Original Study

Low Plasma IL-8 Levels During Chemotherapy Are Predictive of Excellent Long-Term Survival in Metastatic Breast Cancer

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Abstract

Plasma interleukin (IL)-8 levels were monitored in 58 patients with metastatic breast cancer before and during first-line chemotherapy, and changes in the IL-8 levels were correlated with patient survival data. Monitoring plasma IL-8 levels before and during chemotherapy identifies patients with excellent prognosis whose IL-8 levels stay constantly below 16.6 pg/mL.

Background: Interleukin (IL)-8 is a proinflammatory cytokine, and high levels of IL-8 are associated with poor prognosis in many malignancies. The objective of this study was to explore the clinical benefit of monitoring plasma IL-8 levels during breast cancer chemotherapy. **Patients and Methods:** We conducted an exploratory analysis of several circulating proteins, including IL-8, in the plasma. Plasma samples were obtained from 58 metastatic breast cancer patients who took part in a prospective phase 2 first-line bevacizumab chemotherapy trial. Samples were analyzed before therapy, after 6 weeks and 6 months of treatment, and at the final study visit. On the basis of a trajectory analysis of the plasma IL-8 levels, the patients were divided into 3 trajectory groups. **Results:** Plasma IL-8, IL-6, IL-18, matrix metalloproteinase (MMP)-2, MMP-9, YKL-40, resistin, and high-mobility group box 1 (HMGB1) concentrations were measured, and the most pronounced predictor of patient survival was IL-8. On the basis of the trajectory analysis of the IL-8 levels, the majority of patients (n = 35, 60%) belonged to trajectory group 1, and these patients had significantly lower IL-8 levels before and during the entire chemotherapy treatment period than did the patients in the other groups. Trajectory group 1 patients had significantly better overall survival compared to patients in trajectory group 2 (n = 17; age-adjusted HR = 2.45; 95% confidence interval, 1.21-5.97; $P = .012$) and 3 (n = 6; age-adjusted HR = 8.65; 95% confidence interval, 3.16-23.7; $P < .001$). **Conclusion:** Low IL-8 levels during chemotherapy treatment might help identify patients with prolonged survival.

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Introduction

Breast cancer is the most common cause of cancer-related death in women.[1] Currently, patients with human epidermal growth factor 2 (HER2)-negative advanced breast cancer will survive for approximately 2 to 3 years after diagnosis of advanced cancer.[2-5] The disease of most patients will respond to chemotherapy and endocrine therapy, but the cancer will eventually progress. More investigational effort should be expended to find patients with disease that will not respond to current therapies and who are in need of novel investigational treatment options. Furthermore, early

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palliative care improves patient quality of life, symptom management, and even treatment outcomes.[6-8] In particular, patients with chemoresistant cancer might benefit from earlier palliative symptom management if these patients could be better identified.

Interleukin (IL)-8 (alternatively known as CXCL8) is a proinflammatory cytokine.[9] Its complex effects on the tumor microenvironment may result in tumor proliferation, survival, and chemoresistance in malignant disease.[10-14] High IL-8 serum levels and tumor expression are known to be associated with poor patient prognosis in many malignant diseases, including breast cancer.[13,15,16] Even in localized breast cancer, patients with high circulating IL-8 levels have a poorer prognosis than patients with low IL-8 levels.[17,18]

In addition to IL-8, many other cytokines and circulating regulatory factors are associated with breast cancer and are considered to be potential biomarkers for cancer prognosis.[19-34] Serum concentrations of IL-6 and IL-18 are elevated in breast cancer patients,[19,20] and high circulating IL-6 levels are linked to shorter survival in metastatic breast cancer patients than are low circulating IL-6 levels.[21,22] Additionally, IL-6 and IL-18 are associated with chemotherapy resistance.[23,24] Matrix metalloproteinase (MMP)-2 and MMP-9 serum levels are associated with poor overall survival (OS), even in patients with localized breast cancer.[25] YKL-40 (also known as chitinase-3–like protein 1) has been suggested to play a role in cell proliferation, differentiation, inflammation, and tissue remodeling, and has been associated with malignancies with poor survival.[35-37] In patients with either local or advanced breast cancer, high serum YKL-40 levels predict a poor prognosis.[26-28]

Obesity is a known risk factor for breast cancer.[38] Therefore, adipocytokines, including resistin, may be related to breast cancer development and prognosis. Serum resistin levels are known to be elevated in breast cancer patients compared to healthy controls.[29,30] Additionally, compared to low resistin expression, high resistin expression in the primary breast cancer tumor tissue is associated with poorer patient survival and more unfavorable clinicopathologic features of the primary cancer.[31] High-mobility group box 1 (HMGB1) is a ubiquitous nuclear protein that contributes to DNA repair and the stabilization of nuclear homeostasis.[32] HMGB1 is expressed at higher levels in many tumor types compared to healthy tissue,[33] and its expression is associated with many diseases, including cancer.[34]

We conducted an exploratory analysis of multiple plasma cytokines and other circulating proteins. The aim of the study was to identify prognostic markers for metastatic breast cancer. IL-8 levels, a promising biomarker, were explored before and during chemotherapy treatment for their value in predicting patient prognosis. We also measured plasma levels of IL-6, IL-18, MMP-9, MMP-2, YKL-40, resistin, and HMGB1, and investigated their prognostic significance.

Patients and Methods

We conducted a prospective phase 2 trial for metastatic breast cancer patients. The study patients had histologically verified HER2-negative advanced breast cancer and had not received previous chemotherapy in a metastatic setting. A total of 65 patients were enrolled onto the trial at 3 Finnish university oncology clinics between 2009 and 2013 (NCT00979641). The study inclusion criteria, trial design, and clinical results have been published previously.[2] In brief, study patients were treated with a bevacizumab and taxane (paclitaxel or docetaxel) combination as the first-line treatment for metastatic breast cancer. Patients without disease progression continued bevacizumab treatment after the taxane chemotherapy was discontinued. Patients with estrogen receptor–positive breast cancer also received endocrine therapy with bevacizumab maintenance therapy. For second-line therapy after disease progression, the continuation of bevacizumab was optional with chemotherapy. All patients provided written informed consent, and the regional ethics committee of Tampere University Hospital approved the study protocol (R08142M).

