Featured Article

Increased Serum Interleukin-8 in Patients with Early and Metastatic Breast Cancer Correlates with Early Dissemination and Survival

Ina H. Benoy,[1] Roberto Salgado,[1] Peter Van Dam,[1] Katrien Geboers,[1] Eric Van Marck,[1] Simon Scharpé,[2] Peter B. Vermeulen,[1] and Luc Y. Dirix[1]

[1]Translational Cancer Research Group Antwerp, University of Antwerp, General Hospital Sint-Augustinus, Wilrijk, Belgium; and [2]Medical Biochemistry, University of Antwerp, Wilrijk, Belgium

ABSTRACT

Purpose: **The prognostic significance of serum interleukin (IL)-8 was evaluated in patients with metastatic breast cancer. The predictive value of serum IL-8 for the presence of occult metastatic tumor cells in bone marrow aspirates was evaluated in patients with operable and metastatic breast cancer.**

Experimental Design: **Serum IL-8 was measured in healthy controls, patients with operable breast cancer, and patients with untreated, progressive metastatic breast cancer. In 69 patients with either operable or advanced breast cancer, occult cytokeratin-positive cells were counted in bone marrow aspirates.**

Results: **Serum IL-8 levels are increased in 67% (52 of 77) of patients with advanced breast cancer. Overall, these levels are significantly higher in patients with breast cancer compared with healthy volunteers ($P < 0.001$). The IL-8 levels increase significantly in patients with more advanced disease. An elevated serum IL-8 is related to an accelerated clinical course, a higher tumor load, and the presence of liver or lymph node involvement. A multivariate analysis indicates that serum IL-8 is an independent significant factor for postrelapse survival. There was a significant difference between serum IL-8 levels in patients with or without occult cytokeratin-positive bone marrow cells ($P < 0.04$). Serum IL-8 levels also showed an association with the number of these cells ($P < 0.01$).**

Conclusions: **Serum IL-8 is increased in patients with breast cancer and has an independent prognostic significance for postrelapse survival. The observations on the relationship between occult cytokeratin-positive bone marrow cells corroborate the concept of IL-8 acting as a contributor to the process of tumor cell dissemination. Similarly, the relationship between serum IL-8 and nodal stage at presentation deserves further study. These results further expand the concept that inflammation and inflammatory cytokines are critical components of tumor progression.**

INTRODUCTION

The contribution of inflammation and inflammatory cells to the process of tumor progression is increasingly being recognized (1). Several reports have elaborated on the involvement of chemokines in tumor growth, invasion, and metastasis (2). More specifically, interleukin (IL)-8, which bears the glutamic acid-leucine-arginine-positive motif and is therefore also considered an angiogenic chemokine, has received considerable attention (3–5). IL-8 is a member of the CXC chemokine family of related proinflammatory cytokines. IL-8 was originally identified as a chemoattractant for neutrophils (6–8). It is secreted by a variety of stromal cells, *e.g.,* endothelial cells and fibroblasts, and tumor cells, *e.g.,* melanoma, prostate, and endometrial tumor cells. IL-8 binds with high specificity to the CXR-1 and with less specificity to CXR-2 (9). Both receptors are expressed on stromal cells and tumor cells (10). A tumor-promoting role for IL-8 has been proposed in a wide variety of human solid tumors comprising malignant melanoma; non–small-cell lung cancer; malignant mesothelioma; head and neck squamous carcinoma; cervical and endometrial carcinoma; epithelial ovarian carcinoma; gastric, pancreatic, and colorectal carcinoma; hepatocellular carcinoma; androgen-independent prostate adenocarcinoma; renal cell carcinoma; breast cancer; and Kaposi's sarcomas (11–24).

The exact mechanisms by which IL-8 might promote tumor growth remain to be elucidated. An autocrine role of IL-8 modulating survival and proliferation of tumor cells has been suggested (2, 5). In non–small-cell lung cancer cells *in vivo*, blocking of IL-8 with an antibody decreased tumor growth by approximately 40%. *In vitro*, however, no alterations in tumor cell proliferation were observed (25). This is compatible with an IL-8–mediated stromal cell modulation of tumor growth. Autocrine and/or paracrine modulation of tumor growth might be further enhanced by an IL-8 mediated chemoresistance phenotype of the tumor cells (26).

