# Interleukin-6 and interleukin-8 serum levels in prognosis of hormone-dependent breast cancer

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## ARTICLE INFO

## ABSTRACT

*Background:* Increasing amount of evidence points to the importance of immunity in breast cancer. The prognostic value of cytokines and their effect on tumorigenesis remains inconsistent.

*Aim:* To investigate the prognostic significance of IL6 and IL8 and their association with ER and HER2 in estrogen-dependent (ER +) breast cancer.

*Material and methods:* The study included 79 premenopausal women with early and locally advanced ER+ breast cancer. All patients received adjuvant hormonal therapy: tamoxifen alone (56/79) or combination with LHRH agonist goserelin (23/79). IL6 and IL8 serum protein levels were measured by ELISA. Cox proportional hazards regression analysis was implemented for prognostic evaluation of the data categorized based on metastasis outcome.

*Results:* IL6 associated with good ($P = 0.001$, $HR = 0.05$) and IL8 with poor disease outcome ($P = 0.03$, $HR = 2.5$) in the whole group of patients. Multivariate analyses highlighted IL6 as the independent prognostic factor ($P = 0.001$, $HR = 0.0007$). When patients were classified according to ER or HER2 status, IL6 did not have prognostic significance in $ER^{low}$ and $ER^{high}$ subgroups, while IL8 retained prognostic significance only in the $ER^{high}$ subgroup ($P = 0.04$, $HR = 2.8$). IL6 was significant in both HER2− ($P = 0.001$, $HR = 0.05$) and HER2+ subgroups ($P = 0.002$, $HR = 0.04$), while IL8 retained its prognostic significance only in the HER2+ subgroup ($P = 0.001$, $HR = 77.8$).

*Conclusions:* This study contributes to the clarification of the prognostic performance of IL6 and IL8 by providing their first prognostic evaluation in the homogenized ER+ breast cancer patient group. IL6 was indicated as a marker of favorable, whereas IL8 was a marker of unfavorable disease outcome.

## 1. Introduction

Inflammatory cytokines are known to affect tumor growth and have thus been intensively studied as potential prognostic biomarkers in different types of human cancers. IL6 is a pleiotropic cytokine with both pro- and anti-inflammatory properties. It is produced by T and B lymphocytes, monocytes, fibroblasts, endothelial and malignant cells in response to a variety of proinflammatory stimuli such as IL1 and TNF [1]. During acute inflammation, IL6 exerts antiinflammatory effects [2], while in chronic inflammation its actions are proinflammatory [2]. According to Scheller, proinflammatory activities of IL6 are mediated by *trans*-signaling whereas antiinflammatory activities are mediated by classical signaling [3].

IL8 is a pleiotropic proinflammatory cytokine, a potent chemoattractant and activator of neutrophils, monocytes and other immune cells. It is produced by neutrophils, monocytes/macrophages, endothelial and malignant cells in response to a variety of stimuli such as IL1 and TNF, chemical and environmental stressors (chemotherapy agents, hypoxia) and steroid hormones (androgens, estrogens) [4,5]. Literature indicates direct tumor-promoting mitogenic and angiogenic effects of IL8 [6] and indirect effects through chemotactic infiltration and activation of immune cells that secrete growth and angiogenic factors [4].

Hormonal therapy is still the most effective adjuvant treatment for estrogen receptor-positive (ER +) breast cancer patients [7]. Regulatory links between IL6, IL8 and steroid hormones have been thoroughly investigated in breast cancer. The recent study found IL6 to be a major factor in the biology of ER + breast cancer, whereby high serum IL6 levels associated with poor prognosis [8]. According to literature, IL6 is produced by ER − (but not by ER +) breast cancer cells and inhibits the

growth of ER + cells whereas ER − cells are mostly unresponsive [2,9]. IL6 can also increase estrogen levels, both systemically and in breast tumor tissue [2,10]. Concerning IL8, many studies have indicated its high levels in breast carcinoma subtypes with distinctly poor prognosis, such as basal-like (ER–) and HER2-enriched (HER2 + ) [11–13]. Estradiol increases IL8 secretion in normal breast tissue and ER + PR + breast cancer *in vitro* and *in vivo* [14].