Plasma samples were gathered before the initiation of chemotherapy (baseline), after 6 weeks of treatment, after 6 months of treatment, and at the final study visit.

Measurement of Plasma Cytokines

Plasma IL-8, IL-6, IL-18, MMP-2, MMP-9, YKL-40, resistin, and HMGB1 concentrations were measured by enzyme-linked immunosorbent assays (ELISAs) using reagents from BD Biosciences (Erembodegem, Belgium; IL-8), eBioscience (San Diego, CA; IL-6 and IL-18), R&D Systems Europe (Abingdon, UK; MMP-2, MMP-9, YKL-40, resistin), and IBL International (Hamburg, Germany; HMGB1). ELISAs were carried out according to a standard protocol. In brief, for MMP-2, MMP-9, YKL-40, and resistin, a 96-well plate was coated with capture antibody and incubated overnight at 4°C. The wells were washed with phosphate-buffered saline–0.05% Tween 20 and blocked with 1% bovine serum albumin in phosphate-buffered saline, 250 μL per well, for 1 hour at room temperature (RT). The wells were washed, and the standards, and samples diluted in reagent diluent (1% bovine serum albumin in phosphate-buffered saline) were added to the wells and incubated for 2 hours at RT. The wells were washed. Detection antibodies diluted in reagent diluent (with normal goat serum for MMP-9) were added and incubated for 1.5 h at RT. Streptavidin-conjugated to horseradish peroxidase was added after the wash step and incubated for 15 minutes at RT. The wells were washed, and BioFX TMB substrate solution (SurModics, Eden Prairie, MN) was added and incubated for 15 minutes in the dark at RT. After adding 50 μL of stop solution (1 N H_2SO_4), the absorbance of each well was measured at 450 nm with a correction wavelength at 540 nm within 20 minutes with a Victor3 Multilabel Counter (Perkin Elmer, Turku, Finland), and the results were calculated from a standard curve using the smoothed spline method with MultiCalc software (Perkin Elmer). For IL-8, IL-6, IL-18, and HMGB1, ELISAs were performed according to the manufacturer's protocols and then measured and calculated as stated above.

Patient Characteristics

Plasma samples were available from 58 patients (89%). Patient characteristics are listed in Table 1. After taxane discontinuation, patients without disease progression and with hormone receptor–positive disease received endocrine therapy in combination with bevacizumab. Letrozole was the most common endocrine therapy choice (n = 19). The other endocrine therapies included anastrozole (n = 4), exemestane (n = 4), tamoxifen (n = 3), and fulvestrant (n = 3).

Table 1 Baseline Characteristics and Efficacy Results in Plasma Biomarker Population and of Patients With Baseline Samples Available Compared to Overall Study Population

Characteristic	Plasma Biomarker Population (N = 58)	Overall Study Population (N = 65)
Age (y), median (range)	58 (32-75)	57 (32-75)
Menopausal Status		
Premenopausal	9 (15.5)	10 (15.4)
Postmenopausal	49 (84.5)	55 (84.6)
History of early stage disease	52 (89.7)	57 (87.7)
Disease-Free Interval, mo		
≤24	10 (19.2)	11 (16.9)
>24	42 (80.8)	46 (70.8)
Hormone Receptor Status		
ER^+ and/or PR^+	47 (81.0)	53 (81.5)
ER^- and PR^-	11 (19.0)	12 (18.5)
No. of Metastatic Lesions		
≤3	11 (19.0)	14 (21.5)
>3	47 (81.0)	51 (78.5)
Extent of Disease		
<3 sites	36 (62.1)	39 (60.0)
≥3 sites	22 (37.9)	26 (40.0)
Site of Metastatic Disease		
Visceral disease	46 (79.3)	53 (81.5)
Nonvisceral disease	12 (20.7)	12 (18.5)
Overall survival, median (95% CI)	37.5 (25.4-49.6)	35.1 (22.2-50.3)
Progression-free survival, median (95% CI)	11.3 (8.3-14.4)	11.3 (9.7-16.0)
Best Response to Treatment		
Complete response/partial response	38 (71.7)	40 (61.5)
Stable disease	13 (24.5)	15 (23.1)
Progressive disease	2 (3.8)	3 (4.6)

Data are presented as n (%) unless otherwise indicated.
Abbreviations: CI = confidence interval; ER = estrogen receptor; PR = progesterone receptor.

Baseline samples were available from 53 patients. Breast cancer progression was the reason for study discontinuation for most patients (n = 36, 55%). Final plasma samples were available from 50 patients, of whom 24 had disease progression as the reason for treatment discontinuation (48%). The remaining 26 patients discontinued the study treatment as a result of treatment side effects. Plasma samples at week 6 and month 6 were available only from patients who were following the study treatment plan at that time point.

Statistical Analysis

The statistical plan for the analysis was exploratory. IL-8, IL-6, IL-18, MMP-9, MMP-2, YKL-40, resistin, and HMGB1 levels were dichotomized as low or high for each patient using the median value for each molecule as the cutoff value. Additionally, IL-8, IL-6, IL-18, MMP-9, MMP-2, YKL-40, resistin, and HMGB1 levels were divided into 4 groups using the baseline quartile ranges as the cutoff values.

