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# Clinical Significance of Circulating Interleukin-23 as a Prognostic Factor in Breast Cancer Patients

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## ABSTRACT

Little is known about specific IL-23 alterations associated with breast cancer and the data available are still controversial. Therefore, the evaluation of changes in serum IL-23 levels may add further information on the role of this cytokine in breast cancer patients. The aim of this study was to evaluate prospectively the prognostic importance of circulating IL-23 in patients with untreated breast cancer, respect to healthy controls, and the association with clinico-pathological variables. The study involved 50 women diagnosed with stages I–IV breast cancer and 38 healthy controls. Of the 50 breast cancer patients, 37 women were recruited prior to their initial adjuvant chemotherapy and 13 prior to receive first line chemotherapy for metastatic disease. Adjuvant chemotherapy patients were at least in their 4th week post-surgery. IL-23 serum concentrations were measured by a quantitative enzyme immunoassay technique. We found a statistically significant higher systemic cytokine value in women with cancer in comparison with the control group ($14.52 \pm 11.39$ pg/ml vs. $6.35 \pm 4.63$ pg/ml, $P < 0.0001$). Patients with shorter overall survival presented higher IL-23 values, suggesting a negative prognostic correlation. There was no significant differences in IL-23 levels among patients according to the biomolecular characteristics, the different subtypes and the presence of metastatic disease. This work investigated, for the first time, the role of IL-23 in breast cancer patients showing a significant increase respect the control group. However, further validations are needed in larger studies to better investigate the implications of IL-23 increase in these patients. J. Cell. Biochem. 113: 2122–2125, 2012. 

**KEY WORDS:** INTERLEUKIN-23; BREAST CANCER; Th17 CELLS

The variety of biological effects of cytokines and their key role as intercellular mediators by regulating survival, growth, differentiation, and the effector functions of cells, suggest their participation in many pathologic processes, including malignant neoplasms.

It is well accepted that the formation of breast tumors relies not only on oncogenic changes within the epithelial cells, but also on interactions between the tumor cells and the stromal environment.

Inflammation within the tumor microenvironment correlates with increased invasiveness and poor prognosis in many types of cancer, including breast cancer [Goldberg and Schwertfeger, 2010]. Moreover, a growing body of clinical and experimental evidences indicate that the outcome of an immune response toward an evolving breast neoplasm is largely determined by the type of immune response elicited [De Nardo and Coussens, 2007].

In recent decades much attention has focused on the understanding of the role of cytokines in breast cancer. Some cytokines (IL-1, IL-6, IL-11, and TGFβ) stimulate breast cancer proliferation and/or invasion while others (IL-12, IL-18, and IFNs) modulate anti-tumor response. Similarly, high circulating levels of some cytokines seem to be favorable (solubleIL-2R) while others are unfavorable (IL-1b,

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IL-6, IL-8, IL-10, IL-18, and gp130) prognostic indicators [Nicolini et al., 2006].

In addition to Th1 and Th2, various subsets of T cells with regulatory activities have been identified. More recently a subset named Th17 has been defined, involved in the resistance to fungal pathogens, mucosal immunity, several autoimmune diseases, and chronic inflammatory syndromes [Lyakh et al., 2008].

Although several studies have shown the presence of Th17 cells in some types of human cancers and recent evidences suggest that tumor-infiltrating Th17 cells may be a general feature in cancer patients, little is known regarding their generation and regulation within the tumor microenvironment [Su et al., 2010]. This effector T cell subset appears to be also involved in tumor immunology, but plays dual roles in promoting or discouraging cancer development [Zamarron and Chen, 2011].

A range of cytokines, including TGFβ, IL-6, IL-21, IL-23, and IL-1β, have been shown to participate in the generation of Th17 cells [Spolski and Leonard, 2009].

Interleukin-23 (IL-23) has been identified as key factor for maintaining and expanding Th17 inflammatory cell populations [Zamarron and Chen, 2011]. It has a pro-carcinogenic activity, promoting inflammation and angiogenesis within the tumor microenvironment while reducing CD8+ T cell infiltration [Langowski et al., 2006].

