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Cytokine Comparisons Between Women With Breast Cancer and Women With a Negative Breast Biopsy

Debra E. Lyon, PhD, RN [Associate Professor], Nancy L. McCain, DSN, RN, FAAN [Professor], and Jeanne Walter, PhD, RN, FAAMA [Research Associate Professor]
Virginia Commonwealth University School of Nursing, Richmond

Christine Schubert, PhD [Assistant Professor]

Department of Biostatistics, School of Medicine, Virginia Commonwealth University School of Nursing, Richmond

Abstract

Background—Understanding the biological milieu associated with disease states has important implications for biobehavioral research. Cytokines, signaling molecules that mediate and regulate immunity, inflammation, and hematopoiesis, are an important component of the biological milieu associated with breast cancer. Cytokines have been used as biomarkers in research for prognosis and have been associated with symptoms and adverse outcomes in multiple conditions, including breast cancer. To date, however, the examination of cytokine patterns has been limited by traditional laboratory methods. Advances in proteomic technology now permit the characterization of a broader array of cytokines in a single specimen. Because cytokines operate in integrated networks, a more complete understanding will be gained as multiple cytokines can be examined for patterns of response that may be associated with symptoms and prognosis.

Objectives—To use proteomic technology (a) to examine whether there was a difference in cytokine levels and patterns in women with breast cancer compared with controls, (b) to define and compare the receiver operator characteristic curves for standard cytokine classifications, and (c) to identify the best-fitting empirical model of cytokines to distinguish groups of women found to have breast cancer from those with negative biopsies.

Methods—The cytokine levels of 35 women who had been diagnosed recently with breast cancer were compared with 24 women with a suspicious breast mass who were found subsequently to have a negative breast biopsy. Multiplex bead array assays permitted the simultaneous measure of multiple markers in a small volume of serum. Nonparametric procedures were used to determine differences in the median values and the distributions for each cytokine. The receiver operator characteristic curves were defined to identify patterns of cytokines.

Results—There were significantly higher systemic cytokine values in women with cancer in comparison with those in women without cancer for all cytokines measured, with the exception of granulocyte colony-stimulating factor and interferon-gamma. The only significant associations found between cytokines and age or race were increased levels of interleukin-8 ($r = .53$) and macrophage inflammatory protein-1 β ($r = .45$) with increased age in women with a negative biopsy. Three cytokines (granulocyte colony-stimulating factor, interleukin-6, and interleukin-17) distinguished between the breast cancer and no-cancer groups with an exceptionally high areas under the curve (0.981; $SE = 0.017$).

Discussion—Levels of cytokines and their patterns were markedly different in women with breast cancer as compared with those in women who did not have breast cancer. Results from this study highlight the need for further research to examine the levels and patterns of cytokines that may serve as biomarkers in clinical research. Innovations in proteomic technology have implications for expanding biobehavioral research.

Keywords

bioplex; breast cancer; cytokines

Biobehavioral research focuses on the study of interactions among biological factors, behavioral factors, and clinical outcomes including disease progression, symptom management, and quality of life. One of the major concerns when designing a biobehavioral research study is the selection of appropriate biological markers. Although multiple markers have been used to link biological phenomena to behavioral factors and symptoms, accumulating evidence is supporting the association of cytokines with adverse disease outcomes and distressing symptoms in multiple conditions, including breast cancer. To date, however, the examination of cytokines has been limited by traditional laboratory methods that usually measure a single or several cytokines per laboratory specimen. Because cytokines function in complex interrelationships with positive and negative feedback loops, it is important to understand the dynamic relationships among cytokines. Advances in proteomic technology now permit the characterization of a broader array of cytokines in a single specimen and have implications for expanding biobehavioral research.

Background

Cytokines are signaling molecules that mediate and regulate immunity, inflammation, and hematopoiesis (Thomson & Lotze, 2003). Although there are several classification systems for cytokines, a common categorization labels cytokines that promote inflammation as *proinflammatory cytokines*, whereas cytokines that suppress the activity of proinflammatory cytokines and reduce inflammation are called *antiinflammatory cytokines* (Dinarello, 2000). Most biobehavioral research has focused on measures of proinflammatory cytokines as biomarkers.