IL-8 values were clustered by the trajectory analysis originally presented by Nagin.[39] Trajectory groups are clusters of individuals following similar trajectories to an outcome over time.[40] The trajectories were created according to all measurements of IL-8 levels in each patient as a continuous outcome measure. These trajectories are presented in Figure 1. The analyses undertaken were latent class mixture models of quadratic trajectories including a random intercept and concomitant variables. Models were fitted by the FlexMix package[41] of the statistical program R 3.3.0.[42] Relative goodness of fit was assessed using the Bayesian information criteria.

Because of the nonparametric distribution of the IL-8 levels, medians with the confidence interval (CI) of the median are reported. The Mann-Whitney *U* test was used to compare the median IL-8 levels of different baseline characteristics and trajectory groups. Hazard ratios (HR) with 95% CIs were calculated by Cox proportional hazard regression analysis. Multivariable analyses were adjusted for age (continuous), menopause status (premenopausal/postmenopausal), hormone receptor status (negative/positive), presence of visceral metastasis (yes/no), number of metastatic lesions (cutoff of 3 metastatic lesions), and extent of disease (cutoff of 3 metastatic sites). Median OS, median progression-free survival (PFS), and their CIs were calculated by the Kaplan-Meier method. The Wilcoxon signed-rank test was used to compare the baseline, week 6, month 6, and final plasma IL-8 levels between the different trajectory groups. $P < .05$ was considered statistically significant. Statistical analyses were performed by SPSS 23 software (IBM, Armonk, NY).

Results

IL-8 Levels and Patient Baseline Characteristics

There were no statistically significant differences in the baseline IL-8 levels between groups with different baseline characteristics, including menopause status ($P = .104$), hormone receptor status ($P = .152$), number of metastatic lesions ($P = .539$), and presence of visceral disease ($P = .941$). Borderline significantly lower baseline IL-8 levels were observed in patients with <3 metastatic sites compared to the patients with ≥3 metastatic sites (<3 metastatic sites median baseline IL-8: 8.9 pg/mL; 95% CI, 7.8-9.9 pg/mL vs. ≥3 metastatic sites median IL-8: 12.5 pg/mL; 95% CI, 8.0-25.4 pg/mL; $P = .057$).

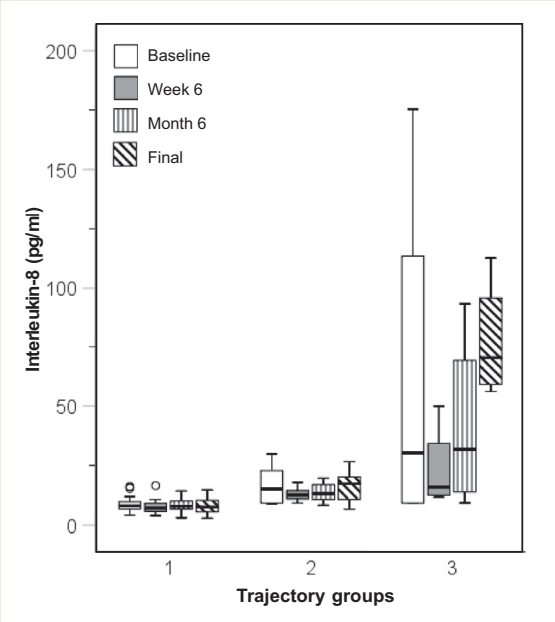
Prognostic Significance of Baseline IL-8 Levels

The patients were divided into two groups (low and high baseline plasma IL-8 level) using a median value of 9.4 pg/mL as the cutoff point. The PFS and OS of these IL-8 groups are listed in Table 2. The high baseline IL-8 group had a significantly shorter OS ($P = .023$).

Trajectory Analysis of IL-8 Levels

The distributions of the 3 trajectory groups are presented in Figure 1 and Table 3. Trajectory group 1 patients had constantly low IL-8 concentrations; the range of IL-8 levels in trajectory group

Figure 1 Interleukin 8 Trajectory Groups. Shown Are Trajectory Groups 1 (n = 35), 2 (n = 17), and 3 (n = 6)



1 was 2.6 to 16.6 pg/mL during the entire treatment period. Trajectory groups 2 and 3 had significantly higher IL-8 levels at baseline, at week 6, at month 6, and at the final study visit compared to trajectory group 1 (Table 3). The final IL-8 levels of trajectory group 3 patients were significantly higher than their month 6 IL-8 plasma levels ($P = .043$). In trajectory group 3, there were no significant changes in the IL-8 levels between the baseline and week 6 and between week 6 and month 6. The changes in the IL-8 levels in trajectory groups 1 and 2 over time were not statistically significant.

The patients belonging to trajectory group 3 with very high IL-8 levels had significantly shorter PFS than the patients belonging to the other groups (Table 4, Figure 2A). No significant differences in PFS were detected between the patients in trajectory groups 1 and 2. In addition, the patients in trajectory groups 2 and 3 had significantly shorter OS than the patients in trajectory group 1 using both an age-adjusted HR and a multivariable Cox model adjusted for age, menopause status, hormone receptor status, presence of visceral metastases, number of metastatic lesions, and extent of the disease (Table 4, Figure 2B).

To further examine the clinical utility of IL-8 levels, a cutoff value of 16.6 pg/mL was found to be useful for finding patients with a significantly more favorable long-term prognosis. All the IL-8 levels in trajectory group 1 remained below 16.6 pg/mL before and during the entire chemotherapy treatment period. A cutoff value of 16.6 pg/mL could identify all of the 35 patients who were categorized into trajectory group 1. In contrast, only one trajectory group 2 patient (1/17, 5.9%) had IL-8 levels constantly below 16.6 pg/mL, and all of the patients in trajectory group 3 had IL-8 levels higher than 16.6 pg/mL before or during chemotherapy treatment. For PFS, the age-adjusted HR was borderline significant for the patients with IL-8 levels higher than 16.6 pg/mL before or during chemotherapy treatment (age-adjusted HR 2.00; 95% CI, 0.97-4.14; $P = .060$), while the multivariable HR was not statistically significant (multivariable HR = 1.91; 95% CI, 0.89-4.09; $P = .094$; Figure 3A). However, the HR for OS was strongly significant for both the age-adjusted and multivariable Cox models for the patients with IL-8 levels higher than 16.6 pg/mL before or during chemotherapy treatment (age-adjusted HR 3.02; 95% CI, 1.60-5.71; $P = .001$, multivariable HR = 3.90; 95% CI, 1.88-8.12; $P < .001$; Figure 3B).

