

## Plasma Free Serotonin as a Marker for Early Detection of Breast Cancer Recurrence

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**Abstract.** Aim: To evaluate the significance of plasma free serotonin (5-hydroxytryptamine) and Ca15.3 for the early detection of breast cancer recurrence. Materials and Methods: Free serotonin and Ca15.3 levels were measured by I-125-Serotonin RIA (DDV Diagnostica, Marburg, Germany) in plasma and an ELISA kit (Roche Diagnostic GmbH, Mannheim, Germany) in serum, respectively in women (N=29) who responded to primary treatment for breast cancer and who were followed-up for recurrence. For analysis, patients were sub-divided according to TNM staging into groups with localized (T1-2N0-M0) and advanced (T1-2N1-2M0-1) disease. The control group were healthy blood donors. Results: Patients with advanced disease had a significantly higher plasma serotonin level than those with localized disease or controls, whereas Ca15.3 levels remained in normal range in all groups. At the time of serotonin measurement, radiological findings were negative for all patients in the localized-disease group, but positive in nine patients in the advanced-disease group. Conclusion: Plasma free serotonin may be used for the early detection of recurrent/metastatic breast cancer disease, but validation on a larger number of patients is needed.

Monoamine serotonin (5-hydroxytryptamine; 5HT) mediates a wide range of (patho)physiological processes, including tumour growth and differentiation (1). In clinical practice, few studies have examined the possible association of circulating

serotonin with prognosis of several types of cancer, but to the best of our knowledge, only serotonin within platelets has been measured (2, 3), while no attempts were made to explore the potentially important pool of free serotonin in plasma. Recently it has been shown that breast cancer cells can synthesize 5HT, which are used to support tumour growth (4). It was suggested that increased tryptophan metabolism via the serotonin pathways could, therefore be linked to malignant progression in breast cancer (4, 5).

The purpose of our prospective study was to evaluate the feasibility of plasma free serotonin for assessing early recurrent breast cancer in comparison with Ca15.3, the most widely used serum-based tumour marker for breast cancer (6).

### Materials and Methods

The study included 29 women (41-80 years old) who responded to primary treatment for breast cancer and were followed-up for recurrence of disease. The patient cohort was subdivided according to TNM (T-tumor, N-node, M-metastases) staging into two groups: localized disease (T1-2N0M0, N=14) and advanced disease (T1-2N1-2M0-1, N=15), all with positivity for estrogen and/or progesterone receptors. The control group was matching group of women without malignant disease (N=9). Measurements of plasma 5HT and serum Ca15.3 levels were performed by commercially available kits: radioimmunoassay (I-125-Serotonin RIA; DDV Diagnostika) and ELISA (Roche-Diagnostics), respectively. For preparation of platelet-poor-plasma samples, all necessary precautions (7) were taken to ensure accurate estimation of very low concentration of serotonin in plasma. No patient was taking medication that could interfere with serotonin metabolism. Ethical approval for the study was obtained from the University Hospital Sestre Milosrdnice Research Ethics Board (S-526); participants in the study signed an informed consent form.

After testing data for normality, groups were compared using the Mann-Whitney *U*-test (5HT data) and one-way ANOVA (Ca15.3 data), with level of significance set at  $p < 0.05$ . GraphPad Prism software (www.graphpad.com) was used throughout.