IL-23 is composed of the p40 subunit of IL-12 and a p19 subunit structurally related to the p35 subunit of IL-12 and is primary secreted by activated dendritic cells and macrophages. However, p19 subunit mRNA is expressed in numerous tissues [Oppmann et al., 2000]. Although IL-23 shares some biological activities with IL-12 [Oppmann et al., 2000], its physiological role today is considered to only expand and maintain the inflammatory Th17 cells [Xu et al., 2010].

Expression of IL-23 is increased in human tumors [Langowski et al., 2006]. The endogenous IL-23 expression has been reported to promote tumor development and growth, through STAT3 activation by inducing inflammatory responses including IL-17 production [Xu et al., 2010]. However, the studies using local and systemic administration of IL-23 have shown that its application at the excessive amount induces antitumor immune responses [Kaiga et al., 2007].

Available data on the role of Th17 cells in breast cancer are still controversial.

It has been evidenced that murine breast cancer cells express functional receptor for IL-22, a Th17 cytokine; moreover, IL-22 inhibits mammary adenocarcinoma EMT6 cell proliferation in vitro and in vivo, by inhibiting signaling pathways such as ERK1/2 and AKT phosphorylation [Weber et al., 2006].

Another study has documented circulating Th17 cells in breast cancer patients and has shown a significant lower number of Th17 cells in peripheral blood of HER-2 positive breast cancer patients respect to healthy controls and HER-2 negative patients [Horlock et al., 2009].

The significant activity of IL-23 on Th17 cells and the recent evidences for its involvement in tumor development and progression have been widely documented. Little is known about specific IL-23 alterations associated with breast cancer. Therefore, the measurement of changes in serum IL-23 levels and the correlation with biological and clinical features may add further information on the role of this cytokine in breast cancer patients.

Accordingly, our study was designed to evaluate prospectively, respect to healthy controls, the independent prognostic importance of circulating IL-23 in patients with untreated breast cancer and the potential association with clinico-pathological variables.

## MATERIAL AND METHODS

### PATIENTS

The study population consisted of 50 consecutive unselected patients with histologically proven and previously untreated stages I–IV breast cancer diagnosed from December 2007 to June 2009 and followed through 2010 and 38 healthy controls.

The following clinico-pathological variables were entered in a database: age (≥18 years), ECOG performance status, menopausal status, histological type, grading, tumor size, node involvement, site of metastases, stage, and molecular characteristics including hormone receptors and HER2 status, Ki67 or MIB-1. Staging was expressed according to the Seventh Edition AJCC TNM Classification. Median age was 57.7 (range 31–85).

Of 50 women with breast cancer included into the study, 37 women were enrolled prior to their initial adjuvant chemotherapy and 13 prior to receive first line chemotherapy for metastatic disease. Adjuvant chemotherapy patients were at least in their fourth week post-surgery.

The most frequent sites of disease in metastatic patients were as follows: lung 31%, liver 23%, bone 23%, lymph nodes 15%, and brain 8%.

According to biomolecular characteristics 28 patients were Luminal A (ER positive, PgR positive, and HER2 negative), 10 patients Luminal B (ER positive, PgR positive, and HER2 positive), 10 patients Triple negative (ER negative, PgR negative, and HER2 negative) and 2 patients HER2 positive (ER negative, PgR negative, and HER2 positive). The different subtypes were defined as: Luminal A (ER and PgR cell staining of either ≥10% by IHC and HER2 negative staining of <3+ by IHC or 2+ staining with no gene amplification by FISH), Luminal B (ER and PgR cell staining of either ≥10% by IHC and HER2 staining 3+ by IHC or 2+ staining with gene amplification by FISH), Triple negative (ER and PgR cell staining of either <1% or <10% by IHC with a range from 0 to 9% and HER2 negative staining of <3+ by IHC or 2+ staining with no gene amplification by FISH) and HER2 positive (ER and PgR cell staining of either <1% or <10% by IHC with a range from 0 to 9% and HER2 staining of 3+ by IHC or 2+ staining with gene amplification by FISH) [Prat and Perou, 2011].