Highest Baseline IL-8 Quartile Level and Prognosis

A very high baseline plasma IL-8 level was also a strong sign of poor prognosis without knowledge of IL-8 levels during treatment. The highest (>18.8 pg/mL) baseline IL-8 level quartile patients had the poorest prognosis in terms of median PFS and OS, at 9.6 months (95% CI, 5.47-13.7 months) and 19.7 months (95% CI, 8.60-30.9 months), respectively (Supplemental Table 1 in the online version). The multivariable HR for PFS was 6.52 (95% CI, 1.58-26.9; $P = .010$) for the highest plasma IL-8 quartile, and the multivariable HR for OS was 8.38 (95% CI, 2.60-27.0; $P < .001$). All of the patients in the highest quartile belonged to trajectory groups 2 (n = 9) and 3 (n = 4). Altogether, a high baseline IL-8 level > 18.8 pg/mL (the highest quartile) could identify 62%

Table 2 Cox Regression Analysis for PFS and OS Grouped by Low or High Baseline IL-8 Levels Using Median as Cutoff Value

Baseline IL-8	pg/mL	No. Patients	No. Events	Adjusted HR 1[a]			Adjusted HR 2[b]		
				HR	95% CI	*P*	HR	95% CI	*P*
PFS									
Low	≤9.4	27	15	1			1		
High	>9.4	26	16	1.44	0.70-2.93	.316	1.32	0.58-3.00	.493
OS									
Low	≤9.4	27	16	1			1		
High	>9.4	26	23	2.14	1.10-4.12	.023[c]	1.65	0.82-3.34	.159

Abbreviations: CI = confidence interval; HR = hazard ratio; IL = interleukin; OS = overall survival; PFS = progression-free survival.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.
[c]Statistically significant.

Table 3 Median IL-8 Levels in 3 Trajectory Groups

Trajectory Group	No. Patients	Baseline IL-8 (pg/mL)		Week 6 IL-8 (pg/mL)		Month 6 IL-8 (pg/mL)		Final IL-8 (pg/mL)	
		Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
1	35	8.05	7.40-9.40	7.30	5.90-9.00	7.60	6.50-8.90	7.90	6.30-9.90
2	17	21.7	12.4-27.5	13.4	11.3-18.0	11.6	8.40-16.1	15.1	7.30-20.2
3	6	38.9	9.30-175	16.2	11.9-50.2	39.4	9.50-93.4	78.9	56.4-113
1 vs. 2 *P*		<.001[a]		<.001[a]		.006[a]		.001[a]	
1 vs. 3 *P*		.002[a]		.002[a]		<.001[a]		<.001[a]	
2 vs. 3 *P*		.199		.332		.009[a]		.001[a]	

Abbreviations: CI = confidence interval of median; IL = interleukin.
[a]Statistically significant.

(13/21) of the patients in the poorer prognosis trajectory groups 2 and 3 with the baseline plasma samples available.

IL-6, IL-18, MMP-9, MMP-2, YKL-40, Resistin, and HMGB1 Baseline Levels as Prognostic Markers for Survival

A Cox regression analysis was also performed for all other measured markers: IL-6, IL-18, MMP-9, MMP-2, YKL-40, resistin, and HMGB1. Using the median and quartile levels as cutoff values, there were no statistically significant differences in PFS using all individual markers (Supplemental Tables 2 and 3 in the online version). Using the median as a cutoff value, a high baseline MMP-9 level was borderline significant for longer OS (multivariable HR = 0.52; 95% CI, 0.26-1.03; $P = .063$). Using the baseline quartile levels as cutoff values, the baseline quartile level of 50% to 75% for MMP-9 was prognostic for OS (multivariable HR = 0.37; 95% CI, 0.13-1.01; $P = .054$), as was the highest baseline quartile MMP-9 level (multivariable HR for OS 0.22; 95% CI, 0.07-0.68; $P = .009$). The highest baseline quartile level of YKL-40 was a sign of poor prognosis in an age-adjusted Cox regression (HR 3.08; 95% CI, 1.10-8.61; $P = .031$). However, in multivariable analysis, the highest baseline level of YKL-40 lost its prognostic significance (multivariable HR = 2.13; 95% CI, 0.65-6.97; $P = .211$). For IL-6, IL-18, MMP-2, resistin, and HMGB1, the median and quartile cutoff level groups revealed no significant OS differences (Supplemental Tables 4 and 5 in the online version).