The aim of this study was to evaluate the prognostic performance of IL6 and IL8, the cytokines with strong regulatory links with the steroid hormones, for the first time in the patient group with hormone-dependent (ER + PR + ) breast cancer. Moreover, the associations of these cytokines with the established breast cancer parameters such as ER and HER2 were also examined.

## 2. Material and methods

### 2.1. Patient group

The study included 79 premenopausal women diagnosed with early and locally advanced hormone receptor-positive breast cancer. All patients received adjuvant hormonal therapy based on ER score ≥ 3 or/ and progesteron receptor (PR) score ≥ 3. Endocrine therapy consisted of tamoxifen alone (56/79) or combination with luteinizing hormone-releasing hormone (LHRH) agonist goserelin (23/79). Taken together, the selection criteria were: locally advanced breast cancer, hormone receptor positivity, administration of hormone therapy and premenopausal status. Surgical removal of a primary tumor was performed on all patients at the Institute of Oncology and Radiology of Serbia, Belgrade. After surgery, histological specimens were examined and classified according to the criteria of AJCC/UICC (American Joint Committee on Cancer/Union International Contre le Cancer) for TNM stage, histological type, tumor grade and receptor status.

This research was approved by the Ethics Committee of the Institute of Oncology and Radiology of Serbia. This study was retrospective, and the course of disease was followed from a tumor-removal surgery up until a distant metastasis occurrence. The follow up ranged from 67 to 135 months with a median of 85 months. HER2 amplification status was based on the criteria of 3+ or 2+/CISH+ scores.

The prospective sample size calculation was based on the pilot experiment with 31 patients. The sample size calculation parameters were: target power of 0.8, the effect size by hazard ratio (HR) of 4, alpha 0.05, variability in standard deviations (SD) of 0.62 and the event rate of 25%. Variability was calculated for each feature as a distance expressed in standard deviations between average values of the two groups stratified according to an actual metastasis outcome. The required numbers were 43 patients with 11 events. The actual average SD distance between groups with and without metastasis was 0.64, the event rate was 25% and average effect size for cytokines was 11.3, resulting in the actual power of 1.0. The actual sample size was 79 patients with 20 events. Calculations were performed by the *stpower cox*, a two-sided test (Stata/MP 13 software, StataCorp, College Station, TX).

Hormone receptor status was determined on formalin-fixed paraffin-embedded tumor tissue sections by IHC. HER2 status (absence or presence of gene amplification) was determined on formalin-fixed paraffin-embedded tumor tissue sections by IHC and chromogenic *in situ* hybridization (CISH). All HER2 + patients received herceptin.

### 2.2. Serum collection

Five milliliters of peripheral blood were taken from all patients postoperatively. Blood samples were centrifuged at 950*g* for 10 min and serums were stored at − 20 °C, in agreement with the manufacturer's instructions for hormonal analyses (estradiol, FSH and LH).

### 2.3. Hormone measurement

Follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol levels were measured by ELISA from serum samples according to the manufacturer's instructions (Human Diagnostics GmbH, Wiesbaden, Germany).

### 2.4. IL6 and IL8 measurement

IL6 and IL8 levels were determined by ELISA from serum samples according to the manufacturer's instructions (Quantikine Human IL6 and Quantikine Human IL8 Immunoassay kits, R&D Systems, Minneapolis, MN).

### 2.5. Prognostic performance evaluation

Categorization of the continuous values measured in serum was achieved by use of the outcome-oriented approach. The log-rank test was employed for the outcome-oriented optimal cutpoint selection with the minimal *P*-value by use of the X-tile 3.6.1 software from Yale University, New Haven, CT [15]. Univariate Cox proportional hazards regression test was performed for comparison of the prognosticated and actual metastasis outcomes. The hazard ratio (HR) designates the effect size by Cox regression, corresponding to metastasis rates in high- and low-risk groups of patients (SPSS version 23, Chicago, IL). Multivariate Cox proportional hazards regression analysis was performed to test for independence of each prognostic factor. Variables categorized by outcome were added to a full model using forward selection entry criterion of *P* < 0.20 in univariate analysis, and removed using backwards elimination per selection stay criterion of P < 0.05 (SPSS).