In persons with cancer, the immune response to tumor cells contributes to an adverse proinflammatory state (Coussens & Werb, 2002). High interleukin (IL)-6 levels have been linked consistently to poorer outcomes in the general population and in cancer populations (Zhang & Adachi, 1999). IL-6 is an autocrine and paracrine growth factor for several cancers, including breast cancer (Balkwill & Mantovani, 2001; Ben-Baruch, 2006). IL-6 may stimulate cancer cell growth and contribute to recurrence and metastasis in breast cancer (Leek & Harris, 2002). Elevated levels of circulating proinflammatory cytokines, including IL-1, IL-6 (Bachelot et al., 2003), IL-8 (Benoy et al., 2004), and IL-18 (Ben-Baruch, 2003), may be associated with shortened survival in persons with cancer, and IL-6 and IL-18 have been correlated with disease stage and progression in breast cancer (Rao, Dyer, Jameel, Drew, & Greenman, 2006). Whereas most studies have found elevated levels of cytokines in patients with cancer, a study of 90 patients with breast cancer and 15 healthy volunteers found no differences in baseline cytokine levels between patients with cancer and the healthy volunteers, as evidenced by plasma levels of IL-1 β , IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor- α (TNF- α) (measured using the Human Inflammatory Cytokine Cytometric Bead Array Kit from Becton Dickinson; Pusztai et al., 2004). However, many participants in that study had levels of cytokines below the measurement threshold. Although not focused on women with breast cancer, Gorelik et al. (2005) found significant differences in serum cytokine levels in 55 patients with early (Stages I–II) ovarian cancer, 55 women with benign pelvic disease,

and 55 healthy women using the Luminex xMAP instrument. Concentrations of IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), and vascular endothelial growth factor were significantly higher in patients with ovarian cancer as compared with those in controls.

Interleukin-17 is comprised of a family of six relatively recently identified potent proinflammatory cytokines (Aggarwal & Gurney, 2002). Interleukin-17A is considered to be a unique CD4⁺ T-cell-derived mediator of angiogenesis (Numasaki et al., 2003) that stimulates vascular endothelial cell migration and regulates the production of a variety of proangiogenic factors, such as TNF- α and vascular endothelial growth factor (Alexandrakis et al., 2006). Interleukin-17A mediates proinflammatory effects by stimulating the release of multiple other cytokines such as IL-6, IL-8, and G-CSFs from epithelial, endothelial, and fibroblastic cells (Fossiez et al., 1996). The overproduction of IL-17A has been found in ovarian cancers (Kato et al., 2001) and in patients with Stages II and III multiple myeloma (Alexandrakis et al.). To date, there have been few studies of IL-17 in breast cancer.

Along with proinflammatory cytokines, hematopoietic cytokines have important roles in individuals with cancer. Colony-stimulating factors are hematopoietic growth factors that stimulate the development of neutrophils and macrophages. Although there have been few studies of the levels of colony-stimulating factors in biobehavioral research, an elevated level of granulocyte monocyte colony-stimulating factor (GM-CSF) was noted in patients with breast cancer compared with that in a benign breast tumor group and a normal control group (Lawicki, Szmikowski, & Wojtukiewicz, 2006).

Cytokines and Symptoms

In addition to adversely affecting disease progression in persons with cancer, elevated levels of proinflammatory cytokines have been associated with distressing symptoms in individuals with breast cancer. Cytokines may play a role in the pathophysiology of neuropsychiatric symptoms through interactions among components of the immune and neuro-endocrine systems. Proinflammatory cytokines have been associated with depression in persons with cancer during treatment (Musselman et al., 2001) and in fatigue in survivors of breast cancer (Bower et al., 2000). Further study of cytokine–brain interactions are ongoing, with the cross-talk between the immune and nervous systems of intense interest in psychoneuro-immunology research (McCain, Gray, Walter, & Robins, 2005).

Although elevated levels of proinflammatory cytokines have been associated with adverse prognoses in individuals with cancer, findings concerning the relationships among proinflammatory cytokines and symptoms have been somewhat inconsistent. The lack of consistency of study findings could be due to the use of a variety of sources of cytokine specimens (serum, plasma, and cells), the differences in cellular activation (stimulated vs. non-stimulated), and the variations in assays which have different levels of sensitivity to high- and low-threshold values. Most studies to date have been limited also to individual or a small number of cytokines by the nature of conventional laboratory assays (enzyme-linked immunosorbent assays or radio immunoassays)—methods that are time intensive and that require considerable quantities of sera or cells to quantify cytokines. Before the advent of multiplex technology, measurement of each cytokine incurred considerable costs in time, money, and sample volume (Kofoed, Schneider, Scheel, Andersen, & Eugen-Olsen, 2006); thus, the systematic examination of cytokine patterns was limited.