Discussion

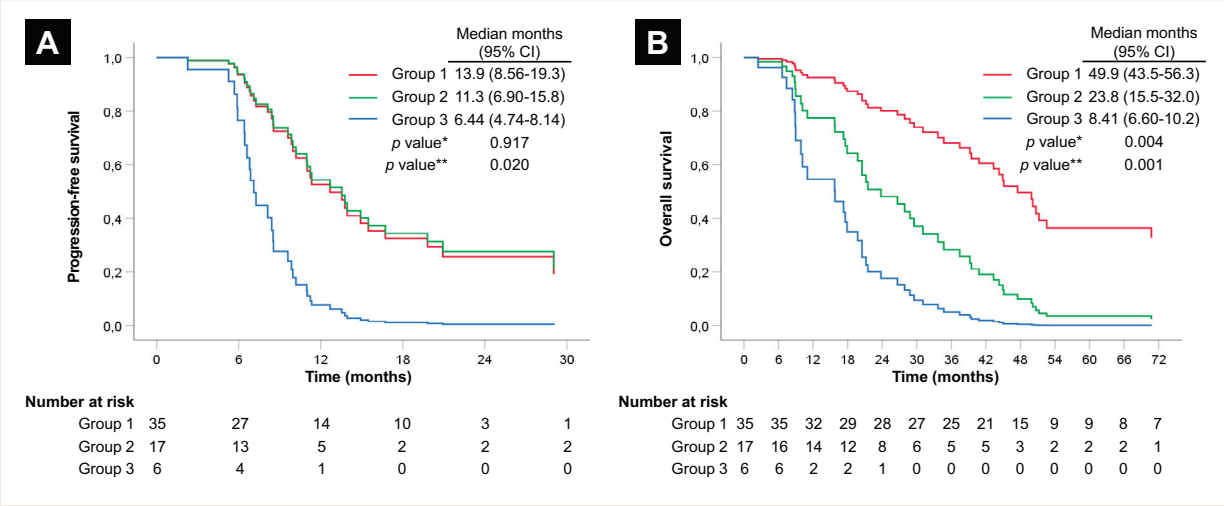
IL-8 level monitoring during chemotherapy for metastatic breast cancer is a promising approach for identifying patients with good prognosis. High baseline plasma IL-8 levels are known to be a poor prognostic marker in breast cancer.[15] However, to our knowledge, our study is novel in its monitoring of plasma IL-8 levels in metastatic breast cancer patients during chemotherapy. We identified a large group of patients belonging to trajectory group 1 (35/58, 60.3%) who had a substantially better prognosis than the rest of the patients. The median OS (50 months) for trajectory group 1 patients was exceptionally good (95% CI, 43.5-56.3 months) in patients with metastatic HER2-negative breast cancer. In contrast, the median OS for trajectory group 2 patients (median OS 24 months; 95% CI, 15.5-32.0 months) was less than half of the OS in the group 1 patients. Interestingly, the remaining 6 patients belonging to trajectory group 3 had exceptionally high IL-8 levels during the entire chemotherapy period, and these patients had a short median OS of 8 months. High IL-8 levels are known to be a sign of chemoresistance.[12,43] The poor survival of our trajectory group 3 patients is a confirmatory finding for the previously reported chemoresistant nature of metastatic cancer with high IL-8 levels.

Table 4 PFS and OS of Patients of 3 Trajectory Groups

Trajectory Group	No. Patients	No. Events	HR 1[a]			HR 2[b]		
			HR	95% CI	*P*	HR	95% CI	*P*
PFS								
1	35	19	1			1		
2	17	9	1.27	0.52-3.08	.589	0.94	0.35-2.54	.917
3	6	6	4.56	1.65-12.6	.003[c]	4.01	1.24-12.9	.020[c]
OS								
1	35	22	1			1		
2	17	15	2.45	1.21-5.97	.012[c]	3.29	1.45-7.45	.004[c]
3	6	6	8.65	3.16-23.7	<.001[c]	7.82	2.27-26.9	.001[c]

Abbreviations: CI = confidence interval; HR = hazard ratio; IL = interleukin; OS = overall survival; PFS = progression-free survival.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.
[c]Statistically significant.

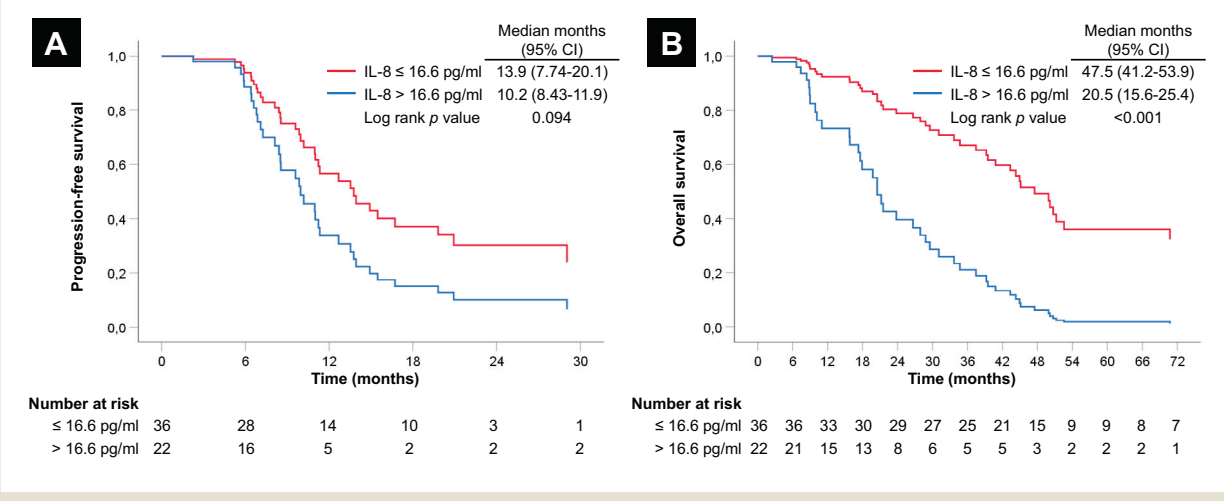
Figure 2 PFS and OS by Multivariable Cox Regression. (A) PFS and (B) OS of 3 Trajectory Groups Using Multivariable Cox Regression Adjusted for Age, Menopause Status, Hormone Receptor Status, Presence of Visceral Metastasis, Number of Metastatic Lesions, and Extent of Disease. Median Survivals and Their Confidence Intervals Were Calculated by Kaplan-Meier Method. *Log-rank *P* Value Between Trajectory Groups 1 and 2. **Log-rank *P* Value Between Trajectory Groups 1 and 3



Abbreviations: OS = overall survival; PFS = progression-free survival.

