# Influence of Circulating Tumour Cells on Production of IL-1α, IL-1β and IL-12 in Sera of Patients with Primary Diagnosis of Breast Cancer Before Treatment

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**Abstract.** *Background: Circulating tumour cells (CTCs) have been found to be a prognostic marker for reduced disease-free survival (DFS), distant DFS, breast cancer-specific survival, and overall survival (OS) before the start of systemic treatment. Determination of CTCs with the CellSearch System (Veridex, Raritan, NJ, USA) is a valuable but time-consuming and costly method. Therefore, the aim of this study was to evaluate cytokine profiles as a marker for CTC involvement. Patients and Methods: Patients chosen for this study were defined as women with breast cancer who agreed to participate in the phase I SUCCESS study. CTC analysis, the blood sampling time points, and the methodology were prospectively designed, and the prognostic value of the CTCs was defined as a scientific objective of the study protocol. A total of 100 patients positive for CTCs and an additional 100 patients negative for CTCs were matched into pairs. Matching criteria were histopathological grading, lymph node status, hormone receptor type, TNM classification and survival vs. tumour associated death. Commercial enzyme-linked immunosorbent assay (ELISA) was used to screen the blood serum samples for the Th1 cytokines: interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin 12 (IL-12), IL-1α, IL-1β, IL-2 and IL-18. The correlation of cytokine levels to the matching criteria listed above were analyzed with the Spearman correlation coefficient and the Mann–Whitney-U rank-sum test. Results: The IL-1α level was significantly lower in the CTC-positive patient group (p=0.043) but significantly higher in the CTC-negative progesterone receptor-positive collective (p=0.029). Furthermore, in patients who survived, significantly higher IL-12p40 levels were found in those with lymph node involvement (p=0.041) and those with triple-negative breast cancer (p=0.043). Of patients who died, those with oestrogen receptor-negative disease had higher IL-1α (p=0.050) and higher IL-1β (p=0.034) levels. Moreover, of those who died, those with triple-negative breast cancer had significantly higher IL-1α levels (p=0.033). In patients with grade 2 tumour, patients with HER2/neu expression had significantly higher IFN-γ levels (p=0.031) and those with no lymph node involvement had significantly higher IL-1α levels (p=0.014). In the collective with grade 3 tumour, patients with progesterone receptor-negative disease had significantly higher IL12p70 concentrations (p=0.048), while those with triple-negative breast cancer had lower IL12p40 levels (p=0.033). Conclusion: Regarding CTC involvement, we speculate that IL-1α might be a marker for the release of tumour cells into the circulation and not into the lymphatic system. In addition, IL-1α like IL-1β appears to be related to CTC release in patients with breast cancer.*

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*Key Words:* Breast cancer, IL-1α, IL-1β, IL-12, circulating tumor cells.

Analyses with the highest level of evidence have established a correlation between poor prognosis and the detection of circulating tumour cells (CTCs) before the start of systemic treatment (1). CTCs in the peripheral blood have been found to be a prognostic marker for reduced disease-free survival (DFS), distant DFS, breast cancer–specific survival, and overall survival (OS) before the start of systemic treatment (1-5). Furthermore, CTC values shortly after commencing therapy provide complementary information concerning the treatment response (5). The SUCCESS study was one of the first trials to determine the strong prognostic association of CTCs with poorer survival in early breast cancer before the start of systemic adjuvant treatment and after adjuvant chemotherapy in a large patient cohort (6).

Nevertheless, the determination of CTCs is time-consuming and costly. Given the increasing significance of cytokine measurement in cancer therapy, the aim of this study was the evaluation of cytokine profiles as a marker for CTC involvement.

T-Lymphocytes and their individual differentiation depending on cytokines and chemokines are a crucial component of the adaptive immune response, presumed to be an important prognostic factor in the presence of breast cancer tumours (7). The differential expression of cytokines that stimulate helper T-lymphocyte maturation can result in stimulation or suppression of critical cell derivation (8, 9). Different interactions lead to the expression of either pro-inflammatory or anti-inflammatory cytokines (10). Therefore our intention was to analyse the distribution of T-helper 1 (Th1), Th2, Th17, regulatory T-cells (Treg) and Th9 cytokines of the T-lymphocyte immune response and reveal differences in cytokine levels in patients with breast cancer of the SUCCESS study group. We studied this with respect to CTC involvement, histopathological grading, lymph node status, hormone receptor type, TNM classification and survival vs. tumour-associated death.

This article focuses on the expression of the Th1 cytokines: Interferon gamma (IFN-$\gamma$), tumour necrosis factor alpha (TNF-$\alpha$), interleukin 12 (IL-12), IL-1$\alpha$, IL-1$\beta$, IL-2 and IL-18. Th1 cytokines have been presumed to play a major role in phagocytic and intracellular defence (8). Analyses imply that patients with breast cancer present specific Th1 expressions that correlate with TNM stage and lymph node involvement. Higher IFN-$\gamma$ values, for instance, correlated with a positive outcome in patients with breast cancer (11), whereas the presence of the IL-1 system was found to correlate inversely with local sex steroid receptor expression, emphasizing a increased malignant behaviour (12). Moreover, IL-12 is assumed to be one of the most critical cytokines for the induction of Th1 responses (8).

The evaluation of TH1 cytokine profiles as a marker for CTC involvement could benefit assessment of the individual risk of patients at the time of primary diagnosis. Correlation of Th1 cytokines to CTC involvement could offer a new clinical prospective in terms of tumour phenotype in order to enhance individualized treatments.