### 2.6. Validation strategies

The confidence intervals (95%CI) of the obtained HRs and *P*-values were corrected for bias by use of the bootstrap internal validation (SPSS or Stata/MP13, StataCorp, College Station, TX).

## 3. Results

The risk of metastasis occurrence prognosticated by IL6 and IL8 serum concentrations was compared with the retrospectively recorded actual metastasis occurrence in each patient by use of the Cox proportional hazards regression statistical evaluation. This procedure has provided an insight into the prognostic value of IL6 and IL8 in breast cancer.

IL6 and IL8 median serum concentrations were indicative of their possible association with the actual metastasis outcome, as these values differed consistently between patient groups with metastasis and without metastasis (Fig. 1). As there were only several metastatic events at each location (Table 1), statistical analysis on the data presented in Fig. 1 was not performed. Medians for IL6 were 1.3, 1.7, 1.1, 1.5 and 2.1 for the patients with metastasis on bones, brain, liver, lungs and without metastasis, respectively. The respective medians for IL8 were 4.2, 4.2, 26.0, 7.8 and 1.3 (Fig. 1).

Clinicopathological parameters at the time of primary diagnosis are presented in Table 1. Their prognostic evaluation together with serum hormones and interleukins is presented in Table 2. Age, grade, FSH, IL6 and IL8 exerted a significant association with distant metastasis occurrence by the criteria of Cox proportional hazards regression test. In univariate analyses, the most pronounced HR of 0.05 was achieved by IL6, followed by 8.0 for the grade, 0.18 for FSH, 0.19 for age and 2.5 for IL8. HR below or above 1.0 indicates an association of a prognostic parameter with good or poor disease outcome, respectively. For instance, HR of 0.05 obtained for IL6 indicated that patients with higher IL6 serum protein values exerted a 20-fold lower risk of incurring a distant metastasis in comparison to patients with lower IL6 values
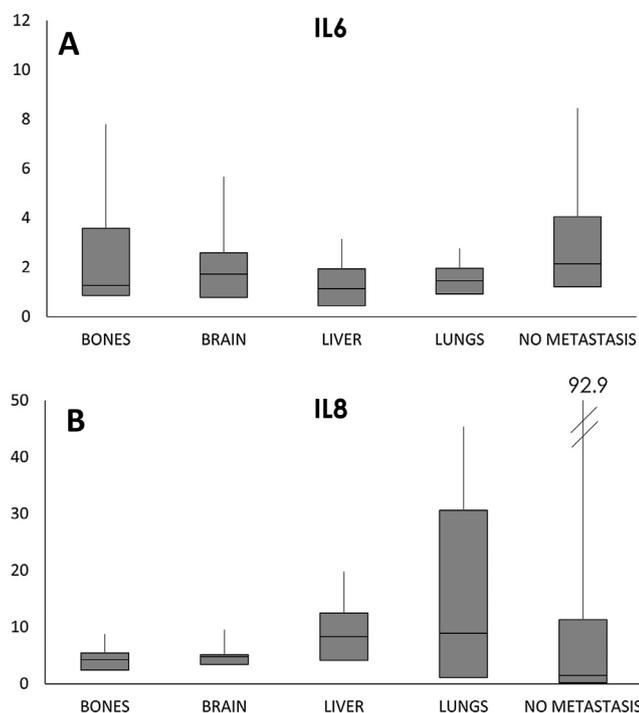
**Fig. 1.** Serum (A) IL6 and (B) IL8 concentrations grouped by metastasis location. Data are represented as box plots displaying medians, 25th and 75th percentiles as boxes, and the standard deviation (SD) of the values as whiskers.

(Table 2; Fig. 2A). Taken together, the results presented in Table 2, Figs. 1 and 2 are compatible with the association of IL6 and IL8 with the low and high metastasis risk, respectively.