In addition to the high IL-8 levels during chemotherapy treatment, exceptionally high baseline IL-8 levels were a strong sign of poor prognosis, even without knowledge of IL-8 levels during treatment. In our study, the IL-8 levels in the highest baseline quartile were above 18.8 pg/mL, and the PFS (multivariable HR = 6.52; 95% CI, 1.58-26.9; $P = .010$) and OS (multivariable HR = 8.38; 95% CI, 2.60-27.0; $P < .001$) of these patients were significantly shorter than those of the patients in the lowest IL-8 quartile group. This result was similar to a previous report that showed that patients with baseline plasma IL-8 levels higher than

Figure 3 PFS and OS Based on Trajectory Analysis. (A) PFS and (B) OS in Patient Population Dichotomized Based on Trajectory Analysis Using All Plasma IL-8 Levels Before and During Chemotherapy Treatment. Red Line Indicates That Plasma IL-8 Levels at Baseline, Week 6, Month 6, and at Study Discontinuation Are Below 16.6 pg/mL. Blue Line Indicates That One or Several Measurements of Plasma IL-8 Levels Are Above 16.6 pg/mL Before or During Chemotherapy Treatment. Multivariable Cox Regression Adjusted for Age, Menopause Status, Hormone Receptor Status, Presence of Visceral Metastasis, Number of Metastatic Lesions, and Extent of Disease. Median Survivals and Their Confidence Intervals Were Calculated by Kaplan-Meier Method



Abbreviations: IL = interleukin; OS = overall survival; PFS = progression-free survival.

the median value of 17.2 pg/mL had shorter survival than patients with lower IL-8 levels ($P = .0045$).[15]

Several studies have been conducted to find a clinically useful biomarker to select patients who might benefit from the addition of the vascular endothelial growth factor A (VEGF-A) antibody bevacizumab to standard chemotherapy for the treatment of metastatic breast cancer.[44-48] In our study, the patients were treated with bevacizumab combined with either paclitaxel or docetaxel chemotherapy as first-line treatment for metastatic breast cancer. It has been shown that IL-8 can promote angiogenesis and may activate vascular endothelial growth factor receptor 2 (VEGFR2).[49] VEGF-A is a ligand for VEGFR2. In our study, very high baseline IL-8 levels were a sign of poor prognosis. Accordingly, in our study, the patients with the highest plasma IL-8 levels at baseline had the shortest treatment benefit. The high baseline plasma levels of proangiogenic IL-8 might be one reason for the lack of benefit from bevacizumab-based therapy. However, because our study did not have a placebo control arm as a comparator, this hypothesis should be tested prospectively in future studies.

The other markers analyzed in our study failed to demonstrate any clear prognostic significance. Zhang and Adachi[21] reported that patients with circulating IL-6 levels higher than the median concentration of 4 pg/mL in their study exhibited poor survival. However, the highest quartile plasma IL-6 cutoff value for our study patients was 3.8 pg/mL, suggesting that most of our study patients had low plasma IL-6 concentrations. This is in accordance with the finding that plasma IL-6 levels were not prognostic in our hands. In addition, the limited patient population in our study might partly explain why the other tested circulating markers had no prognostic value.

The plasma analyses in our study were exploratory and were performed retrospectively. In the future, it would be useful to monitor plasma IL-8 levels prospectively in clinical trials involving metastatic breast cancer patients. IL-8 levels are known to correlate with the tumor burden in many malignant diseases.[50] Rising IL-8 levels during treatment could be a sign of chemoresistance, and it therefore might be beneficial to refer patients with rising IL-8 levels to new treatment modalities. It might be worthwhile to study whether patients with high plasma concentrations of the proinflammatory cytokine IL-8 would benefit from novel immunotherapies. In an unselected metastatic breast cancer population, the response rates to immunotherapies have been low.[51] However, in a report of novel immunotherapies, a clear association was seen between the treatment response and IL-8 levels in melanoma and non–small-cell lung cancer patients.[52] Nevertheless, the correlation between high IL-8 levels and the response rates to immunotherapies in metastatic breast cancer remains unexplored.

Conclusion

Low plasma IL-8 levels during chemotherapy in metastatic breast cancer patients are a clear sign for excellent long-term prognosis. We found that patients with constantly low plasma IL-8 levels had a better prognosis than the patients with plasma IL-8 levels higher than 16.6 pg/mL. Plasma IL-8 levels might therefore be useful for the selection of patients with excellent prognosis and those who might be suitable for less intensive radiologic imaging and follow-up visits.

Clinical Practice Points

- High circulating IL-8 levels are associated with poor prognosis in patients with advanced breast cancer and are related to chemoresistance.
- Metastatic breast cancer patients with constantly low plasma IL-8 levels during first-line chemotherapy have an excellent long-term prognosis.
- Very high baseline plasma IL-8 levels are associated with significantly shorter PFS and OS.
- Monitoring circulating IL-8 levels during first-line chemotherapy might be beneficial to distinguish good-prognosis patients who might be suited to less intensive treatment and follow-up schedules.
- Patients with very high plasma IL-8 levels either at the beginning of chemotherapy treatment or during therapy for metastatic breast cancer should be followed more intensively because of the chemoresistant nature of their disease.
- In the future, whether patients with high plasma IL-8 levels and therefore poor prognosis might benefit from novel treatment modalities, ie, immunologic therapy, should be prospectively explored.

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Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

Supplemental tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2019.03.006.