## Materials and Methods

*SUCCESS study design.* SUCCESS was a prospective, randomized adjuvant study comparing three cycles of fluorouracil–epirubicin–cyclophosphamide (FEC; 500/100/500 mg/m$^2$) followed by three cycles of docetaxel (100 mg/m$^2$) every 3 weeks vs. three cycles of FEC followed by three cycles of gemcitabine (1,000 mg/m$^2$ d1,8) docetaxel (75 mg/m$^2$) every 3 weeks. Following the completion of chemotherapy, the patients were further randomized to receive either 2 or 5 years of bisphosphonate therapy with zoledronate. Hormone receptor-positive women received suitable endocrine treatment.

Eligible patients were defined as women with breast cancer (stages pT1–T4, pN0–N3, M0) who were approved to participate in the phase I SUCCESS study (www.success-studie.de).

The research questions associated with CTC analysis, the blood sampling time points, and the methodology were prospectively designed, and the prognostic value of the CTCs was described as a scientific objective of the study protocol. The study was permitted by 37 German ethical boards (lead ethical board: LMU, Munich) and conducted in agreement with the Declaration of Helsinki.

*Blood sample collection for CTC enumeration.* Blood samples for CTC enumeration were taken from 2,090 consecutive patients after complete resection of the primary tumour and before adjuvant chemotherapy after written informed consent was acquired. Sixty-four patients were excluded because of test failure or a time interval of more than 96 h between the blood collection and sample preparation. A follow-up evaluation after chemotherapy and before the commencement of endocrine or bisphosphonate treatment was available for a subgroup of 1,492 patients (see homepage: http://www.success-studie.de).

The method was conducted as described by the SUCCESS Study group (6). CTCs were investigated using the CellSearch System (Veridex, Raritan, NJ, USA). Peripheral blood was drawn into three CellSave tubes (30 ml), sent at room temperature to the central laboratory at the University of Munich, and examined within 96 hours of collection.

The patient samples were then centrifuged for 10 minutes at $800 \times g$. The plasma was removed, and a dilution buffer was added. This combination was overlaid on 6 ml of Histopaque (Sigma, Steinheim, Germany) and centrifuged for 10 minutes at $400 \times g$. Subsequently, 7.5 ml of this sample enclosing the buffy coat was treated on the CellTracks AutoPrep system using the CellSearch Epithelial Cell Kit (Veridex). After immuno-magnetic enrichment with an antibody to epithelial cell adhesion molecule (EpCAM), the cells were marked with fluorescent anti-cytokeratin (CK8,18,19–phycoerythrin) and anti-CD45 (CD45–allophycocyan) antibodies, and 4,6-diamidino-2-phenyl-indole-dihydrochloride was used to identify the intact cells.

*Patients included.* In this study, 200 patients of the SUCCESS study were included and assigned into two groups: 100 Patients were CTC-positive (group 1) and the other 100 patients were CTC-negative (group 2). These two groups were then framed and investigated accordingly. Patients from the respective groups were then matched into pairs of two according to histopathological grading, lymph node involvement, hormone receptor type, TNM classification and survival vs. tumour associated death. Out of 200 patient samples that were investigated, 160 patients were still alive at the last observation after the end of therapy and 40 patients had died from their tumour during therapy. The groups investigated included 98 patients with tumour graded G2 and 102 patients graded G3. Matching criteria of the 200 patient collective did not allow patients with grade G1. Tumour stage of the anamnestic diagnosis was classified according to the TNM classification, which was conducted accordingly to the WHO system (13). The matching of patients was performed according to the criteria at the time of primary diagnosis. The histopathological grading was classified according to the Bloom and Richardson system classification (14).

*Detection of CTCs and cytokine determination.* The identification and enumeration of CTCs were achieved using CellTracks Analyzer

II. CTCs were defined as nucleated cells lacking CD45 and expressing cytokeratin. All positive samples were assessed by two independent investigators. Samples with a minimum of one CTC per 30 ml of blood were regarded as CTC-positive.

The blood from 84 persons with no clinical evidence of malignant disease was processed blinded and used as a negative control. Four of these negative controls (4.9%) contained cells that fit the definition of epithelial cells and which could be interpreted as CTCs (one control had one, two controls had two, and one control had three epithelial cells). For the measurement of cytokines a commercial enzyme-linked immunosorbent assay (ELISA) was used to screen the blood serum samples for the Th1 cytokines: IFN-γ, TNF-α, IL-12, IL-1α, IL-1β, IL-2 and IL-18. The ELISA kits used were acquired by Meso Scale Discovery® (Rockville, MD, USA). We used anti-species multi-array 96-well plates for the development of a sandwich immunoassay (see Figure 1). The 10 spot Multi Spot plates were pre-coated with capture antibodies on independent and well defined spots that enabled us to immobilize a primary capture antibody against the protein of interest - specific for each cytokine (IFN-γ, TNF-α, IL-12, IL-1α, IL-1β, IL-2 and IL-18). Standards and samples were also added to the appropriate wells. A standard curve was run with each assay. We firstly added the blood serum, calibrator and control then incubated plates at room temperature with shaking for 2 h. After removing excess sample from the well with wash buffer, we added the solution containing the detection (anti-target) antibody conjugated with electrochemiluminescent labels over the course of two incubation periods. During incubation, where time slots differed in each test, the target present in the sample bound to the capture antibody immobilized on the working electrode surface by the anti-species antibody. Recruitment of the labelled detection antibody by the bound target completed the sandwich. After a second shaking and incubation (time differed for each test), wash buffer was used to remove the entire unbound enzymes and an MSD Read Buffer was added to produce the appropriate chemical environment for electrochemiluminescence. We then loaded the plate into an MSD instrument (MESO QuickPlex SQ 120) for analysis where voltage applied to the plate electrodes caused the captured labels to emit light. The instrument measured the intensity of the emitted light to present a quantitative measure of the amount of the protein of interest that was present in the sample (15, 16) (see homepage: www.mesoscale.com).