To further develop the science of using cytokines as biomarkers to link potential biological processes and associated behavioral variables and symptoms, standardized measures that promote replicability and generalizability are needed. Cytometric-bead-based assays, a proteomic technology, enable simultaneous measurements of multiple analytes in sample volumes too small for traditional immunoassays (Morgan et al., 2004). The aims of this study

were to use cytometric-bead-based immunoassays (a) to examine whether there was a difference in cytokine levels and patterns in women with breast cancer compared with those in controls, (b) to define and compare the receiver operator characteristic (ROC) curves for standard cytokine classifications, and (c) to identify the best-fitting empirical model of cytokines to distinguish the breast cancer from the negative biopsy groups. The ROC curve is an efficient way to evaluate the predictive ability of a parameter with continuous values (Campbell, 1994) and is used to evaluate the discriminating power of a diagnostic test or statistical model (Liu, Li, Cumberland, & Wu, 2005).

Methods

Participants

The study involved 59 stored serum specimens of 35 women diagnosed with Stages I–III breast cancer and 24 women with suspicious breast masses who were found subsequently to have a negative breast biopsy. The participants were recruited from two university health systems in the mid-Atlantic region. Of the 35 women diagnosed with cancer, 31 women were recruited prior to their initial adjuvant chemotherapy (chemotherapy after surgery) and 4 women were recruited prior to receiving neoadjuvant chemotherapy (chemotherapy before surgery). Serum samples were obtained prior to the participants' first chemotherapy. Adjuvant chemotherapy patients were those who were at least in their 4th week postsurgery (lumpectomy or mastectomy). Samples from patients in the second group were obtained prior to a breast biopsy necessitated by a suspicious mammogram or ultrasound scan. All participants gave informed consent to the respective protocols, which were approved by the university's institutional review board.

Sample Collection and Assays

A sample of 10 μ l of serum was obtained from cryopreserved peripheral blood specimens. Handling and processing of specimens were similar for both groups of participants. Blood samples had been collected without anticoagulant into serum separator vacutainers and allowed to coagulate for 20 to 30 min at room temperature. Sera were separated by centrifugation, and all specimens were aliquoted immediately, frozen, and stored in a -70°C freezer.

The Bio-Plex[®] (Bio-Rad, Hercules, CA) multiplex assay system is a flexible, easy-to-use system that permits the simultaneous analyses of up to 100 different biomolecules in a single microplate well. Bio-Plex assays contain dyed beads conjugated with monoclonal antibodies specific for a target protein or peptide such as a cytokine. Each of the 100 spectrally addressed bead sets can contain a capture antibody specific for a unique target protein. The antibody-conjugated beads are allowed to react with a sample and a secondary, or detection, antibody in a microplate well to form a capture-type "sandwich" immunoassay. Multiplex assays can be created by mixing bead sets with different conjugated antibodies to test simultaneously for many analytes in one sample.

After incubation, the contents of each microplate well are drawn into the Bio-Plex array reader, and precision fluidics align the beads in a single file through a flow cell, where two lasers excite the beads individually. High-speed digital signal processors and Bio-Plex Manager software (Bio-Rad, Hercules, CA) record the fluorescent signals simultaneously for each bead, translating the signals into analyte concentration data for each assay. Daily system calibration and assay validation were performed using standardization methods developed by the manufacturer. Bio-Plex offers greatly improved sensitivity (10 pg/ml), reliability, recovery, and reproducibility in comparison with conventional enzyme-linked immunosorbent assays. For this study, the Bio-Plex Human 17-Plex was used. This standardized kit includes coupled beads, detection antibodies, and standards for the detection of IL-1 β , IL-2, IL-4, IL-5, IL-6,

IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, granulocyte-macrophage colony-stimulating factor, interferon-gamma (IFN- γ), monocyte chemoattractant protein-1, macrophage inflammatory protein-1 β , and TNF- α .