IL-8 expression is enhanced by vascular endothelial growth factor (VEGF)-A, and both are modulated by hypoxia (27–29). VEGF is one of the most prominent hypoxia-induced growth factors involved in autocrine-mediated survival and paracrine-mediated endothelial cell survival and proliferation. More angiogenesis concurs with a higher vessel density, thus an increased propensity for metastasis, and enhanced paracrine

Received 4/26/04; revised 6/30/04; accepted 7/21/04.
Grant support: Fund for Scientific Research Flanders "Kredieten aan Navorsers" grant 1.5.158.03. I. Benoy is a Research Assistant of the Fund for Scientific Research Flanders.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: Luc Y. Dirix, Oncology Center Gasthuiszusters van Antwerpen, Oosterveldlaan 24, 2610 Wilrijk, Belgium. Phone: 32-3-4433648; Fax: 32-3-4433009; E-mail: luc.dirix@pandora.be.

growth stimulation of the tumor cells. Haraguchi *et al.* (19) have documented a high IL-8 concentration in veins draining colorectal tumors. This suggests that circulating IL-8 is derived, at least in part, from the primary tumor; whether it is produced by tumor cells, stromal cells, or both was not investigated. Interestingly, a positive association between the IL-8 concentration in the veins draining the tumor and mean vascular density was observed; this suggests that circulating IL-8 might mirror intratumoral, ongoing hypoxia-mediated angiogenesis. Considering the occurrence of both IL-8 receptors on both tumor cells and endothelial cells, enhanced expression of IL-8 might give rise to an additional stimulus for ongoing angiogenesis supporting tumor growth (10). Similar observations have been made in non–small-cell lung cancer and melanoma (11, 12, 30).

Additionally, IL-8 was primarily known to be chemotactic for neutrophils. Inflammatory infiltrates have been associated with enhanced tumor growth and worse survival (31). This might be attributed to the release of angiogenic growth factors by neutrophils and macrophages (32).

The role of IL-8 in breast cancer was investigated by examining the differential expression of the IL-8 receptors on endothelial and tumor cells in breast cancer tissue. All breast cancer cells expressed the IL-8 receptors CXR-1 and CXR-2, whereas only 50% of the benign breast tissue samples expressed either CXR-1 or CXR-2. Moreover, the endothelial cells also expressed both IL-8 receptors (10). This suggests that autocrine and paracrine interactions between the stromal cells and tumor cells might be mediated by IL-8. IL-8 mRNA transcripts have been measured in normal breast tissue and in both stromal and tumor cells of neoplastic breast tissue (23). Although no correlation was observed between the expression of IL-8 mRNA and clinicopathological variables such as tumor grade, patient age, or nodal status, a significantly higher expression was measured in neoplastic tissue compared with normal tissue.

Serum IL-8 measurements in breast cancer are scarcely documented. Yokoe *et al.* (33) have measured serum IL-8 in 12 heavily pretreated patients with recurrent breast cancer and reported a small increase of IL-8 in those patients with refractory progressive disease and almost no decrease in those with partial response or no change after systemic therapy.

We prospectively compared serum IL-8 levels in healthy controls, patients with localized breast cancer, and patients presenting with untreated and thus progressive metastatic disease. Furthermore, static clinicopathological variables, *e.g.,* hormone receptor status, and more dynamic variables such as tumor doubling time were recorded, and associations with IL-8 were studied. The prognostic importance of serum levels of IL-8 was also documented in these patients. Serum IL-8 was correlated with the presence or absence of micrometastasis in bone marrow.

PATIENTS AND METHODS

Patients and Therapy. This report concerns three groups of individuals. The control group consisted of 27 healthy female volunteers. Samples from this group were collected in 2000 and 2001. The first patient group consisted of 64 patients with breast cancer presenting with operable disease. The second group included 77 patients presenting in our clinic with untreated metastatic breast cancer. These patients were seen between May 1998 and March 2001. A third patient group includes 69 patients with untreated breast cancer (43 of the 64 patients with operable disease and 26 of the 77 patients with advanced disease). These 43 patients with localized disease were all those who consented to have a preoperative bone marrow aspiration, and the 26 patients from the advanced disease cohort are those who presented with non-pretreated metastatic breast cancer and consented to have a pretreatment bone marrow aspiration.