*Statistical analysis.* Statistical analysis was accomplished using SPSS 22.0 (IBM Corp., Armonk, NY, USA).

We evaluated the relationship between each cytokine of the Th1 group (IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-12 and IL-18) and each matching criterion (CTC-positive *vs.* CTC-negative; survival *vs.* death; grade 2 *vs.* grade 3; lymph node involvement *vs.* no lymph node involvement; triple hormone receptor-positive *vs.* triple hormone receptor-negative; progesterone receptor-positive *vs.* progesterone receptor-negative; oestrogen receptor-positive *vs.* oestrogen receptor-negative; HER2/neu receptor-positive *vs.* HER2/neu receptor-negative) by using the non-parametric Spearman correlation coefficient. Each parameter to be considered was required to have a *p*-value of less than 0.05. Statistically significant results for the Spearman correlation coefficient were then assessed with the non-parametric Mann-Whitney-U rank-sum test. Moreover, variables were examined by the use of box-plot analysis. All statistical tests were considered significant at $p<0.05$.

## Results

*CTC-positive vs. CTC-negative patients.* In the CTC-positive patient group, patients with no lymph node involvement had high levels of IL-1α, whereas those with lymph node involvement expressed low levels of IL-1α (Spearman correlation coefficient *p*=0.042, Mann–Whitney *U*-test *p*=0.043; Figure 1a).

On the other hand, analysis of the CTC-negative collective showed that patients with progesterone receptor-negative disease had higher levels of IL-1β in comparison to those with progesterone receptor-positive disease (Spearman correlation coefficient *p*=0.028, Mann–Whitney *U*-test *p*=0.029; Figure 1b).

However, statistical analysis found no significant difference according to the presence or absence of CTCs in patients with breast cancer in regard to the levels of IFN-γ, TNF-α, IL-2 and IL-18.

*Patient survival.* The box plot analysis of the collective of patients who remained alive revealed that patients with lymph node involvement also had higher levels of IL-12p40. (Spearman correlation coefficient *p*=0.041, Mann–Whitney *U*-test *p*=0.041; Figure 2a). Statistically significant correlations were also proven for patients with triple hormone receptor-negative compared to -positive breast cancer, with the former displaying significantly higher levels of IL-12p40. (Spearman correlation coefficient *p*=0.042, Mann–Whitney *U*-test *p*=0.043; see Figure 2b).

In comparison, in the group of patients who died, patients with oestrogen receptor-negative tumour had higher levels of IL-1α and IL-1β (Spearman correlation coefficient *p*=0.049, Mann–Whitney *U*-test *p*=0.050 for IL-1 α; Spearman correlation coefficient *p*=0.032, Mann–Whitney *U*-test *p*=0.034 for IL-1β; Figure 3b and c respectively). A significant correlation was also found for patients with triple hormone receptor-negative breast cancer having higher levels of IL-1α. (Spearman correlation coefficient *p*=0.031, Mann–Whitney *U*-test *p*=0.033; Figure 3a).

Statistical examination confirmed no significant difference regarding the survival or death of patients with breast cancer in regard to the levels of IFN-γ, TNF-α, IL-2, and IL-18.

*Grading 2 vs. grade 3 tumour.* In the collective with grade 2 tumour, patients with no lymph node involvement had higher levels of IL-1 α (Spearman correlation coefficient *p*=0.013, Mann–Whitney *U*-test *p*=0.014; Figure 4a). Patients with grade 2 HER2/neu-positive breast cancer also had higher levels of IFN-γ (Spearman correlation coefficient *p*=0.030, Mann–Whitney *U*-test *p*=0.031; Figure 4b).