Descriptive Statistical Analyses

Because the cytokine data were skewed, nonparametric procedures (i.e., the Wilcoxon test and the Kolmogorov–Smirnov test) were used to determine differences in the median values and the distributions, respectively, for each cytokine between women with and without cancer. Interpretations focused on the central tendency of the data, as represented by the median values. Statistical analyses were done using SAS version 9.1.3, and the significance level was set at $\alpha < .05$. Logistic regression was used to determine how well the groups of cytokines were able to distinguish between women with and without cancer. Using the 17-Plex cytokine panel five groups of cytokines were considered for analysis: Type 1 cytokines (IL-2, IL-12, TNF- α , and IFN- γ), Type 2 cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13), proinflammatory cytokines (IL-1 β , IL-6, IL-8, IL-17, TNF- α , and IFN- γ), anti-inflammatory cytokines (IL-4, IL-10, and IL-12), and a best-fitting empirical model created through stepwise procedures. Each cytokine measure was log transformed to satisfy normality assumptions of the logistic regression. Finally, ROC curves of the five groups were plotted, and corresponding areas under the curve (AUC) were determined. The ROC curve is an efficient way to evaluate the predictive ability of a parameter with continuous values (Campbell, 1994). ROC curves may be used to classify individuals into one of two categories such as cancer or no-cancer groups. Each of these five groups of cytokines was compared with one another to determine which group might best distinguish between women with and women without breast cancer.

Results

Demographic and clinical characteristics of the study sample are presented in Table 1. The combined sample for this analysis included 43 Caucasian women and 15 African American women; there were 2 Asian women and 1 Hispanic woman, all three of whom had cancer. The cancer group did not differ significantly in mean age from the no-cancer group. There was more ethnic diversity in the cancer group than the no-cancer group. Descriptive statistics for each measured cytokine are presented in Table 2. The women with cancer had significantly higher median cytokine values than women without cancer for all cytokines measured except for G-CSF and IFN- γ . The remaining 15 medians were significantly different, as were the distributions. Data were stratified further by two ethnic groups, non-Hispanic White and non-Hispanic Black women. There were no significant differences in any cytokine between races within the cancer or no-cancer groups. In addition, there were no significant associations between cytokines and age, either overall or stratified by race or cancer status, with the exception of increased levels of IL-8 ($r = .53$) and macrophage inflammatory protein-1 β ($r = .45$) with increased age in women with a negative biopsy.

Resulting p values for each cytokine included in the model and the AUC for the associated ROC curve (plotted in Figures 1 and 2) are listed. Type 1 cytokines as a group had a fairly large AUC (0.83; $SE = 0.051$) but not quite as high as the Type 2, proinflammatory, or anti-inflammatory cytokines (Table 2). Two cytokines, log(IL-4) and log(IL-10), were significant predictors in both the Type 2 and anti-inflammatory models, and log(IL-17) was a significant predictor in the proinflammatory model. All three of these models had large AUC values, with the proinflammatory model having the highest (0.985; $SE = 0.015$).

The stepwise model was used to consider all cytokines measured to determine if there were combinations of these that might distinguish between women with and without cancer. The resulting model included the log values of IL-6, IL-17, and G-CSF. All of the terms in the stepwise model were significant. IL-6 had not been significant in either the Type 2 or

proinflammatory models, but IL-17 was significant in the proinflammatory model. G-CSF was not classified under any of the first four traditional groups of cytokines but, coupled with IL-6 and IL-17, created a diagnostic model for distinguishing between the cancer and no-cancer groups. This empirical model used fewer cytokines and had an exceptionally high AUC (0.981; $SE = 0.017$).

The ROC curves for all the five groups are plotted in Figures 1 and 2. Comparing traditional groupings (Type 1, Type 2, proinflammatory, and anti-inflammatory), Type 1 cytokines performed least favorably and proinflammatory cytokines performed most favorably in distinguishing between women with and without cancer (Figure 1). Comparing the best of these four ROC curves with the empirical model (Figure 2), the stepwise model contained fewer cytokines and performed similarly with the proinflammatory cytokines in distinguishing between the groups (Figure 3).