Clinicopathological Variables. The following clinicopathological variables were entered in a database for all patients: age, menopausal status, Karnofsky performance status, histologic type, tumor size, nodal status, and hormone receptor expression. For the 77 metastatic breast cancer patients, the extent of disease as assessed by clinical and imaging studies was also added to the database, along with previous adjuvant chemotherapy and endocrine treatment, disease-free interval, current therapy, presence of liver involvement, presence of lung metastases, pleural effusion, ascites, bone involvement, number of organs involved, estimation of tumor load, and tumor growth kinetics.

Tumor Load. An estimation of the extent of disease was attempted as described previously (34). For bone lesions, a whole body technetium scan at the time of data collection was used: (*a*) limited disease was defined as one to two hot spots, (*b*) moderate tumor load was defined as three to five separate hot spots, and (*c*) high tumor load was defined as more than five separate hot spots. For liver involvement, a standard contrast-enhanced computed tomography scan was used, and for pulmonary lesions, a standard contrast-enhanced computed tomography scan or a standard X-ray was used. These were scored as follows: (*a*) small volume of disease, $<10\%$ of the estimated organ volume was involved with disease; (*b*) moderate volume of disease, 10–25% of the estimated organ volume was involved with disease; and (*c*) large burden, more than one quarter of either the liver or lung was estimated to be replaced by tumor. Two investigators scored each patient separately. These different categories, although arbitrarily chosen, were predefined. A true validation of the assessment of tumor load was not performed.

Tumor Progression Kinetics. For the majority of patients, no clear progression kinetics were available. For 37 patients, this was the case. Tumor measurements were available at two different time points before the start of therapy. The time interval separating these two assessments ranged between 6 and 14 weeks. The majority of these assessments were made radiologically, and if numerous lesions were considered as target lesions, the most rapidly growing lesion determined the progression kinetics. In one patient with a supraclavicular node metastasis, assessment was made clinically by measuring nodal size. Disease progression was considered to be rapid when estimated tumor doubling time (duplication of surface area) in any lesion was <3 months, assuming linear progression kinetics.

Blood Collection. Blood was collected in a serum separator tube (Vacutainer; Becton Dickinson, San Jose, CA) and allowed to stand for 30 minutes at room temperature to ensure full clotting. All samples were subsequently centrifuged at $3{,}000 \times g$ for 5 minutes, and the supernatant was aliquoted and

stored at −80°C until further analysis. Plasma samples were collected in sterile tubes containing sodium citrate and centrifuged and stored in the same manner. Serum IL-8 concentration was determined with an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). VEGF and basic fibroblast growth factor (bFGF) measurements were performed as described previously (34). The half of the detection limit value of the patient samples was used for statistical analysis in case the measured values did not reach the detection limit of the assay. The detection limits of the assays were 10.0 pg/mL for IL-8, 0.22 pg/mL for bFGF, and 9 pg/mL for VEGF. Within-assay reproducibility has been tested previously (35).

Bone Marrow Preparation and Immunostaining. After providing signed informed consent, patients underwent bone marrow aspiration from the posterior iliac crest, a procedure approved by the institutional ethical boards. In brief, 9 mL of bone marrow aspirate were collected in heparin-coated tubes. After centrifugation using a Ficoll-Paque density gradient, the mononucleated interphase cells were isolated and washed twice with PBS. Cells (5×10^5 cells per spot) were centrifuged onto Superfrost-coated glass slides. Micrometastatic cancer cells were then detected immunohistochemically using the Epimet Cell Detection Kit (Baxter Inc., Unterschleissheim, Germany). Cells were identified as disseminated epithelial cells according to the European International Society for Hematotherapy and Graft Engineering Working Group for Standardization of Tumor Cell Detection (36). A total of 2 million cells per patient were screened microscopically by two independent observers. Results were expressed as the number of positive cells per million MNC.

Fourteen patients with a hematologic malignancy who underwent bone marrow sampling for diagnostic purposes were considered as appropriate negative control patients.