In patients with grade 3 tumours, those with progesterone receptor-negative disease had higher levels
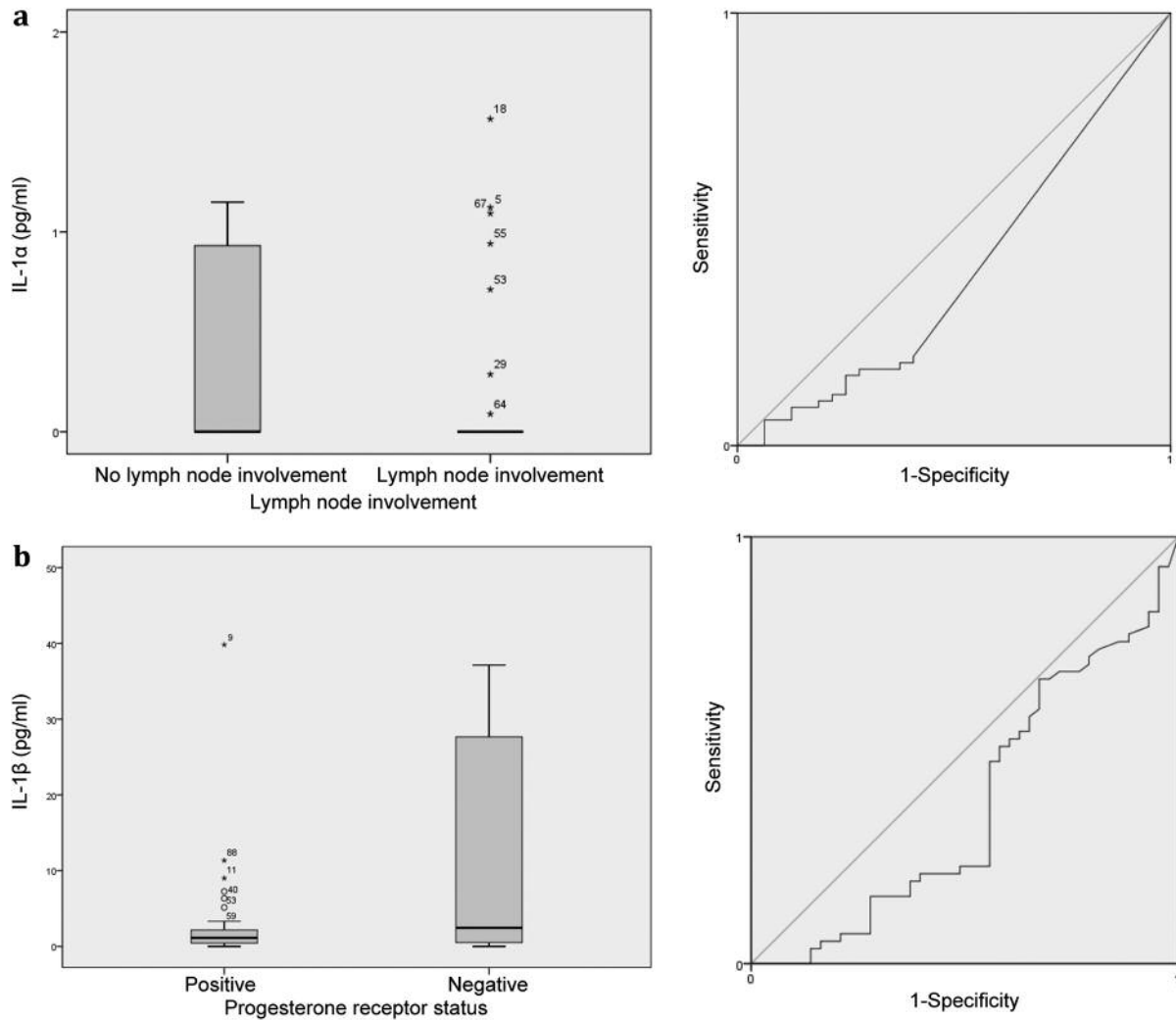
Figure 1. *a: Left panel: Box plot analysis of interleukin 1-alpha (IL-1α) expression in sera of patients with circulating tumour cell (CTC)-positive breast cancer. IL-1α release was significantly higher in patients without lymph node metastasis compared to patients with lymph node involvement (p=0.043). Right panel: Receiver operator curve analysis of sensitivity versus specificity gave an area under the curve (AUC) of 0.402. b: Left panel: Box plot analysis of interleukin 1 beta (IL-1β) expression and patients with CTC-negative disease. IL-1β release was significantly higher in those with progesterone receptor-negative compared to those with progesterone receptor-positive breast cancer (p=0.029). Right panel: Receiver operator curve analysis of sensitivity versus specificity gave an AUC of 0.372. Boxes indicate the 25th and 75th percentiles, with a horizontal line at the median and bars display the 5th and 95th percentiles. Circles specify values more than 1.5 box lengths. Asterisks specify values (marked with a number) more than 3.0 box lengths from the 75th percentile.*

of IL-12p70 (Spearman correlation coefficient $p=0.048$; Mann–Whitney *U*-test $p=0.048$; Figure 5a). Patients with grade 3 tumours had a significantly higher IL-12p40 release in those with triple hormone receptor-positive status compared to those with triple-negative disease (Spearman correlation coefficient $p=0.032$; Mann–Whitney *U*-test $p=0.033$; Figure 5b). None of the other cytokines tested revealed differences in expression patterns in terms of tumour grading.

*Lymph node involvement.* Statistical analysis demonstrated no significant difference concerning lymph node involvement or no lymph node involvement in patients with breast cancer with and without the presence of CTCs in respect to the cytokine levels of IFN-γ, TNF-α, IL-12, IL-1α, IL-1β, IL-2 and IL-18.