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Supplemental Table 1 PFS and OS for Study Patients Grouped by Baseline IL-8 Quartile

Baseline IL-8	pg/mL	No. Patients	No. Events	HR 1[a]			HR 2[b]		
				HR	95% CI	*P*	HR	95% CI	*P*
PFS									
<Q25	<7.7	13	5	1			1		
Q25-Q50	7.7-9.4	14	10	2.12	0.70-6.39	.180	2.15	0.53-8.65	.279
Q50-Q75	9.4-18.8	13	7	1.34	0.42-4.27	.618	0.99	0.20-4.76	.995
>Q75	>18.8	13	9	5.22	1.62-16.8	.006[c]	6.52	1.58-26.9	.010[c]
OS									
<Q25	<7.7	13	6	1			1		
Q25-Q50	7.7-9.4	14	10	2.70	0.95-7.69	.062	3.46	1.08-11.0	.035[c]
Q50-Q75	9.4-18.8	13	10	2.29	0.81-6.46	.115	1.64	0.51-5.28	.406
>Q75	>18.8	13	13	7.44	2.62-21.1	<.001[c]	8.38	2.60-27.0	<.001[c]

Abbreviations: CI = confidence interval; HR = hazard ratio; IL = interleukin; OS = overall survival; PFS = progression-free survival; Q = quartile.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.
[c]Statistically significant.

Supplemental Table 2 Cox Regression Analysis for PFS Grouped by Low or High Baseline IL-6, IL-18, MMP-9, MMP-2, YKL-40, Resistin, and HMGB1 Levels Using Median as Cutoff Value

Baseline	Value	No. Patients	No. Events	HR 1[a]			HR 2[b]		
				HR	95% CI	*P*	HR	95% CI	*P*
IL-6	pg/mL								
Low	≤1.8	27	15	1			1		
High	>1.8	26	16	0.84	0.40-1.73	.637	0.44	0.18-1.07	.071
IL-18	pg/mL								
Low	≤99.2	27	17	1			1		
High	>99.2	26	14	0.60	0.29-1.25	.176	0.71	0.31-1.60	.411
MMP-9	ng/mL								
Low	≤76.4	27	18	1			1		
High	>76.4	26	13	0.72	0.35-1.47	.370	0.56	0.25-1.29	.177
MMP-2	ng/mL								
Low	≤244.5	27	15	1			1		
High	>244.5	26	16	0.91	0.42-1.95	.810	0.80	0.37-1.74	.585
YKL-40	ng/mL								
Low	≤60.3	27	16	1			1		
High	>60.3	26	15	1.26	0.60-2.65	.536	0.951	0.40-2.22	.909
Resistin	ng/mL								
Low	≤13.4	27	15	1			1		
High	>13.4	26	16	1.44	0.69-2.99	.325	1.13	0.53-2.39	.749
HMGB1	ng/mL								
Low	≤7.1	27	15	1			1		
High	>7.1	26	16	1.28	0.60-2.71	.512	1.27	0.59-2.71	.535

Abbreviations: CI = confidence interval; HMGB1 = high-mobility group box 1; HR = hazard ratio; IL = interleukin; MMP = matrix metalloproteinase; PFS = progression-free survival.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.

Supplemental Table 3 PFS Analysis by Cox Regression for Study Patients Using Baseline IL-6, IL-18, MMP-9, MMP-2, YKL-40, Resistin, and HMGB1 Quartile Levels as Cutoff Values

Baseline	Value	No. Patients	HR 1[a]			HR 2[b]		
			HR	95% CI	*P*	HR	95% CI	*P*
IL-6	pg/mL							
<Q25	<0.7	15	1			1		
Q25-Q50	0.7-1.8	12	0.85	0.30-2.40	.767	1.38	0.33-5.65	.652
Q50-Q75	1.8-3.8	14	0.57	0.21-1.57	.286	0.31	0.09-1.05	.060
>Q75	>3.8	12	1.23	0.43-3.55	.692	1.02	0.27-3.80	.973
IL-18	pg/mL							
<Q25	<53.5	13	1			1		
Q25-Q50	53.5-99.2	14	1.23	0.45-3.36	.676	1.11	0.40-3.13	.831
Q50-Q75	99.2-264.3	14	0.65	0.24-1.75	.401	0.70	0.24-2.01	.516
>Q75	>264.3	12	0.65	0.23-1.87	.432	0.81	0.24-2.69	.733
MMP-9	ng/mL							
<Q25	<49.6	13	1			1		
Q25-Q50	49.6-76.4	14	1.02	0.40-2.60	.963	0.84	0.28-2.47	.756
Q50-Q75	76.4-129.6	13	0.82	0.31-2.18	.700	0.68	0.24-1.92	.474
>Q75	>129.6	13	0.61	0.20-1.89	.396	0.34	0.09-1.30	.118
MMP-2	ng/mL							
<Q25	<218.8	13	1			1		
Q25-Q50	218.8-244.5	14	1.35	0.46-3.90	.579	1.43	0.44-4.70	.548
Q50-Q75	244.5-284.0	13	1.05	0.34-3.18	.927	0.96	0.27-3.36	.959
>Q75	>284.0	13	1.15	0.35-3.75	.807	1.05	0.31-3.46	.935
YKL-40	ng/mL							
<Q25	<38.3	13	1			1		
Q25-Q50	38.3-60.3	14	0.99	0.35-2.78	.995	1.68	0.54-5.28	.368
Q50-Q75	60.3-113.3	13	1.30	0.42-3.98	.640	1.24	0.42-3.70	.688
>Q75	>113.3	13	1.23	0.43-3.49	.698	1.03	0.27-3.89	.962
Resistin	ng/mL							
<Q25	<11.4	13	1			1		
Q25-Q50	11.4-13.4	14	1.04	0.36-3.00	.931	1.43	0.40-5.06	.572
Q50-Q75	13.4-15.6	13	1.69	0.60-4.73	.315	2.06	0.61-6.99	.242
>Q75	>15.6	13	1.30	0.46-3.67	.609	1.01	0.33-3.06	.982
HMGB1	ng/mL							
<Q25	<5.1	13	1			1		
Q25-Q50	5.1-7.1	14	1.27	0.45-3.61	.643	0.74	0.24-2.30	.610
Q50-Q75	7.1-9.7	13	1.87	0.60-5.83	.281	2.29	0.62-8.45	.213
>Q75	>9.7	13	1.24	0.42-3.68	.688	0.76	0.24-2.35	.640

Abbreviations: CI = confidence interval; HMGB1 = high-mobility group box 1; HR = hazard ratio; IL = interleukin; MMP = matrix metalloproteinase; PFS = progression-free survival; Q = quartile.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.