The median value for IL6 was 1.7 pg/mL in the group prognosticated as high-risk and 2.2 pg/mL in the group prognosticated as low-risk, while for IL8 the values were 4.2 pg/mL and 1.3 pg/mL, for FSH 17.5 IU/L and 18.6 IU/L, for LH 10.5 IU/L, 15.6 IU/L, and for estradiol 35.02 pg/mL and 37.4 2 pg/mL, respectively (not shown). A significant positive correlation was found between FSH and LH levels, as well as age and LH levels (Spearman's rank order correlation test, $P < 0.001$ and $P = 0.03$, respectively). The significant negative correlation was found between FSH and estradiol levels ($P = -0.006$). There was no significant correlation between serum IL6 and IL8 or serum ER/PR/LH/FSH hormones and IL6/IL8 levels (not shown).

Multivariate Cox proportional hazards regression analysis of the metastasis risk highlighted IL6, FSH and age as the independent prognostic factors. The coefficient and HR revealed IL6 as the most influential variable (Table 3).

Kaplan-Meier estimator plots illustrate the prognostically significant associations of IL6 and IL8 in the whole group and ER$^{high}$, HER2+ subgroups (Fig. 2). Patients with prognosticated higher risk are designated on the lower line as their probability of remaining metastasis-free is lower. Dotted lines indicate the patient subgroup with lower cytokine values, below the cutoff value which separates the prognosticated low- and high-risk groups by the measured cytokine values (Fig. 2). Taken together, a dotted line below a full line indicates an association with prognosticated lower risk (Fig. 2A), while a dotted line positioned above indicates an association with the prognosticated higher risk (Fig. 2B, 2C, 2D).

When patients were classified according to ER status, IL6 lost its prognostic significance in ER$^{low}$ and ER$^{high}$ subgroups, while IL8 remained significantly associated with a poor disease outcome in the ER$^{high}$ subgroup ($P = 0.04$, HR = 2.8; Table 4; Fig. 2C). When patients were classified according to HER2 status, IL6 remained prognostically significant in both HER2− and HER2+ subgroups ($P = 0.001$, HR = 0.05 and $P = 0.002$, HR = 0.04, respectively; Table 4) whereas

**Table 1**
Clinicopathological parameters at the time of primary diagnosis.

| Clinicopathological parameters | n | % |
|---|---|---|
| *Age (years)* | | |
| < 44 (median) | 39 | 49.4 |
| ≥ 44 | 40 | 50.6 |
| *Menopausal status* | | |
| Premenopausal | 79 | 100 |
| Postmenopausal | 0 | 0 |
| *Tumor size (cm)* | | |
| < 2 | 34 | 43.0 |
| ≥ 2 | 44 | 55.7 |
| NA | 1 | 1.3 |
| *Nodal status* | | |
| N0 | 34 | 43.0 |
| N+ | 45 | 57.0 |
| *Histological type* | | |
| IDC | 34 | 43.0 |
| ILC | 26 | 32.9 |
| other types | 19 | 24.1 |
| *Tumor grade* | | |
| G1 | 12 | 15.2 |
| G2 | 58 | 73.4 |
| G3 | 3 | 3.8 |
| NA | 6 | 7.6 |
| *Estrogen receptor status* | | |
| ERlow | 39 | 49.4 |
| ERhigh | 40 | 50.6 |
| *Progesterone receptor status* | | |
| PR$^{low}$ | 15 | 19.0 |
| PR$^{high}$ | 57 | 72.1 |
| NA | 7 | 8.9 |
| *HER2 status* | | |
| HER2− | 61 | 77.2 |
| HER2+ | 17 | 21.5 |
| NA | 1 | 1.3 |
| *Metastasis location* | | |
| Bone | 8 | 40.0 |
| Lung | 4 | 20.0 |
| Brain | 5 | 25.0 |
| Liver | 3 | 15.0 |

Abbreviations: n, number of patients; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER$^{low}$: ER score = 2–6; ER$^{high}$: ER score = 7 and 8; PR$^{low}$: PR score = 3 or 4; PR$^{high}$: PR score > 4; HER2−: HER2 gene not amplified; HER2+: HER2 gene amplified by the criteria of 3+ or 2+/CISH+ scores; NA, data not available.