*Hormone receptor type presence vs. absence.* Furthermore, the statistical analysis also verified no significant difference regarding the presence or absence of single hormone receptor
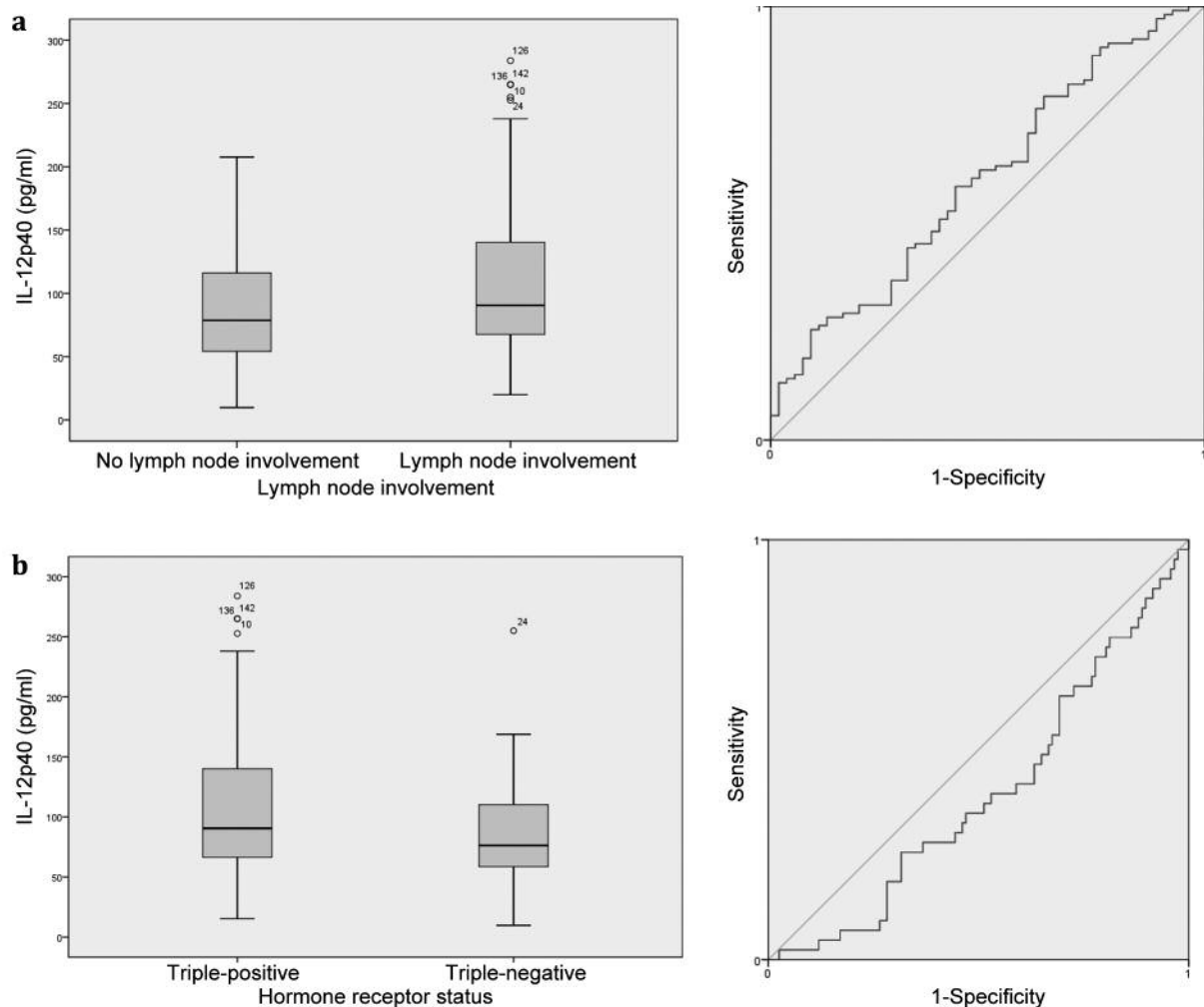
Figure 2. *Box plot analysis of interleukin 12p40 (IL-12p40) expression in sera of patients who remained alive [regardless of circulating tumour cell (CTC) status] according to lymph node involvement (a) and hormone receptor status (b). IL-12p40 release was significantly higher in patients who remained alive and had lymph node metastasis compared to patients without lymph node involvement (p=0.041) (a, left panel), and in patients with triple-positive hormone receptor status compared to those with triple-negative hormone receptor disease (p=0.043) (b, left panel). Receiver operator curve analysis of sensitivity versus specificity gave an area under the curve of 0.599 (a, right panel) and 0.395 (b, right panel) respectively. Boxes indicate the 25th and 75th percentiles, with a horizontal line at the median and bars display the 5th and 95th percentiles. Circles specify values more than 1.5 box lengths. Asterisks specify values (marked with a number) more than 3.0 box lengths from the 75th percentile.*

types (progesterone receptor, oestrogen receptor, HER2/neu) in patients with breast cancer with and without the presence of CTCs in regards to the levels of IFN-γ, TNF-α, IL-12, IL-1α, IL-1β, IL-2 and IL-18.

*Triple positive vs. triple negative*. No statistically significant correlation was demonstrated with the general comparison of triple-negative to triple-positive breast cancer with and without the presence of CTCs in respect to the cytokine levels of IFN-γ, TNF-α, IL-12, IL-1α, IL-1β, IL-2 and IL-18.