Supplemental Table 4 Cox Regression Analysis for OS Grouped by Low or High Baseline IL-6, IL-18, MMP-9, MMP-2, YKL-40, Resistin, and HMGB1 Levels Using Median as Cutoff Value

Baseline	Value	No. Patients	No. Events	HR 1[a]			HR 2[b]		
				HR	95% CI	*P*	HR	95% CI	*P*
IL-6	pg/mL								
Low	≤1.8	27	19	1			1		
High	>1.8	26	20	1.10	0.57-2.12	.771	1.12	0.52-2.39	.771
IL-18	pg/mL								
Low	≤99.2	27	18	1			1		
High	>99.2	26	21	1.38	0.72-2.63	.319	1.21	0.60-2.43	.588
MMP-9	ng/mL								
Low	≤76.4	27	21	1			1		
High	>76.4	26	18	0.73	0.38-1.37	.330	0.52	0.26-1.03	.063
MMP-2	ng/mL								
Low	≤244.5	27	20	1			1		
High	>244.5	26	19	0.96	0.47-1.93	.910	1.42	0.66-3.05	.362
YKL-40	ng/mL								
Low	≤60.3	27	18	1			1		
High	>60.3	26	21	1.87	0.94-3.73	.071	1.41	0.66-2.99	.370
Resistin	ng/mL								
Low	≤13.4	27	19	1			1		
High	>13.4	26	20	1.13	0.60-2.12	.701	1.09	0.56-2.13	.784
HMGB1	ng/mL								
Low	≤7.1	27	20	1			1		
High	>7.1	26	19	0.93	0.47-1.86	.850	1.19	0.57-2.48	.626

Abbreviations: CI = confidence interval; HMGB1 = high-mobility group box 1; HR = hazard ratio; IL = interleukin; MMP = matrix metalloproteinase; PFS = progression-free survival.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.

Supplemental Table 5 OS Analysis by Cox Regression for Study Patients Using Baseline IL-6, IL-18, MMP-9, MMP-2, YKL-40, Resistin, and HMGB1 Quartile Levels as Cutoff Values

Baseline	Value	No. Patients	HR 1[a]			HR 2[b]		
			HR	95% CI	*P*	HR	95% CI	*P*
IL-6	pg/mL							
<Q25	<0.7	15	1			1		
Q25-Q50	0.7-1.8	12	2.66	1.04-6.77	.039[c]	1.64	0.48-5.60	.426
Q50-Q75	1.8-3.8	14	1.51	0.59-3.88	.386	0.92	0.31-2.65	.878
>Q75	>3.8	12	2.29	0.82-6.39	.111	2.23	0.76-6.54	.142
IL-18	pg/mL							
<Q25	<53.5	13	1			1		
Q25-Q50	53.5-99.2	14	0.67	0.26-1.73	.415	0.52	0.19-1.43	.205
Q50-Q75	99.2-264.3	14	0.80	0.31-2.00	.635	0.65	0.25-1.66	.369
>Q75	>264.3	12	1.62	0.66-3.96	.290	1.20	0.42-3.39	.729
MMP-9	ng/mL							
<Q25	<49.6	13	1			1		
Q25-Q50	49.6-76.4	14	0.50	0.20-1.21	.128	0.39	0.14-1.06	.067
Q50-Q75	76.4-129.6	13	0.50	0.20-1.23	.133	0.37	0.13-1.01	.054[c]
>Q75	>129.6	13	0.48	0.19-1.22	.128	0.22	0.07-0.68	.009[c]
MMP-2	ng/mL							
<Q25	<218.8	13	1			1		
Q25-Q50	218.8-244.5	14	1.93	0.75-4.99	.170	1.81	0.59-5.52	.295
Q50-Q75	244.5-284.0	13	1.21	0.41-3.56	.728	1.77	0.52-5.97	.356
>Q75	>284.0	13	1.73	0.61-4.91	.300	2.16	0.74-6.29	.158
YKL-40	ng/mL							
<Q25	<38.3	13	1			1		
Q25-Q50	38.3-60.3	14	1.56	0.56-4.32	.386	2.00	0.70-5.74	.195
Q50-Q75	60.3-113.3	13	1.97	0.63-6.06	.238	2.10	0.68-6.41	.191
>Q75	>113.3	13	3.08	1.10-8.61	.031[c]	2.13	0.65-6.97	.211
Resistin	ng/mL							
<Q25	<11.4	13	1			1		
Q25-Q50	11.4-13.4	14	1.10	4.31-2.83	.835	0.97	0.30-3.08	.966
Q50-Q75	13.4-15.6	13	1.22	0.47-3.13	.673	1.26	0.46-3.45	.645
>Q75	>15.6	13	1.17	0.45-3.03	.744	0.95	0.35-2.55	.919
HMGB1	ng/mL							
<Q25	<5.1	13	1			1		
Q25-Q50	5.1-7.1	14	1.81	0.73-4.44	.195	1.65	0.65-4.21	.288
Q50-Q75	7.1-9.7	13	1.22	0.46-3.24	.679	1.95	0.67-5.63	.217
>Q75	>9.7	13	1.35	0.49-3.68	.550	1.34	0.48-3.72	.568

Abbreviations: CI = confidence interval; HMGB1 = high-mobility group box 1; HR = hazard ratio; IL = interleukin; MMP = matrix metalloproteinase; PFS = progression-free survival; Q = quartile.
[a]HR adjusted for age.
[b]HR adjusted for age, menopause status, hormone receptor status, presence of visceral metastasis, number of metastatic lesions, and extent of disease.
[c]Statistically significant.