**Table 2**
Prognostic performance of the examined classifiers.

| Parameter | P-value$^*$ | HR | 95% CI$^*$ |
|---|---|---|---|
| Age | 0.001 | 0.19 | 0.06–0.43 |
| Tumor size | 0.99 | 0.99 | 0.61–1.45 |
| Nodal status | 0.88 | 1.04 | 0.94–1.79 |
| Type | 0.57 | 0.86 | 0.48–1.6 |
| Grade | 0.05 | 8.0 | 1.0–78.5 |
| HER2 | 0.79 | 0.85 | 0.19–2.4 |
| FSH | 0.001 | 0.18 | 0.07–0.44 |
| LH | 0.08 | 0.37 | 0.03–1.1 |
| E2 | 0.06 | 0.27 | 0.02–0.86 |
| IL6 | 0.001 | 0.05 | 0.04–0.05 |
| IL8 | 0.03 | 2.5 | 1.0–8.8 |

$^*$ Univariate Cox proportional hazards regression test, bootstrap corrected.

IL8 remained prognostically significant in HER2+ ($P = 0.001$, HR = 77.8; Table 4; Fig. 2D) but not in HER2− subgroup of patients (Table 4).
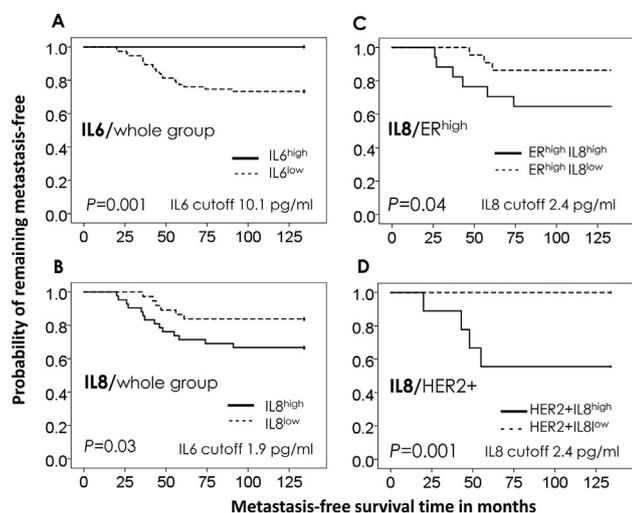
Fig. 2. Kaplan-Meier prognostic analysis of IL6 and IL8 in the entire patient group. Plots represent (A), serum IL6 levels over the entire patient group (B), serum IL8 levels over the entire patient group (C), serum IL8 levels in the ER$^{high}$ subgroup (D), serum IL8 levels in the HER2+ subgroup. Upper and lower curves represent the IL6$^{high}$/IL6$^{low}$ or IL8$^{high}$/IL8$^{low}$ patient subgroups defined by an optimal outcome-oriented cutoff. The exact cutoff values are indicated for each plot. Patients with higher cytokine values above the cutoff are plotted on solid lines, while lower values are presented on dotted lines. A wider separation between upper and lower curves indicates better prognostic performance. P-values were calculated by the Cox proportional hazards regression test. Metastasis incidences in patient subgroups prognosticated as low- and high-risk were respectively: (A) 0/27%, (B) 16/33%, (C) 14/35% and (D) 0/44%. Metastasis-free survival time in months is indicated on the x-axis.

**Table 3**
Multivariate Cox proportional hazards regression analysis.

| Parameter | Coefficient | P-value[*] | HR | 95% CI[*] |
|---|---|---|---|---|
| Age | −1.4 | 0.002 | 0.25 | 0.07–0.73 |
| FSH | −1.0 | 0.004 | 0.37 | 0.00–1.33 |
| IL6 | −11.6 | 0.001 | 0.0007 | 0.0001–0.90 |

  [*] Bootstrap corrected.