Statistical Analysis. Statistical analysis was performed with Graphpad Prism version 2.0 (Graphpad Software, Inc., San Diego, CA). Half the detection limit value of the patient samples was used for statistical analysis in case the measured values did not reach the detection limit of the assay. The detection limit of the Il-8 assay was 10 pg/mL. Comparisons of continuous variables were performed with the Mann-Whitney *U* test. The relation between continuous variables was analyzed with a Spearman rank correlation analysis. Means and SDs are given. $P < 0.05$ was considered significant. Survival curves were plotted by the Kaplan-Meier method, and log-rank comparison was performed to compare differences in survival. Multivariate analysis was performed with Cox regression to discern which variables yield independent predictive value on survival.

Ethical Committee. The local ethical committee approved this study, and written informed consent was obtained from all patients involved.

RESULTS

Serum Interleukin-8 in Female Volunteers and Breast Cancer Patients. In 27 healthy volunteers the mean serum IL-8 was 5.4 pg/mL (median, 5.0 pg/mL; 95% confidence interval, 4.826–5.989) with a range from 5.0 to 10.8 pg/mL (Fig. 1). In the 64 patients with operable disease, serum IL-8 increased to a mean value of 8.3 pg/mL (median, 5.0 pg/mL).

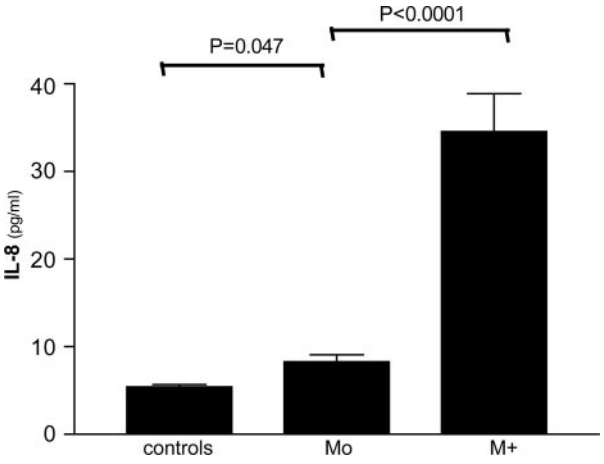

Fig. 1 Serum IL-8 levels in healthy volunteers (*controls*), operable breast cancer patients (*M0*) and patients with progressive metastatic breast cancer (*M+*).

The difference in serum IL-8 between the controls and patients with operable breast cancer is significant ($P = 0.047$). Serum IL-8 increased in patients with progressive metastatic breast cancer to a mean value of 34.54 pg/mL (median, 17.2 pg/mL) ranging from 5.0 to 211.8 pg/mL. Again, this differed significantly from both controls ($P < 0.001$) and patients with operable disease ($P < 0.001$).

Serum Interleukin-8 in Patients with Metastatic Breast Cancer (Univariate Analysis). More than 67% (52 of 77) of patients with metastatic breast cancer had increased serum IL-8 levels. Within this group, serum IL-8 levels increased gradually, according to tumor load ($P < 0.0001$) and tumor progression kinetics ($P < 0.0114$; Table 1). Patients with liver or lymph node involvement also had higher IL-8 levels compared with those without liver ($P < 0.0004$) or lymph node ($P = 0.0068$) involvement. This association between serum IL-8 and liver metastasis was independent of tumor load. Serum IL-8 did not differ according to the number of metastatic sites involved or the presence or absence of a pleural effusion. With regard to clinicopathological data at initial diagnosis, a striking correlation between nodal status and serum IL-8 was observed ($P < 0.0068$). There were no associations between serum IL-8 and age at diagnosis, T stage at presentation, hormone receptor status, menopausal status, histology, adjuvant chemotherapy or endocrine treatment, or disease-free interval.

Serum Interleukin-8 and Prognosis in Patients with Metastatic Breast Cancer. Univariate and multivariate analyses were performed in patients to evaluate the prognostic importance of IL-8 (Fig. 2). Serum samples were taken from untreated patients at the time of diagnosis. When dichotomized, using the median values of IL-8 (17.2 pg/mL), survival was significantly shorter for patients with IL-8 levels above this median value in univariate analysis ($P = 0.0045$). The following variables were entered for multivariate logistic regression analysis: tumor load, progression kinetics, number of sites involved, IL-8 values, T stage, N stage, hormone receptor status, menopausal status, histology, liver involvement, pleural effusion, and

Table 1 Serum IL-8 levels in patients with untreated metastatic breast cancer.