The most common chemotherapy used was anthracycline and taxanes-based regimen, whether in adjuvant or in metastatic setting according to current guidelines for breast cancer treatment.

The clinical and pathological characteristics of the patients are summarized in Table I.

### BLOOD MEASUREMENTS

Venous blood was sampled at the moment of diagnosis before the initiation of systemic therapy and was collected after a overnight

TABLE I. Clinical–Pathological Characteristics

| | |
|---|---|
| No patients | 50 |
| Median age | 57.7 (range 31–85) |
| Adjuvant stage | 37 |
| Metastatic disease | 13 |
| Main metastatic sites | 31% Lung, 23% Liver, 23% Bone, 8% Brain, 15% Lymph nodes |
| Luminal A | 28 |
| Luminal B | 10 |
| Triple Negative | 10 |
| HER2 positive | 2 |

fast. Serum obtained from peripheral blood was allowed to clot at room temperature for 2 h, separated by centrifugation at $1,000 \times g$ for 15 min and stored at $-20^{\circ}C$ until use.

Thirty-eight sex and age-matched healthy donors were recruited as controls. Each subject gave a written informed consent to the study, which was approved by the local ethical committee.

### ASSAY METHOD

IL-23 serum concentrations were measured by a quantitative enzyme immunoassay technique. The assay was performed by using a commercially available kit (R&D System Europe, Abingdon, UK); a microplate reader capable of measuring absorbance at 450 nm (correction wavelength set at 540 nm) was used to measure the intensity of color developed in each well. The minimum detectable dose was 6.8 pg/ml.

### STATISTICAL ANALYSIS METHODS

The statistical analysis was performed with MedCalc (version 7.3.0.1). Data were presented as mean ± SD. Differences between data series were analyzed by the Mann–Whitney test.

Receiver operating characteristics (ROC) analysis was employed to calculate the area under the curve (AUC) for IL-23 and to find the best cut-off value for the identification of the progression to end-point. Kaplan–Meier curves were generated to assess survival in subjects with IL-23 values above and below the optimal cut-off. Statistical significance was set at $P < 0.05$.

## RESULTS

IL-23 levels in patients were significantly higher than those in controls ($14.522 \pm 11.39$ vs. $6.34 \pm 4.63$ pg/m; $P < 0.0001$ (Fig. 1).

There was no significant differences in IL-23 levels among patients according to the biomolecular characteristics as hormone receptors status and HER2 expression, the different subtypes (Luminal A, Luminal B, Triple negative, HER2 positive) and the presence of metastatic disease.

Patients with shorter overall survival presented higher IL-23 values, suggesting a negative prognostic correlation. ROC analyses were therefore undertaken in order to define the diagnostic profile of IL-23, considering death as status variable. Results showed an AUC of 0.714, whereas the best IL-23 cut-off value was 9.073 pg/ml with a sensitivity of 62% and a specificity of 86.8%.

Kaplan–Meier survival curves in patients with IL-23 levels above and below the optimal cut-off are presented in Figure 2. In subjects with IL-23 values above 9.073 pg/ml, the mortality was increased ($P = 0.034$; Log-Rank Test).

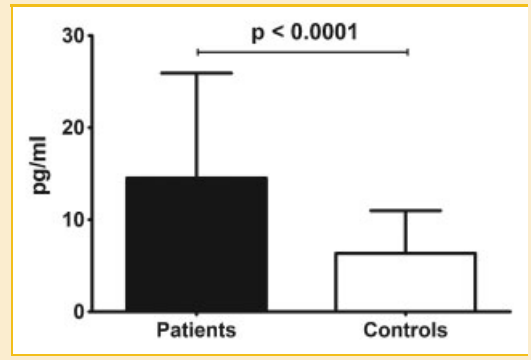


Fig. 1. Bars represent means ± standard deviations. IL-23 levels in patients were significantly higher than those in controls. Differences were assessed using Mann–Whitney test.