**Table 4**
Prognostic performance of IL6 and IL8 in subgroups of patients classified according to their receptor status.

| Parameter | P-value[*] | HR, 95%CI[*] | P-value[*] | HR, 95%CI[*] |
|---|---|---|---|---|
| | | ER$^{low}$ | | ER$^{high}$ |
| IL6 | 0.35 | 1.7; 0.48–8.3 | 0.15 | 1.6; 0.04–8.7 |
| IL8 | 0.06 | 2.7; 0.80–11.0 | 0.02 | 3.8; 0.99–110 |
| | | HER2− | | HER2+ |
| IL6 | 0.001 | 0.05; 0.04–0.05 | 0.002 | 0.04; 0.03–0.05 |
| IL8 | 0.2 | 1.8; 0.67–8.2 | 0.001 | 77.8; 33–602 |

Abbreviations: ER$^{low}$: ER score = 2–6; ER$^{high}$: ER score = 7 and 8; HER2–: HER2 gene not amplified; HER2+: HER2 gene amplified by the criteria of 3 + or 2+/CISH + scores.
  [*] Cox proportional hazards regression test, bootstrap corrected.

## 4. Discussion

This study investigates the association of cytokines with the metastasis outcome in the ER+ breast cancer patient group. Furthermore, we also searched for clues which might help explain the hormonal therapy unresponsiveness which is manifested by metastasis occurrence in hormonally treated ER+ patients.

Endocrine therapy for hormone-dependent (ER+) breast cancer consists of tamoxifen alone or a combination with LHRH agonist [16].

Goserelin is the LHRH agonist that results in an initial stimulation of gonadotrophin (FSH and LH) release, followed by a fall in gonadotrophin secretion and a subsequent decrease in circulating estrogen to postmenopausal levels [17]. The significant positive correlation between FSH and LH concentrations and a significant negative correlation between FSH and estradiol levels reported in this study were expected and might reflect ovarian physiology under hormonal therapy [18]. Thereby, lower estradiol coinciding with higher FSH levels indicates premenopausal ovarian suppression which was achieved by hormonal therapy administered to all patients [19]. Furthermore, in this study, FSH and age associated with a good disease outcome, in agreement with the previous finding of ER+ breast cancers being strongly affected by menopausal status and age [20]. The absence of significant correlations between IL6/IL8 serum levels and ER/PR/LH/FSH or lymph node involvement was also in line with previous findings obtained in breast carcinoma tissue [21], but in disagreement with the report of IL6 and IL8 serum levels strongly correlating with lymph node metastasis, ER and HER2 expression [22]. In the later study by Ma et al., the levels of IL8 were also significantly higher in ER− than ER+ and HER2+ than HER2− patients, neither of which could be observed in our current investigation [22]. These discrepancies may be attributed to the differences in patient groups, as the above study specifically selected only the ductal type of carcinoma, with mixed ER− and ER+ tumors [22], while our current study included only ER+ tumors, but with mixed breast carcinoma type.

The prognostic association of IL6 with breast cancer outcome is rather controversial, as this cytokine has been reported to mark good, as well as poor outcome. In this study, IL6 was characterized as a marker of good prognosis, in accordance with the previous clinical study in the patient group unmatched for ER [21]. Antitumor action of IL6 was also demonstrated in vitro [2,9]. However, several studies also describe the opposite prognostic association of IL6 as its high serum levels indicated poor prognosis and diminished response to endocrine therapy [23,24]. Serum levels of IL6 and IL8 reportedly correlate with the clinical stage of the disease in a mixed group of ER− and ER+ patients [22,25]. Bachelot et al. identified serum IL6 as an independent adverse prognostic marker of overall survival [26]. Their patient group was also mixed, consisting of ER− and ER+ patients. Also, in advanced stage patients, elevated IL6 levels independently associated with poor prognosis [27]. Yet, in the ER+ patient group similar to ours, Won et al. recently reported that serum IL6 did not have any prognostic value [28]. Taken together, the above discrepancies in prognostic characterization of serum IL6 might be primarily attributed to differences in the studied breast cancer patient groups.