Discussion

These findings of significant differences in the cytokine levels between a group of women diagnosed with breast cancer and a group who received a negative breast biopsy report are quite different from those reported by Pusztai et al. (2004), who did not find a difference between patients with breast cancer and controls. The significant group differences in cytokine levels in our study share similarities with a recent study of serum cytokines in ovarian cancer by Gorelik et al. (2005), who found significant differences in serum cytokine levels in women with ovarian cancer as compared with controls. The elevated level of GM-CSF in the cancer group in this study is similar with the statistically significant increase in the levels of GM-CSF in patients with breast cancer compared with patients with benign breast tumor and the normal control group examined by Lawicki et al. (2006). Because colony-stimulating factors or hematopoietic growth factors have received little examination as biological markers in nursing research, further study is needed to define and characterize their patterns.

The addition of G-CSF to the empirical model may be surprising because there were no significant differences in G-CSF values between the two groups (Table 1). In fact, $\log(\text{G-CSF})$ in the regression model by itself was not significant, and its resulting ROC curve had an AUC of less than a chance value (0.48). However, once the effects of IL-6 and IL-17 were accounted for in the model, G-CSF was significant. Higher values of $\log(\text{G-CSF})$ were associated with the negative biopsy group compared with the women with cancer. In other words, G-CSF was not a significant predictor by itself. Instead, its effects were masked by the relationships between other cytokines, and once the levels of these cytokines were adjusted for, differences were observed in the levels of G-CSF between the two groups.

The stepwise model reported here is unique in that it contains only three cytokines, with very high AUC (0.981). This three-cytokine empirical model performed better than a model comprised of GM-CSF and CA 15-3, the most widely used serum marker for breast cancer (Duffy, 2001). Lawicki et al. (2006) examined the diagnostic utility of M-CSF and CA 15-3 to distinguish benign breast tumor and patients with breast cancer. They found AUC values for M-CSF and CA 15-3 of 0.801 and 0.785, respectively.

There are a few notable limitations to this study. A relatively small sample of patients with breast cancer was used, which limited the ability to examine accurately the relationships between stage of cancer and cytokine measures. There was no normal control sample to compare with samples from women with breast cancer and women with a benign biopsy. The type of surgery and time since surgery could have affected cytokine values in women with breast cancer. In addition, several women in the breast cancer group had not yet had surgery. However, in a study of 45 women with breast cancer (Stages I and II) who had either a

mastectomy or segmental resection, serum levels of IL-1 β decreased to baseline in 5 days, indicating a rapid return to baseline values after surgery (Mettler et al., 2004). All women with breast cancer were at least in their 4th week postsurgery. In addition to some variation in the surgical status of the women with breast cancer, baseline characteristics of study participants such as exercise, diet, and other factors that potentially affect cytokine values were not considered in the analysis.

Conclusions

Although understanding the impact of individual behavioral and genetic differences on immunologically relevant processes (Cohen, 2004) is now considered an important part of biobehavioral research, there has been less focus on understanding the disease-related immunological context in studies of ill populations. The striking differences found in the cytokine profiles indicate that immune parameters are quantitatively different in women with breast cancer compared with women who were found to have negative breast biopsies. Because both groups were living in a stressful situation (one group had been diagnosed with cancer, the other group was waiting for biopsy results to rule out cancer), there might be physiological differences in the cytokine profiles beyond what might be seen for immune responses to perceived stress. The cytokine pattern in women with cancer reveals an inflammatory milieu, which, in itself, warrants further investigation.

The current findings suggest that the use of single cytokines or of only traditional proinflammatory and anti-inflammatory subsets could reduce the potential utility for using cytokines to examine patterns of response to biobehavioral interventions. A broader focus on cytokine patterns may be necessary to better understand both within-subject and group-level differences over time.

Further expanding the use of cytokines as biomarkers is important for capturing the dynamic changes in integrated networks that may help to understand the reciprocal relationships that are important in biobehavioral research. The results of this study highlight the need for a broader conceptualization of cytokines than the traditional proinflammatory and anti-inflammatory classifications when using cytokines as biomarkers. Although this study was not powered to provide clinical prognostic utility, the findings highlight the need for a further study of the patterns of cytokines in women with breast cancer.

As biobehavioral research is undertaken, it is critical that investigators use precise methods and have appropriate control groups. Descriptive studies of immune function of virtually all groups are needed to define normal levels and expected changes over time that may be clinically significant. Although there are difficulties entailed in incorporating biomarkers such as levels of cytokines in research, nurse investigators have the requisite disciplinary perspective and knowledge to take a leadership role in further developing biobehavioral research.

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FIGURE 1.