## Discussion

In this study, we described Th1-derived cytokines in the sera of patients with breast cancer with and without CTCs. The group of patients diagnosed with CTCs and no lymph node involvement had significantly enhanced IL-1α levels. IL-1α is a Th1 cytokine derived from tumour cells. It is hypothesized that cell senescence increases IL-1α expression and creates a microenvironment that is conducive to metastatic disease progression in patients with cancer (17). Therefore IL-1α might be involved in
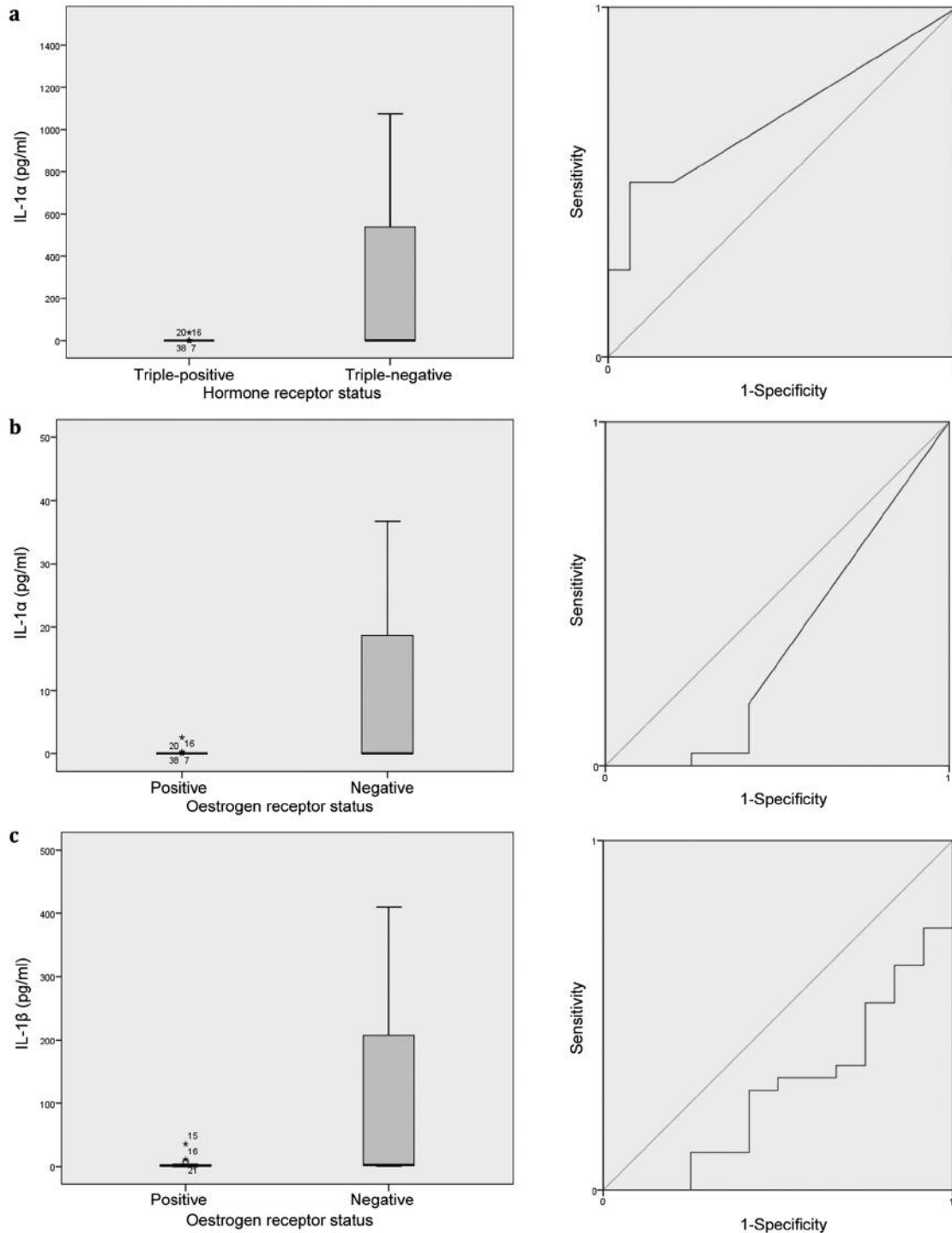
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Figure 3. *a: Box plot analysis of interleukin 1-alpha (IL-1α) expression in sera of patients who died from their disease [regardless of circulating tumour cell (CTC) status] according to triple hormone receptor status (a) and oestrogen receptor status (b) and of IL-1β according to oestrogen receptor status (c). Significantly higher IL-1α release was found in patients who died with triple-negative hormone receptor status compared to patients with triple-positive hormone receptor (p=0.031) (a, left panel), and in those with oestrogen receptor-negative compared to those with oestrogen receptor-positive cancer (p=0.049) (b, left panel). Similarly, we identified significantly increased IL-1β release in those with oestrogen receptor-negative compared to those with oestrogen receptor-positive cancer (p=0.034) (c, left panel). Receiver operator curve analysis of sensitivity versus specificity gave an area under the curve of 0.688, 0.350 and 0.286 (right panel, a, b and c, respectively). Boxes indicate the 25th and 75th percentiles, with a horizontal line at the median and bars display the 5th and 95th percentiles. Circles specify values more than 1.5 box lengths. Asterisks specify values (marked with a number) more than 3.0 box lengths from the 75th percentile.*
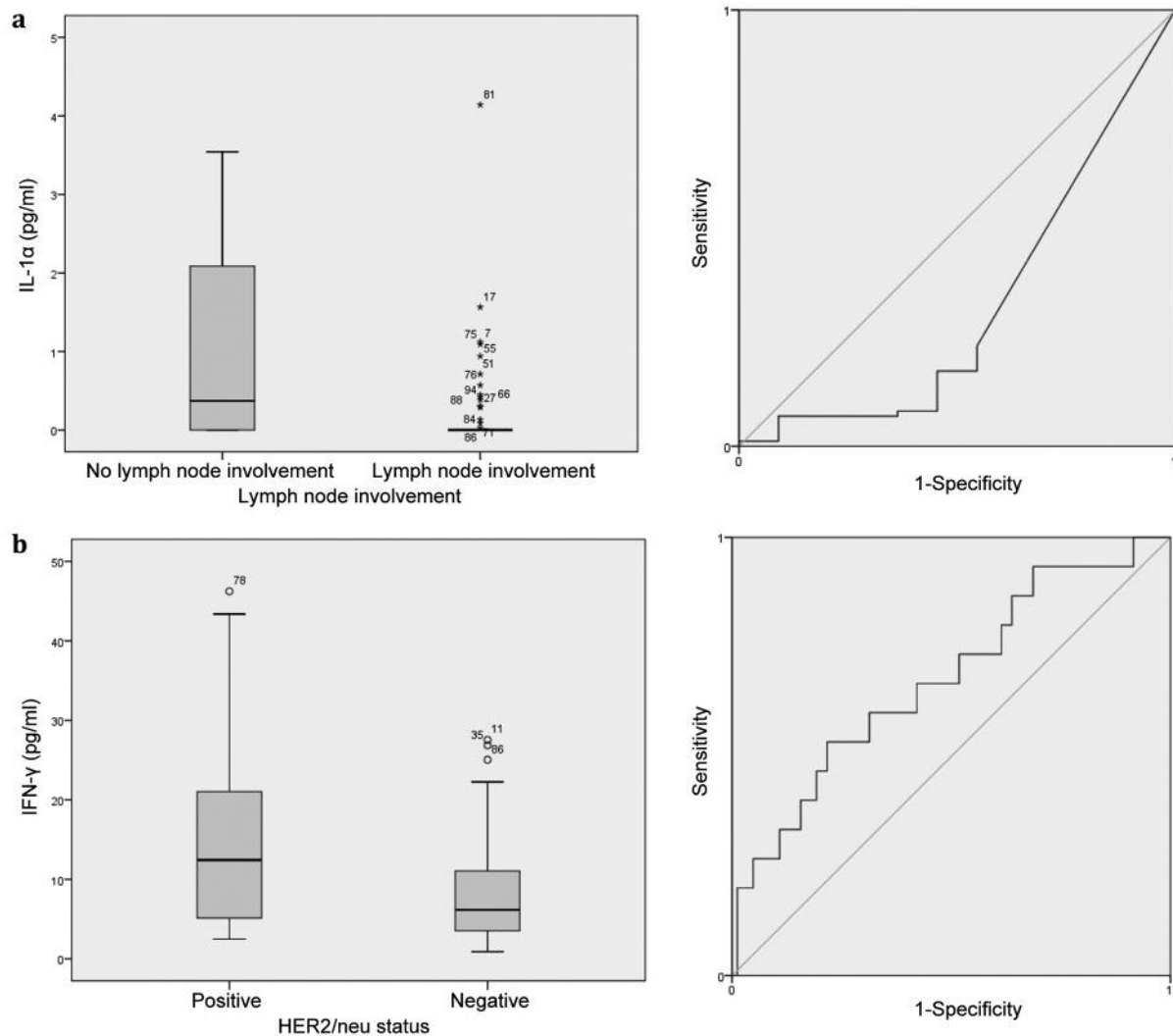
Figure 4. *Box plot analysis of interleukin 1-alpha (IL-1α) (a) and interferon gamma (IFN-γ) (b) expression in sera of patients with grade 2 (G2) tumour [regardless of circulating tumour cell (CTC) status]. IL-1α release was significantly higher in the G2 patient collective with no lymph node metastasis compared to patients with lymph node involvement (p=0.013) (a, left panel). Significantly enhanced IFN-γ release was shown in the G2 patient collective with HER2/neu receptor-positive compared to HER2/neu receptor-negative breast cancer (p=0.030) (b, left panel). Boxes indicate the 25th and 75th percentiles, with a horizontal line at the median and bars display the 5th and 95th percentiles. Circles specify values more than 1.5 box lengths. Asterisks specify values (marked with a number) more than 3.0 box lengths from the 75th percentile. Receiver operator curve analysis of sensitivity versus specificity gave an area under the curve of 0.322 (a, right panel) and 0.676 (b, right panel), respectively.*