We show here that serum IL8 associated with a poor disease outcome in line with our recent study measuring intratumoral IL8 levels in hormone-dependent (ER+PR+) breast cancer [29] and other studies of systemically untreated early breast cancer patients over a short-term [35] and long-term follow-up [36]. Previous reports are generally highly consistent in characterizing IL8 as a marker of poor outcome [25,30–32]. IL8 was also found to be overexpressed in tumor cells in comparison to normal surrounding tissue [34]. This can be explained by the fact that IL8 and also IL6 are produced not only by immune cells [5] but also by malignant cells [35] and by tumor-associated stromal cells [36–38]. Therefore, it may be speculated that the increased IL6 and IL8 serum levels measured in this study originated in large part from the tumor itself. This is compatible with several studies indicating that serum IL8 levels might be of diagnostic use in breast cancer [31,33] and that serum levels of interleukins 6 and 8 were significantly elevated in patients with larger tumors and increased metastatic potential [41]. Such intratumoral production of IL6 and IL8 might reflect the metastatic risk based on the fact that breast cancer stem cells and IL6/IL8 production are linked by syndecan-1 as their common regulator [39,40].

Breast cancer is mainly classified by expression of ER and HER2. In this study, when patients were grouped according to ER staining

intensity, IL6 lost its prognostic significance in both ER$^{low}$ and ER$^{high}$ subgroups, while IL8 remained at least marginally prognostically significant in both subgroups. Such grouping of patients based on ER intensity did not reveal any differences in IL6 and IL8 prognostic performance and thus did not provide any clues pointing to the estrogen-dependence of IL6 and IL8 prognostic effects.

When patients were classified according to HER2 status, IL6 associated with good disease outcome in both HER2− and HER2+ subgroups, suggesting that its prognostic effect was independent of HER2. On the other hand, IL8 exerted prognostic significance only in the HER2+ subgroup, with its HR increasing from 2.5 to 78. This indicated a possibility that prognostic performance of IL8 in large part depended on HER2. Other known links between IL8 and HER2 include the report of IL8 coinciding with HER2 in breast cancer [12,42,43]. Moreover, epidermal growth factor (HER2 ligand) potently upregulates IL8 secretion by breast tumor cells and this effect is amplified by estrogen and progesterone [44]. Haim et al. also showed that epidermal growth factor and estrogen upregulate the transcription and secretion of IL8 by breast tumor cells in an additive manner [45].

Estrogen ablation in ER+ patients impedes the growth of malignant cells. However, hormone therapy resistance is a common phenomenon with a rate approaching 50%, manifested by metastasis occurrence. Such resistance might be explained by estrogen receptor or its downstream signaling disfunction, modification of cell cycle or other mechanisms [7]. As all patients were prescribed endocrine therapy in the current study, those without metastasis could be considered as hormone-sensitive and those with metastasis as refractory. Taken together, in addition to marking good and poor prognosis, IL6 and IL8 might also respectively mark sensitivity and resistance to endocrine therapy.

The advantages of this study include the first use of breast cancer patient group which is uniformly positive for estrogen and progesterone receptors. Such design has reduced group inhomogeneity which could mask or alter the prognostic performance of IL6 and IL8. Moreover, the creation of ER$^{low}$, ER$^{high}$, HER2+ and HER2− subgroups has offered an insight into ER or HER2-dependency of the IL6/IL8 prognostic performance. Furthermore, the possibility of noninvasive and repeated measurements of serum-derived prognostic factors presents an important advantage for clinical use. Additionally, the statistical reliability was enhanced by the use of bootstrap as the bias-correction method. The main limitation of this study was the relatively small sample size of 79 patients, although this number exceeded the requirement set by the prospective sample size analysis.

In conclusion, the presented study contributes to clarification of the previously inconsistent prognostic performance of IL6 and provides the first prognostic evaluation of IL8 in the patient group homogenous for ER and PR. The results also suggest that IL6 and IL8 might be considered as respective markers of sensitivity and resistance to endocrine therapy. Clinical applicability is based on relevance for the breast cancer immunotherapy research and importance of prognosis for identification of patients with high risk of metastasis occurrence which may benefit from more aggressive treatments.

## Funding

## Conflict of interest

None.

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