	N	Il-8 (pg/mL)	*P*
Tumor load			
1	18	13.82 ± 25.25	
2	34	30.83 ± 37.59	*<0.0001*
3	16	76.28 ± 59.96	
Progression			
<3	26	54.31 ± 57.56	*0.0114*
>3	11	14.81 ± 15.36	
No. of organs involved			
1	21	28.12 ± 46.54	0.11
>1	56	39.95 ± 45.52	
T stage			
T_1/T_2	56	38.73 ± 49.92	0.35
T_3/T_4	12	19.93 ± 19.67	
N stage			
N_0	31	30.25 ± 46.79	*0.0068*
N_1	37	48.24 ± 54.60	
Hormone receptor status			
+	56	48.77 ± 60.57	0.20
−	18	32.27 ± 40.41	
Menopausal status			
Premenopausal	27	35.76 ± 47.75	0.52
Postmenopausal	48	42.08 ± 52.04	
Histology			
IDA	59	37.45 ± 48.95	0.42
ILA	12	36.70 ± 31.47	
Liver status			
M_0	37	24.34 ± 39.52	*0.0004*
M+	40	48.18 ± 48.64	
Pleural effusion			
No	63	34.84 ± 45.70	0.07
Yes	14	45.21 ± 47.0	

NOTE. *N* = number of patients. Values for IL-8 are the mean ± SD. Significant *P* values are italicized.

Abbreviations: IDA, invasive ductal adenocarcinoma; ILA, invasive lobular adenocarcinoma.

differentiation grade. Serum IL-8 was shown to have independent prognostic significance ($P = 0.007$).

Serum Interleukin-8 and Micrometastasis in Breast Cancer Patients. Using a cytokeratin staining for the detection of occult epithelial cells in bone marrow aspirates, we were unable to retain any positive staining cell in the bone marrow aspirates of 14 patients with benign and malignant hematologic conditions (data not shown). In the bone marrow aspirates of 69 patients with different stages of breast cancer, cytokeratin-positive cells could be detected in 24 patients (35%). The number of positive cells ranged between 0.5 and 122 per million mono nuclear cells. Patients with positive bone marrow involvement had increased serum IL-8 levels compared with those without bone marrow involvement ($P = 0.0334$). The significance of this relationship persisted when only the 43 patients with locoregional disease were considered. In these patients, serum and plasma VEGF and serum bFGF were also determined, and neither showed any relationship with the presence of cytokeratin-positive cells (data not shown). In these 43 patients, a trend was observed between nodal status, as evaluated by routine histology, and serum IL-8 ($P = 0.078$). In the group of 69 patients, serum IL-8 correlated with the actual number of cytokeratin-positive cells ($r = 0.32$; $P = 0.0082$).

DISCUSSION

Members of the chemokine family have been observed to contribute to both growth and progression of different types of human cancer. Chemokines are divided into two major subfamilies (CC and CXC) based on the position of their NH_2-terminal cysteine residues, and they bind to seven transmembrane domain G-protein–coupled receptors, whose two major subfamilies are designated CCR and CXCR. Muller *et al.* (37) provided convincing evidence that a leukocyte chemoattractant receptor named CXCR4 and its ligand, CXCL12 (SDF1α), together govern the pattern of breast cancer metastasis in an animal model. IL-8 is a member of the CXC chemokine family; it is chemotactic for leucocytes and activates leukocytes. IL-8 also has mitogenic, angiogenic, and motogenic properties in different cancer models. Green *et al.* (23) evaluated differences in mRNA transcripts for 13 different cytokines between 56 normal breast tissues and 73 neoplastic breast tissues using reverse transcription-polymerase chain reaction. The only correlation in this study was a higher IL-8 mRNA level in the neoplastic breast tissue. De Larco *et al.* (38) showed a strong correlation between the metastatic potential of breast carcinoma cell lines and their ectopic expression of IL-8. They continued to show that increased IL-8 expression in metastasis-prone cell lines is possibly caused by an atypical epigenetic mechanism, whereby upstream CpG methylation, rather than promoter methylation, results in increased IL-8 production (39). More recent data have suggested that IL-8 plays a major role in the predilection of breast cancer for metastasis to bone and that IL-8 expression in breast cancer relates to estrogen receptor negativity (40, 41).

