients included in this study.

Figure 1 shows plasma TGF-beta1 levels in breast cancer patients, stages I/II, III and IV (median value: 2.1 ng/ml, range: 0.13-8.48 ng/ml, n=52) relative to the healthy donors (median value: 1.32 ng/ml, range: 0.41-4.93, n=36). These data indicate that circulating levels in breast cancer patients were significantly elevated ($p<0.05$) when compared with healthy donors.

Figure 2 shows association between elevation of plasma TGF-beta1 and progressive stages of disease, as observed by the statistically higher TGF-beta1 level for the stage III (median value: 2.30 ng/ml, range: 0.7 -3.54 ng/ml, n=9, $p<0.05$) and for stage IV (median value: 2.46 ng/ml, range 0.13 - 8.48 ng/ml, n=33, $p<0.05$) compared with stage I / II (median value: 0.89 ng/ml, range: 0.37-1.60 ng/ml, n=10).

Table 1. Clinicopathological features of breast cancer patients included in this study

	Parameter	No
Patients	**Age:** Range	37-74
	Median value	51
	Menopausal status	
	Premenopausl status	10
	Postmenopausal status	42
Tumor	**Clinical stage**	
	I / II	10
	III	9
	IV	33
	Histological type*	
	IDC	15
	ILC	23
	Unknown IC	14
	Histological grade	
	G1	15
	G2	23
	G3	14
	Unknown G	13
	Steroid receptor status	
	ER+	22
	ER-	24
	PR+	20
	PR-	22
	Unknown ER	6
	Unknown PR	5
	Site of distant metastasis**	
	BM+	17
	BM-	16
	VM+	17
	VM-	17

* IDC - invasive ductal carcinoma, ILC - invasive lobular carcinoma, IC - unknown type of invasive carcinoma;
** BM - bone metastasis, VM - viscral metastasis

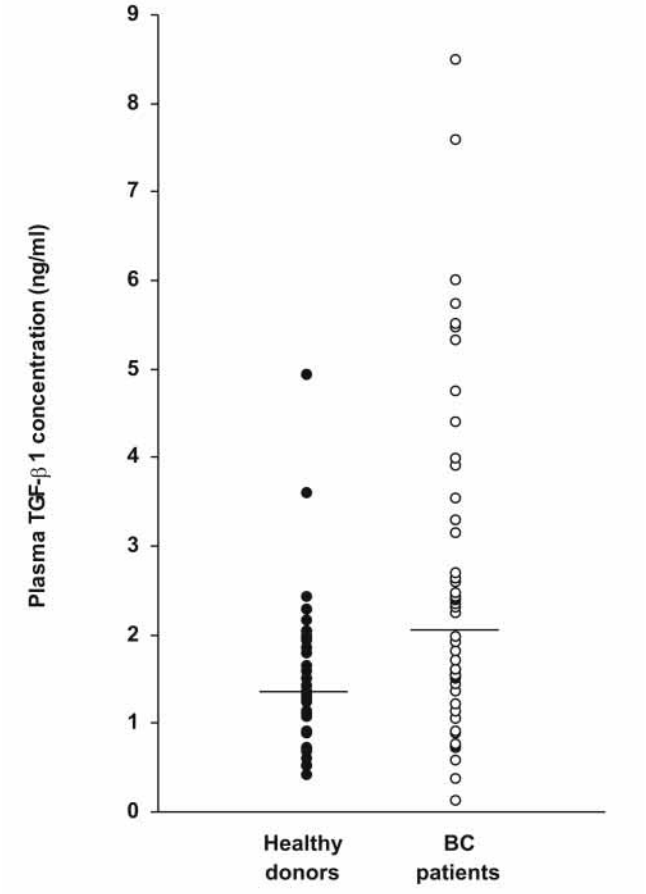

Figure 1. Plasma TGF-beta1 concentrations of breast cancer patients (BC), stages I/II, III and IV relative to the healthy donors. Horizontal bars represent median value