circulation-specific metastasis and not metastasis *via* the lymphatic system. This hypothesis is furthermore supported by increased levels of IL-1α in patients with grade 2 tumours and no lymph node involvement. These findings identify IL-1α as a crucial factor for the ability of senescent cells to generate a tissue microenvironment that stimulates cancer expansion (18). The abnormal increased expression of IL-1α in less differentiated breast cancer cells with no lymph metastasis might be responsible for local invasiveness and malignant behaviour.

Significantly enhanced IL-1α levels were also found in patients with triple hormone receptor-negative breast cancer who died from their disease. Furthermore, increased IL-1α and IL-1β levels were shown for patients who died from oestrogen receptor-negative breast cancer. It is conjectured that IL-1α expressed on malignant cells stimulates antitumour immunity, while IL-1β that originates from the microenvironment or malignant breast cancer cells activates inflammation that increases invasiveness and induces tumour suppression (12, 19, 20). Therefore, one can suggest that
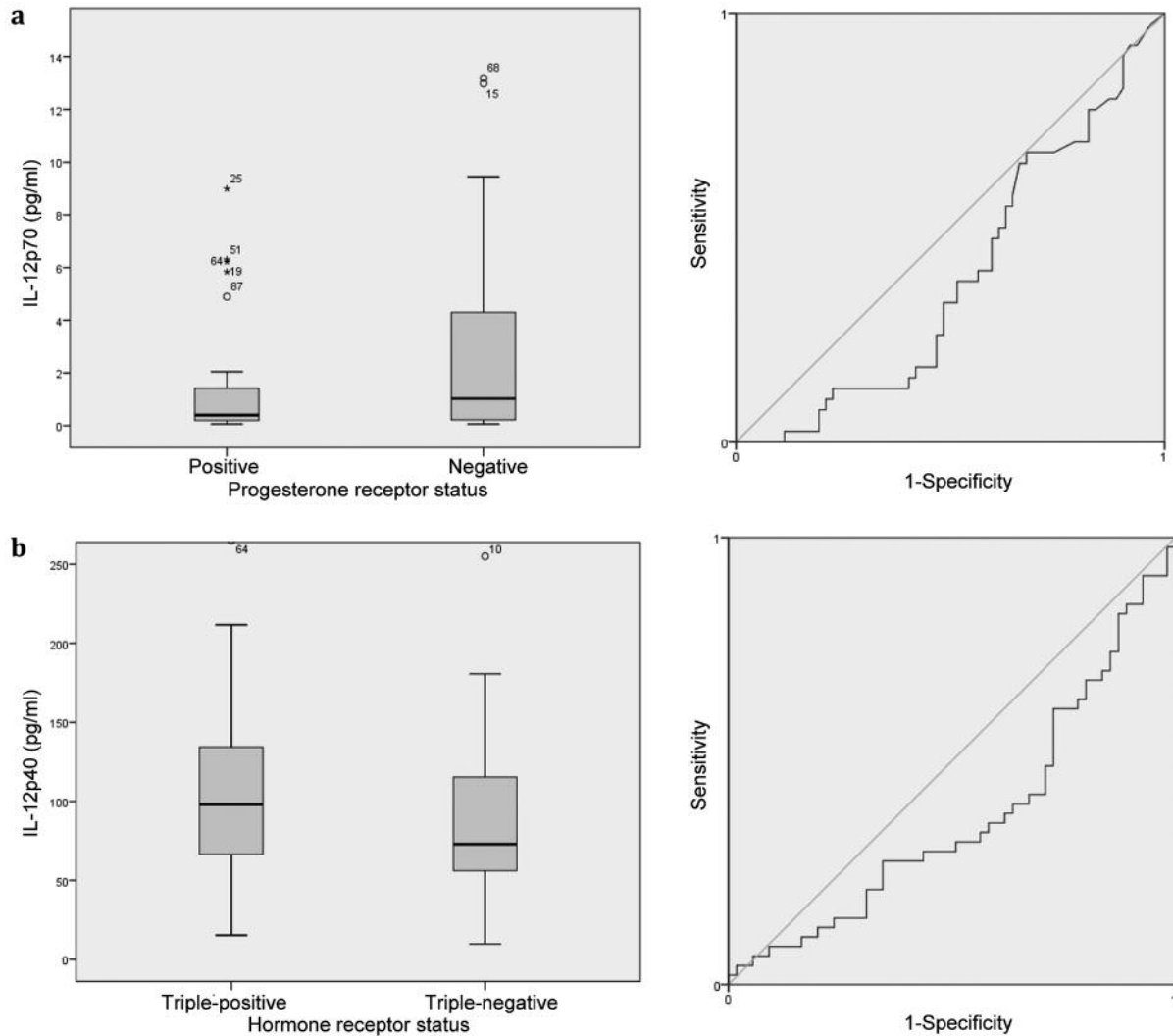
Figure 5. *a: Box plot analysis of interleukin 12p70 (IL-12p70) (a) and IL-12p40 expression in sera of patients with grade 3 (G3) tumour (regardless of circulating tumour cell (CTC) status). IL-12p70 release was significantly higher in patients with progesterone receptor-negative compared to those with progesterone receptor-positive breast cancer (p=0.048) (a, left panel). IL-12p40 release was significantly higher in those with triple-positive compared to patients with triple-negative breast cancer (p=0.032) (b, left panel). Boxes indicate the 25th and 75th percentiles, with a horizontal line at the median and bars display the 5th and 95th percentiles. Circles specify values more than 1.5 box lengths. Asterisks specify values (marked with a number) more than 3.0 box lengths from the 75th percentile. Receiver operator curve analysis of sensitivity versus specificity gave an area under the curve of 0.384 (a, right panel) and 0.377 (b, right panel), respectively.*