In patients with breast cancer, data on circulating levels of IL-8 are scarce, and the prognostic relevance of circulating IL-8 has not been reported. The comparative analysis of the IL-8 profile in healthy volunteers, patients with local disease, and those with progressive metastatic disease demonstrates that healthy volunteers have significantly lower levels of IL-8 than both patient groups. Moreover, these findings also indicate that advanced disease gives rise to higher circulating levels of IL-8.

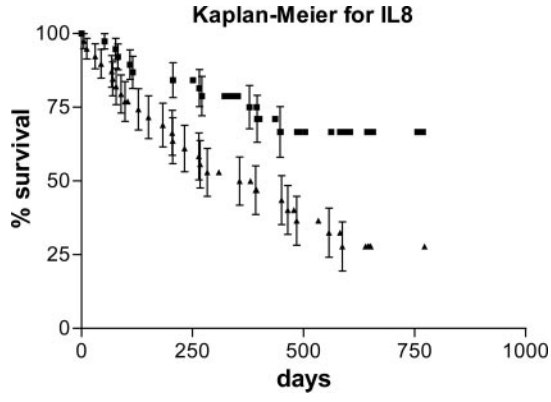

Fig. 2 Overall postrelapse survival of 77 patients with metastatic breast cancer. ■, IL8 < med; ▲, IL8 > med. Log rank test: p = 0.0045; N = 77.

This study clearly defines the independent prognostic significance of circulating IL-8 in patients presenting with metastatic breast cancer. It is important to remember that this cohort of patients was unselected and untreated, with the exception of adjuvant therapy, for their disseminated disease. This is important because breast cancer cells treated with cytotoxic agents have an altered cytokine expression profile exhibiting higher IL-8 levels, leading to enhanced chemoresistance of the tumor cells. Our data suggest that both an increased tumor bulk and a more malignant phenotype of the tumor may account for the gradual rise in circulating levels of IL-8.

This is elaborated by the highly significant association between higher tumor load and circulating IL-8, confirming the previous association seen in other tumors, *e.g.,* melanoma and renal cell carcinoma, and by the consistent finding of high IL-8 being positively associated with the kinetics of progression. A shorter clinical tumor doubling time, considered as a measure of the malignant phenotype, as well as tumor bulk correlated with circulating IL-8 levels. Although a higher tumor load does not necessarily correlate with the quantity of tumor cells, the notion that both tumor cells and stromal cells produce IL-8 lends further evidence for this statement. Moreover, the higher IL-8 levels found in patients with liver metastasis suggest that metastatic tumor cells continue to produce IL-8. However, this might also be in accordance with a decreased clearance of IL-8 by hepatic cells. Nevertheless, the gradual rise of IL-8 with tumor progression and the association of IL-8 with tumor load and tumor doubling time suggest that serum IL-8 mirrors both tumor cell mass and tumor aggressiveness. When analyzing clinicopathological variables at presentation, higher IL-8 levels concurred with nodal metastasis. It is tempting to speculate in this case that IL-8 contributed to early metastasis in these patients. In the 43 patients with operable breast cancer, a trend was observed between nodal status, as evaluated by routine histology, and serum IL-8 ($P = 0.078$). We failed to observe a relation between serum IL-8 and hormone receptor status, both in each separate cohort and when both cohorts were analyzed together. In summary, these observations concur with the notion that increased serum IL-8 in patients with metastatic breast cancer is associated with a worse clinical outcome. When corrected for other variables, an independent prognostic significance was retained after multivariate logistic regression analysis. Additionally, high circulating IL-8 levels correlated with poor response to systemic chemotherapy in patients with epithelial ovarian cancer. Yokoe *et al.* (33) described the occurrence of sustained higher circulating IL-8 levels in those breast cancer patients who did not respond to therapy. Investigations considering chemoresponsiveness in patients with low and high IL-8 levels are ongoing.

We furthermore analyzed whether serum IL-8 would predict for the presence of occult tumor cells in bone marrow. In a group of 69 patients with different stages of breast cancer who consented to have their marrow aspirated before any type treatment, cytokeratin-positive cells were detected in 24 patients (35%). Increased serum IL-8 predicted for the presence of cytokeratin-positive cells in bone marrow ($P = 0.0334$). The significance of this relationship persisted when only the 43 patients with locoregional disease were considered. Other angiogenic cytokines (serum VEGF, plasma VEGF, and bFGF) failed to show any such relation. In these 69 patients, serum IL-8 strongly correlated with the actual number of cytokeratin-positive cells ($r = 0.32$; $P = 0.0082$).