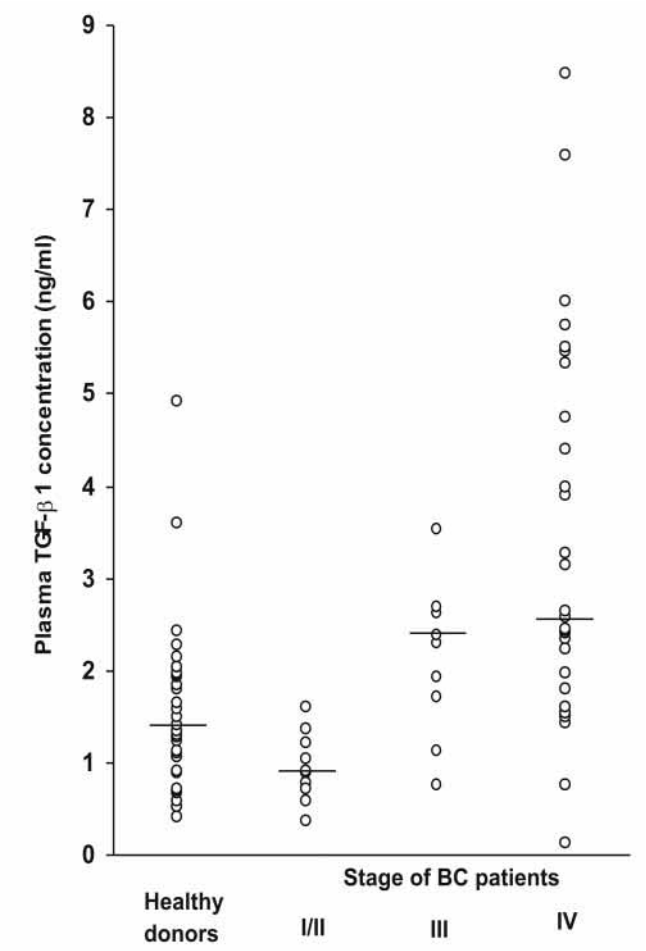

Figure 2. Relationships between plasma TGF-beta1 concentrations in breast cancer patients' stages I/II, III and IV. Horizontal bars represent median values

DISCUSSION

TGF-beta1 has a complex role in carcinogenesis. Decreased expression of the type II TGF-beta receptor, in hyperplasia, is associated with increased risk for invasive breast cancer, and in invasive breast cancer correlates with higher tumor grade and proliferative capacity. However, advanced tumors overexpress TGF-beta1 ligand, suggesting pro-oncogenic role in advanced disease and this increased production of the ligand is often associated with the loss of growth inhibitory response (7). Considering that, during the progression breast cancer, at some stage, a switch in TGF-beta1 action happens. If we could find increased expression of TGF-beta1 in early stages (I/II), it would be useful for the host, but not for the tumor itself, considering on the existence of functional receptors, in general. For that reason, it is unlikely that malignant cells in that stage would synthesize such factor.

We have analyzed a group of 52 breast cancer patients and found statistically significant elevation in plasma levels TGF-beta1 relative to healthy donors. To exam if it is possible to determine the stage of disease during which this elevation happens (and consequently switching in TGF-beta1 action), we divided our patients in clinical stages. Initially significant increase of plasma TGF-beta1 levels were found in stage III, i.e. in locally advanced breast cancer, which is characterized not only by increasing tumor mass but also by spreading of tumor to surrounding tumor stroma. The importance of stroma-epithelial interactions is well established in tumorogenesis and likely involves autocrine and paracrine action of multiple growth factors, including TGF-beta1, which is expressed in both stroma and epithelium (3). Thus, tumor cells, which produce TGF-beta1, could manipulate stromal cells to assist in their malignancy. The net result, of these changes, i.e. increased production of the TGF-beta1 within tumor and stroma is the elevation of plasma levels in

this stage.
Further elevations that we found in advanced/metastatic (stage IV) breast cancer contribute the progression of the disease. In response to increased production of TGF-beta1, the tumor cells become more invasive and metastasize to distant organs, as a result of TGF-beta1 mediated stimulation of angiogenesis, cell motility, suppression of immune system and increased interaction with extracellular matrix.

CONCLUSION

Most tumor markers have a low sensitivity in early stages of disease and therefore cannot be used in screening or diagnosis. The application of plasma tumor markers is useful mainly during the follow-up of cancer patients and especially in monitoring of advanced disease.
It would be of great value to have circulating tumor marker that reliably predicts prognosis, independent of TNM stage. Circulating tumor markers might be useful clinically in several situations. As we know, prognostic value of some factor is to indicate tumor burden, growth rate and/or metastatic potential. Prognostic role of TGF-beta1 is not fully understood, but from these results we could say that it could be a marker for monitoring patients disease course, as well as for understanding the biology of breast cancer.

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REFERENCES

1. Hayes DF, Trock B, Harris AL. Assessing the clinical impact of prognostic factors: When is "statisticaly significant" clinicaly useful?. Breast Cancer Res Treat 1998;52:305-19.

2. Blobe GC, Schiemann WP, Lodish HF. Role of Transforming grrowth factor beta in human disease. New Eng J Med 2000;342:1350-8.

3. Welch DR, Wei LL. Genetic and epigenetic regulation of human breast cancer progression and metastasis. Endocrine Related Cancer 1998;5:155-97.

4. Reiss M, Barcellos-Hoff MH. Transforming growth factor beta and breast cancer. Working hypothesis. Breast Cancer Res Treat 1997;45:81-95.

5. Wakefield LM, Letterio JJ, Chen T, Danielpour D, Allison RSH, Pai LH et al. Transforming growth factor - β1 circulates in normal human plasma and is unchanged in advanced metastatic breast cancer. Clin Cancer Res 1995;1:129-36.

6. Ivanović V, Todorović-Raković N, Demajo M, Nešković-Konstantinović Z, Subota V, Ivanišević-Milovanović O et al. Elevated plasma levels of transforming growth factor beta1 (TGF-β1) in patients with advanced breast cancer: association with disease progression. Eur J Cancer 2003;39:454-61.

7. Reiss M. TGF-β and cancer. Microbes and Infection 1999;1:1327-47.