Percentage difference in cytokines between cancer and negative biopsy groups. G-CSF = granulocyte colony-stimulating factor; IFN- γ = interferon-gamma; IL = interleukin; TNF- α = tumor necrosis factor-alpha; GM-CSF = granulocyte monocyte colony-stimulating factor; MIP-1 β = macrophage inflammatory protein; MCP-1 = monocyte chemoattractant protein-1.

FIGURE 2.

Receiver operator characteristic curves for Th1, Th2, proinflammatory, and anti-inflammatory cytokines. AUC and *SEs* are as follows: Th1 (IL-2, IL-12, TNF- α , and IFN- γ): AUC = 0.83; *SE* = 0.051; Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13): AUC = 0.97; *SE* = 0.021; proinflammatory (IL-1 β , IL-6, IL-8, IL-17, TNF- α , and IFN- γ): AUC = 0.985; *SE* = 0.015; anti-inflammatory (IL-4, IL-10, and IL-12): AUC = 0.964; *SE* = 0.023. Th1 = Type 1 cytokine; Th2 = Type 2 cytokine; AUC = areas under the curve; IL = interleukin; TNF- α = tumor necrosis factor-alpha; IFN- γ = interferon-gamma.

FIGURE 3.

Receiver operator characteristic curve for the stepwise model of the best combination of cytokines (IL-6, IL-17, and G-CSF; AUC = 0.981; SE = 0.017). IL = interleukin; G-CSF = granulocyte colony-stimulating factor; AUC = areas under the curve.

TABLE 1

Characteristics of the Sample

Variable	Cancer (<i>n</i> = 35)	Negative biopsy (<i>n</i> = 24)
Age, <i>M</i> (<i>SD</i>), (years)	48.5 (7.9)	49.9 (10.9)
Ethnicity		
Non-Hispanic White	23 (67)	18 (75)
Non-Hispanic Black	8 (24)	6 (25)
Hispanic	1 (3)	0
Asian or Pacific Islander	2 (6)	0
Stage of cancer		
I	8 (23)	
II	18 (52)	
III	5 (14)	
IV	0	
Neoadjuvant	4 (11)	

Note. The neoadjuvant group had not yet been staged. Values are in *n* (%) unless otherwise noted.

TABLE 2
Cytokine Values for the Breast Cancer and Negative Biopsy Groups (pg/ml)

Variable	Percentile		
	25th	50th	75th
No-cancer group (<i>n</i> = 24)			
Age (years)	43.0	48.5	53.5
IL-1 β	44.00	49.00	55.50
IL-2	27.00	36.75	56.75
IL-4	197.25	236.75	316.50
IL-5	21.00	23.00	24.50
IL-6	35.25	50.75	75.75
IL-7	53.65	63.00	69.00
IL-8	96.25	108.70	130.00
IL-10	63.70	76.50	87.00
IL-12 (p70)	61.75	65.00	70.25
IL-13	29.50	34.50	38.25
IL-17	118.00	130.00	142.75
G-CSF	69.75	85.50	115.00
GM-CSF	76.25	96.00	127.50
IFN- γ	28.00	34.00	43.00
MCP-1	129.25	178.75	230.20
MIP-1 β	1034.00	1416.40	1745.50
TNF- α	103.25	133.00	158.00
Cancer group (<i>n</i> = 35)			
Age (years)	44.00	50.00	54.00
IL-1 β	52.00	64.50	118.00
IL-2	48.00	119.00	340.50
IL-4	383.00	481.50	554.50
IL-5	23.00	26.00	33.00
IL-6	77.50	138.00	226.00
IL-7	78.00	90.00	97.00
IL-8	179.00	226.00	340.00
IL-10	108.50	121.00	137.00
IL-12 (p70)	74.00	87.00	94.00
IL-13	41.00	47.00	53.00
IL-17	146.00	165.50	184.00
G-CSF	75.50	85.00	105.00
GM-CSF	154.00	221.50	278.00
IFN- γ	36.00	38.00	46.00
MCP-1	348.00	663.00	923.00
MIP-1 β	2987.00	4237.50	7309.00
TNF- α	124.00	176.00	191.00

Note. IL = interleukin; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte monocyte colony-stimulating factor; IFN- γ = interferon-gamma; MCP-1 = monocyte chemoattractant protein-1; MIP-1 β = macrophage inflammatory protein; TNF- α = tumor necrosis factor-alpha.