This observation renders clinical support to the observations made by De Larco *et al.* (38, 39). It is reasonable to equate the presence of disseminated tumor cells in bone marrow as the precursor of actual bone metastases. The role of IL-8 in the predilection of breast carcinoma cells for bone tissue metastasis has been pioneered by Bendre *et al.* (40). These investigators have shown that recombinant IL-8 induces the expression of RANKL mRNA and protein in osteoblastic cells and stimulates formation of bone-resorbing osteoclasts, even in the absence of RANKL. In this model, the role of parathyroid hormone-releasing protein would then be a critical event later on, to stimulate the vicious cycle of bone destruction.

In summary, this study renders clinical support for the role of IL-8 in clinical progression of human breast cancer, both at early stage and in patients with advanced disease. Furthermore, it increases the evidence for the critical role of inflammation and its mediators in the process of tumor progression.

ACKNOWLEDGMENTS

We are grateful to Hilde Elst and the personnel of the laboratory of clinical chemistry from the General Hospital Sint-Augustinus for excellent technical assistance.

REFERENCES

1. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.

2. Wang JM, Deng X, Gong W, Su S. Chemokines and their role in tumor growth and metastasis. J Immunol Methods 1998;220:1–17.

3. Strieter RM, Kunkel SL, Elner VM, et al. Interleukin-8. A corneal factor that induces neovascularization. Am J Pathol 1992;141:1279–84.

4. Koch AE, Polverini PJ, Kunkel SL, et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science (Wash DC) 1992; 258:1798–801.

5. Lindley IJD. Interleukin-8. In: Mire-Sluis A, Thorpe R, editors. Cytokines. London: Academic Press; 1998. p. 125–40.

6. Sozzani S, Locati M, Allavena P, Van Damme J, Mantovani A. Chemokines: a superfamily of chemotactic cytokines. Int J Clin Lab Res 1996;26:69–82.

7. Yoshimura T, Matsushima K, Tanaka S, et al. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci USA 1987;84:9233–7.

8. Walz A, Peveri P, Aschauer H, Baggiolini M. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem Biophys Res Commun 1987;149:755–61.

9. Rossi D, Zlotnik A. The biology of chemokines and their receptors. Annu Rev Immunol 2000;18:217–42.

10. Miller LJ, Kurtzman SH, Wang Y, et al. Expression of interleukin-8 receptors on tumor cells and vascular endothelial cells in human breast cancer tissue. Anticancer Res 1998;18:77–81.

11. Huang S, Mills L, Mian B, et al. Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. Am J Pathol 2002;161: 125–34.

12. Yuan A, Yu CJ, Luh KT, et al. Aberrant p53 expression correlates with expression of vascular endothelial growth factor mRNA and interleukin-8 mRNA and neoangiogenesis in non-small-cell lung cancer. J Clin Oncol 2002;20:900–10.

13. Galffy G, Mohammed KA, Dowling PA, et al. Interleukin 8: an autocrine growth factor for malignant mesothelioma. Cancer Res 1999; 59:367–71.

14. Chen Z, Malhotra PS, Thomas GR, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. Clin Cancer Res 1999;5:1369–79.

15. Fujimoto J, Sakaguchi H, Aoki I, Tamaya T. Clinical implications of expression of interleukin 8 related to angiogenesis in uterine cervical cancers. Cancer Res 2000;60:2632–5.

16. Fujimoto J, Aoki I, Khatun S, Toyoki H, Tamaya T. Clinical implications of expression of interleukin-8 related to myometrial invasion with angiogenesis in uterine endometrial cancers. Ann Oncol 2002;13:430–4.

17. Davidson B, Goldberg I, Kopolovic J, et al. Expression of angiogenesis-related genes in ovarian carcinoma: a clinicopathologic study. Clin Exp Metastasis 2000;18:501–7.

18. Kitadai Y, Haruma K, Sumii K, et al. Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. Am J Pathol 1998;152:93–100.

19. Haraguchi M, Komuta K, Akashi A, et al. Elevated IL-8 levels in the drainage vein of resectable Dukes' C colorectal cancer indicate high risk for developing hepatic metastasis. Oncol Rep 2002;9:159–65.