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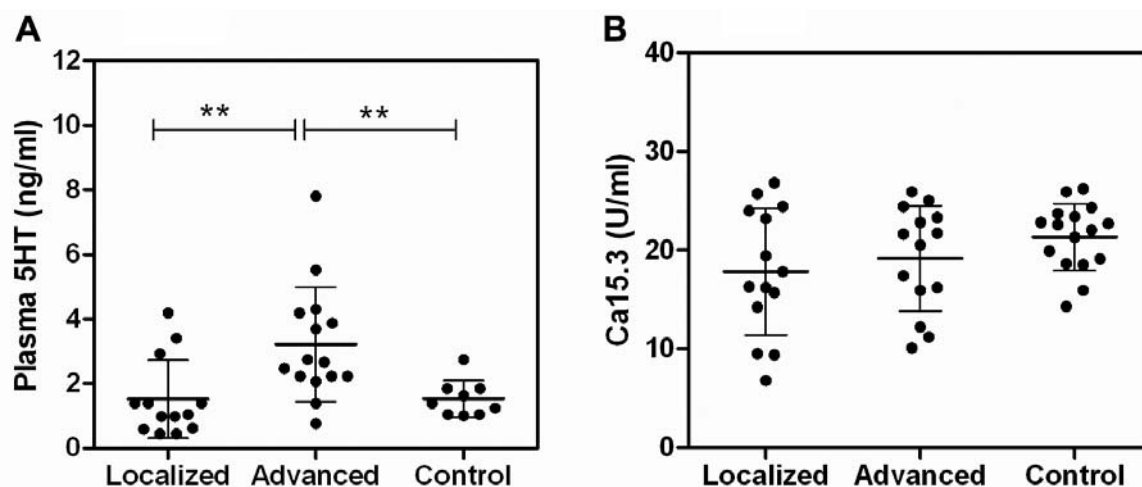


Figure 1. Plasma free serotonin (A) and serum Ca15.3 (B) in patients with localized (T1-2N0-M0, n=14) and advanced (T1-2N1-2M0-1, n=15) stages of breast cancer, and in a control group without malignant disease. Individual values with means indicated by a heavy line and SDs by bars. \*\**p*<0.01.

## Results

Patients with advanced disease had significantly higher plasma 5HT level than those with localized disease and the controls (means ±SD: localized: 1.41±1.23, advanced: 3.21±1.77, controls: 1.53±0.56 ng 5HT/ml; Figure 1A). On the other hand Ca15.3 measurements remained within the normal range (localized: 17.81±6.44, advanced: 19.16±5.32, controls: 21.33±3.37 U/ml, Figure 1 B). Limit of detection (LOD) of the analytical methods used for serotonin is 0.293 ng/ml (range 0.49-500 ng/ml) and for Ca15.3 <1.00 U/ml.

Radiological findings were positive in nine patients in the advanced group.

## Discussion

Our data show that plasma free serotonin may for detect early appearance of recurrent disease in patients of the advanced group who, at the time of 5HT measurement, had Ca15.3 values within normal range and evident disease on imaging (computed tomography-CT, bone scan).

The ideal tumour biomarker does not currently exist, and there is a constant quest for novel or companion markers for use in clinical practice. Ca15.3, a common serum-based marker for monitoring breast cancer, is increased in about 10% of patients with stage I and 75% of patients with stage IV disease, but its most important clinical application relates to monitoring therapy in patients with advanced disease (6). Serial determination of Ca15.3 after primary treatment for breast cancer can pre-clinically detect recurrent/metastatic disease with lead times of about 2-9 months before evidence-based radiological findings (6). A low concentration of Ca15.3, however, does not exclude disease recurrence.

Direct involvement of the serotonin system in breast cancer has recently been reported, showing that increased protein expression of serotonin biosynthetic enzyme, accompanied by aberrant control of 5HT receptors, favours malignant progression of disease (4). Increased enzymatic degradation of the serotonin precursor tryptophan, consistently shown in breast cancer tissue, also provides a substrate for increased serotonin synthesis (5). Extensive expression of both enzymes, tryptophan hydroxylase 1 (TPH1) and indoleamine 2,3-dioxygenase (IDO), correlates with advanced disease stage and may be linked to poor prognosis (5). Recently it was suggested that 5HT-receptor sub-types expressed in breast cancer cells may have utility as diagnostic/prognostic markers (8).

Our results in this preliminary study indicate that in addition to 5HT signalling from the local microenvironment, 5HT derived from blood plasma might also be involved in breast cancer progression. These preliminary data suggest that monitoring free serotonin in plasma from patients with breast cancer may be useful for early detection of disease recurrence. Confirmation is, however, needed in a larger patient cohort.

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