## DISCUSSION

Despite several studies have confirmed the important role played by Th17 cells in the pathogenesis of human chronic inflammatory and autoimmune diseases and recent studies have shown the presence of these cells in human tumors, their functional role in cancer growth and progression is still unknown. IL-23, a heterodimeric cytokine discovered in 2000, is involved in the induction of Th17 cells [Lyakh et al., 2008].

The levels of Th17 cells were found significantly increased also in peripheral blood of patients with advanced ovarian carcinoma and in the tumor tissues of patients with advanced ovarian, pancreatic, and renal cell carcinoma [Kryczek et al., 2007].

Increased number of Th17 cells was also observed in tumor-draining lymph nodes of patients with advanced gastric cancer. Furthermore, in these patients the serum concentrations of IL-17 and IL-23 cytokines were significantly elevated [Zhang et al., 2008].

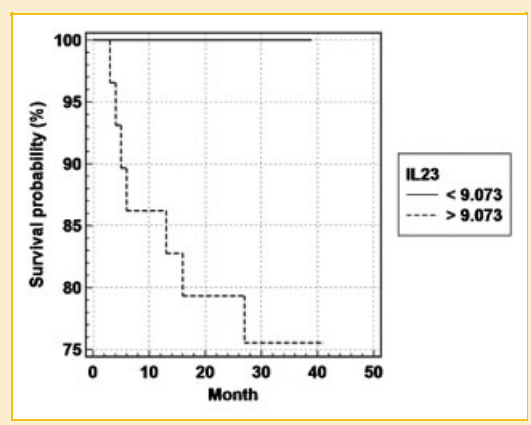


Fig. 2. Kaplan–Meier survival curves in patients with IL-23 levels above and below the optimal cut-off (9.073 pg/ml). In subjects with IL-23 values above the optimal cut-off mortality increases ($P = 0.034$; Log-Rank Test).

These data suggest that the development of tumor-infiltrating Th17 cells is a general feature of cancer.

Since Th17 cells are expanded and maintained by IL-23, this cytokine could be an indirect marker of Th17 cells presence.

We previously demonstrated higher IL-23 serum levels in patients affected by colorectal cancer respect to healthy controls; the increased cytokine levels did not correlate with the severity of disease, tumor removal, and chemotherapeutic treatment [Adamo et al., 2011].

To our knowledge no data are present in literature about the involvement of serum IL-23 in breast cancer. This work investigated, for the first time, the role of IL-23 in breast cancer showing significant higher serum levels in patients with breast cancer respect to healthy controls.

Because these patients were prospectively enrolled at the time of diagnosis and had not received any form of medical treatment, no confounding variables for serum IL-23 can be discerned.

The study was not designed to evaluate the clinical usefulness of IL-23 as a tumor marker. We focused mainly on the prognostic importance of IL-23 and provide biological reasons on how IL-23 might promote tumor growth.

Our data indicate, for the first time, that measurement of IL-23 may be of important prognostic value in the assessment of survival. In fact, we have observed a higher mortality in patients with more elevated levels of IL-23. Even if the exact relationship between disease progression and cytokine values is far from being completely understood, it is noteworthy that we detected different outcomes correlated to different IL-23 levels and the Kaplan–Meier curves suggest a trend.

This study has been limited by small sample sizes and the different stages of disease (37 patients in the adjuvant setting and 13 with metastatic breast cancer) as we have enrolled unselected consecutive patients. Consequently, significant differences were not recorded in IL-23 levels in correlation to the presence of metastatic disease.

Further validations are needed in larger studies to better investigate the clinical implications of IL-23 serum levels increase and to define the correlation with Th17 cells number in blood and/or tumor tissues. Moreover, these findings highlight the need for further research to examine the levels and patterns of cytokines and their implications as biomarkers in breast cancer patients.

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