20. Ren Y, Poon RT, Tsui HT, et al. Interleukin-8 serum levels in patients with hepatocellular carcinoma: correlations with clinicopathological features and prognosis. Clin Cancer Res 2003;9:5996–6001.

21. Inoue K, Slaton JW, Eve BY, et al. Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. Clin Cancer Res 2000;6:2104–19.

22. Slaton JW, Inoue K, Perrotte P, et al. Expression levels of genes that regulate metastasis and angiogenesis correlate with advanced pathological stage of renal cell carcinoma. Am J Pathol 2001;158:735–43.

23. Green AR, Green VL, White MC, Speirs V. Expression of cytokine messenger RNA in normal and neoplastic human breast tissue: identification of interleukin-8 as a potential regulatory factor in breast tumours. Int J Cancer 1997;72:937–41.

24. Masood R, Cai J, Tulpule A, et al. Interleukin 8 is an autocrine growth factor and a surrogate marker for Kaposi's sarcoma. Clin Cancer Res 2001;7:2693–702.

25. Smith DR, Polverini PJ, Kunkel SL, et al. Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. J Exp Med 1994; 179:1409–15.

26. Duan Z, Feller AJ, Penson RT, Chabner BA, Seiden MV. Discovery of differentially expressed genes associated with paclitaxel resistance using cDNA array technology: analysis of interleukin (IL) 6, IL-8, and monocyte chemotactic protein 1 in the paclitaxel-resistant phenotype. Clin Cancer Res 1999;5:3445–53.

27. Rofstad EK, Halsor EF. Hypoxia-associated spontaneous pulmonary metastasis in human melanoma xenografts: involvement of microvascular hot spots induced in hypoxic foci by interleukin 8. Br J Cancer 2002;86:301–8.

28. Belperio JA, Keane MP, Arenberg DA, et al. CXC chemokines in angiogenesis. J Leukocyte Biol 2000;68:1–8.

29. Kunz M, Hartmann A, Flory E, et al. Anoxia-induced up-regulation of interleukin-8 in human malignant melanoma. A potential mechanism for high tumor aggressiveness. Am J Pathol 1999;155:753–63.

30. Ugurel S, Rappl G, Tilgen W, Reinhold U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J Clin Oncol 2001;19: 577–83.

31. Coussens LM, Werb Z. Inflammation and cancer. Nature (Lond) 2002;420:860–7.

32. Chen JJ, Yao PL, Yuan A, et al. Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. Clin Cancer Res 2003;9:729–37.

33. Yokoe T, Iino Y, Morishita Y. Trends of IL-6 and IL-8 levels in patients with recurrent breast cancer: preliminary report. Breast Cancer 2000;7:187–90.

34. Dirix LY, Salgado R, Weytjens R, et al. Plasma fibrin D-dimer levels correlate with tumour volume, progression rate and survival in patients with metastatic breast cancer. Br J Cancer 2002;86:389–95.

35. Salgado R, Benoy I, Weytjens R, et al. Arterio-venous gradients of IL-6, plasma and serum VEGF and D-dimers in human cancer. Br J Cancer 2002;87:1437–44.

36. Borgen E, Naume B, Nesland J, et al. Standardization of the immunocytochemical detection of cancer cells in BM and blood. I. Establishment of objective criteria for the evaluation of immunostained cells. Cytotherapy 1999;1:377–88.

37. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature (Lond) 2001;410:50–6.

38. De Larco JE, Wuertz BR, Rosner KA, et al. A potential role for interleukin-8 in the metastatic phenotype of breast carcinoma cells. Am J Pathol 2001;158:639–46.

39. De Larco JE, Wuertz BR, Yee D, Rickert BL, Furcht LT. Atypical methylation of the interleukin-8 gene correlates strongly with the metastatic potential of breast carcinoma cells. Proc Natl Acad Sci USA 2003;100:13988–93.

40. Bendre MS, Gaddy-Kurten D, Mon-Foote T, et al. Expression of interleukin 8 and not parathyroid hormone-related protein by human breast cancer cells correlates with bone metastasis in vivo. Cancer Res 2002;62:5571–9.

41. Freund A, Chauveau C, Brouillet JP, et al. IL-8 expression and its possible relationship with estrogen-receptor-negative status of breast cancer cells. Oncogene 2003;22:256–65.