increased expression of IL-1α and IL-1β in patients with a triple-negative hormone status who died, especially in terms of negative oestrogen receptor status, is potentially responsible for the severity of tumour malignancy and local invasiveness, ultimately resulting in the death of those patients. We can consequently also infer that the manipulation of IL-1α and IL-1β in malignant cells or in the tumour microenvironment could offer new approaches in terms of cancer therapy for patients with triple-negative breast cancer. Furthermore, significantly enhanced IL-1β

expression in patients diagnosed as having progesterone receptor-negative breast cancer not having CTCs might indicate the involvement of IL-1β in local invasiveness rather than circulation, leading to less favourable outcome.

Regardless of CTC status, in patients who survived, we identified significantly enhanced IL-12p40 release in combination with a triple-positive hormone receptor status and lymph node metastasis. It is hypothesized that IL-12 has an anti-angiogenetic effect and the ability to induce a T-cell-based antitumour immune response capable of eliminating

disseminated cancer cells (21, 22). It has already been implied that intratumoural treatment of patients with breast cancer with IL-12 in combination with other cytokines leads to infiltration by polymorphonuclear cells, dendritic cell antigen presentation and CD8^{+} T-cells, with consequent tumour regression. Furthermore, it has been shown that local IL-12 therapy stimulates specific antitumor T-cells in lymph nodes resulting in a memory immune response (23), and is even capable of eradicating disseminated tumour cells (20, 24). An enhanced IL-12p40 level in this collective of survivors with triple-positive hormone receptor status therefore indicates a natural advantage, most probably responsible for local apoptosis, eradicating distant disease and thus enhancing survival. However, in our study, patients with lymph node metastasis, indicating a highly malignant phenotype, nevertheless survived. The increased IL-12p40 expression in these patients emphasises antitumour immune response in distant metastatic lesions in lymph nodes, increasing a favourable outcome. Moreover, we identified significantly enhanced IL-12p40 release in the patient collective with grade 3 triple-positive breast cancer. A pattern of increased IL-12p40 expression together with a significant increase in the total number of lymphocytes, CD4+, CD8+, natural killer cells and C-reactive protein has been described to be of clinical benefit, as opposed to progression, in hormone-responsive metastatic breast cancer (25). One can suppose an association exists between triple-positive breast cancer and enhanced IL-12p40 expression, furthermore responsible for counteracting the malignant invasiveness of a poorly differentiated tumour. Moreover, the significantly enhanced IL-12p70 release in patients with grade 3 progesterone receptor-negative disease suggests a direct association between IL-12p70 and the absence of progesterone receptor in poorly differentiated tumours.

Patients with grade 2 HER2/neu receptor-positive breast cancer, regardless of CTC status, had a significantly higher IFN-γ expression. IFN-γ is purportedly responsible for promoting apoptosis by enhancing cytotoxic T-lymphocyte activity and influencing p53 expression, associated with a protective role against cancer (11, 26). It is furthermore presumed that IFN-γ genetic polymorphisms might even be significantly related to a higher risk of breast cancer (27). Therefore, significantly enhanced IFN-γ expression in patients with less differentiated (grade 2) and HER2/neu receptor-positive breast cancer may enhance tumour apoptosis, resulting in a more favourable outcome.

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