Short Communication

Serum Insulin-Like Growth Factor-I and Platelet-Derived Growth Factor as Biomarkers of Breast Cancer Prognosis

Patrizia Pasanisi,[1] Elisabetta Venturelli,[1] Daniele Morelli,[2] Luigi Fontana,[3] Giorgio Secreto,[1] and Franco Berrino[1]

[1]Department of Preventive and Predictive Medicine and [2]Unit of Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy and [3]Division of Food Science, Human Nutrition and Health, Istituto Superiore di Sanitá, Rome, Italy and Division of Geriatrics and Center for Human Nutrition, Washington University School of Medicine, St. Louis, Missouri

Abstract

Epidemiologic studies have shown that growth factors and inflammatory mechanisms may affect breast cancer risk and prognosis. The present analysis on 110 postmenopausal breast cancer patients tested if serum insulin-like growth factor I (IGF-I), platelet-derived growth factor (PDGF), fructosamine, and C-reactive protein, a serum marker of inflammation, are associated with breast cancer relapse. The risk of adverse events after 5.5 years of follow-up was examined by Cox proportional hazards modeling, controlling for hormone receptor status, stage at diagnosis, and for body weight and serum testosterone level, which were known to significantly affect prognosis. PDGF and, to a lesser extent, IGF-I were positively but not significantly associated with the risk of breast cancer recurrence. By combining PDGF and IGF-I, however, the adjusted hazard ratio of recurrence among the women with both PDGF and IGF-I levels > their median values (respectively, 9.3 and 174.4 ng/mL) was 6.4 (95% confidence interval, 1.5-26.7) compared with the women with PDGF and IGF-I levels ≤ their median values. Fructosamine and C-reactive protein were not associated with recurrences. The results suggest that PDGF may be an important prognostic factor for breast cancer and that IGF-I may increase the risk of recurrence in the presence of high PDGF levels. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1719–22)

Introduction

Western diet rich in saturated and partially hydrogenated fat, refined carbohydrates, and animal protein is associated with high prevalence of overweight/obesity, metabolic abnormalities, and high serum levels of hormones and growth factors that may affect both breast cancer risk (1, 2) and prognosis (1, 3-5). Overweight is associated with inflammation (6), and there is experimental and epidemiologic evidence that inflammatory mechanisms that are mediated by specific dietary components and caloric intake (7) may contribute to breast cancer etiology (8, 9).

In the present study, we followed up women enrolled into a previously published dietary intervention trial aimed at reducing sex-hormone levels in postmenopausal breast cancer patients (3, 10) to test if the baseline serum levels of serum insulin-like growth factor I (IGF-I), platelet-derived growth factor (PDGF), fructosamine, and serum markers of inflammatory response [C-reactive protein (CRP)] are associated with breast cancer relapse.

Received 7/20/07; revised 2/21/08; accepted 4/14/08.

Grant support: The Diet and Androgens 2 study was financed by the Europe Against Cancer Programme of the European Union and the Italian Association for Cancer Research.

Requests for reprints: Patrizia Pasanisi, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, Milan, 20133 Italy. Phone: 39-02-23903513; Fax: 39-02-23903516.
E-mail: patrizia.pasanisi@istitutotumori.mi.it

doi:10.1158/1055-9965.EPI-07-0654

Patients and Methods

Patients. One hundred and ten women (mean age ± SD, 56.8 ± 5.6 y) operated for breast cancer at least a year previously (4.6 ± 4.4 y on average), not undergoing chemotherapy, with at least one ovary, and with no clinical evidence of disease recurrence, volunteered to participate in a dietary intervention study (Diet and Androgens 2) that required the modification of their usual diet over a year to reduce insulin and sex-hormone levels (3, 10). The patients had to be postmenopausal, defined as absence of menses for at least 2 y and by a postmenopausal gonadotropin and estrogen profile.

The women were requested to reduce their sources of refined carbohydrates and saturated fats and to increase their intake of whole grain cereals and pulses. All patients signed an informed consent, and the study was approved by the Institutional Review Board and the Ethics Committee of the Milan National Cancer Institute.

Compliance was monitored through repeated 24 h dietary diaries and anthropometric measurements. None of the participants abandoned the study. They significantly reduced body weight, serum insulin and testosterone levels, and the prevalence of metabolic syndrome (5, 10). They were followed up for 5.5 y, and recurrences and contralateral breast cancer were registered. High baseline levels of testosterone and body weight were significantly associated with breast cancer prognosis (3).

Blood Sampling and Laboratory Measurements. Baseline blood samples were collected at recruitment, before any dietary modification.

Blood samples were collected between 8 a.m. and 9 a.m. after overnight fasting to reduce intraindividual variation in hormone levels and were stored at −80°C. The technicians analyzing the serum samples were blinded to the outcome recurrence status of the patients. A quality control serum sample was measured at the beginning and at the end of the batch. Serum growth factor levels were measured in duplicate through commercial radioimmunoassay kits from Biosource for IGF-I and enzyme immunoassay from R&D for PDGF. We decided to measure the PDGF-AB isoform because its circulating levels are much higher than those of the other PDGF isoforms. Furthermore, PDGF-AB binds to PDGF receptors with nearly 10-fold higher affinity than does PDGF-AA or PDGF-BB (11).

Fructosamine and CRP were assayed with a Hitachi modular automatic analyzer (Roche).

The coefficients of intraassay and interassay variation, respectively, were: 2.8 and 5.3% for a mean IGF-I level of 304 ng/mL, 3.6 and 7.5% for a mean PDGF level of 25.9 ng/mL, 0.9 and 2.9% for a mean fructosamine level of 288 μmol/L, and 1.34 and 5.7% for a mean CRP level of 0.5 mg/L.

Statistical Analysis. The statistical analysis focused on the risk of breast cancer recurrences (local relapse, distant metastasis, and contralateral breast cancer) in relation to the baseline levels of the variables under study. The means of the baseline serum levels of IGF-I, PDGF, CRP, and fructosamine in patients who recurred were compared with those of patients who did not. The prognostic effect of all these parameters on risk of recurrence was assessed by hazard ratios (HR) and 95% confidence intervals (95% CI) through the Cox proportional hazards model. The following covariates were considered as potential confounders: age (in tertiles), years since diagnosis, extent of disease at diagnosis, as size of the primary (T1, T2, or more) and axillary node status (N-, N+), and estrogen and progesterone receptor status (positive, negative). Because 38% of the women were under tamoxifen treatment, which decreases IGF-I levels (12), we considered current tamoxifen treatment (yes or no) as a potential confounder. T, N, and hormone receptor status proved to be significant confounders and were retained in the analytic model. Because our previous results showed that body weight and serum testosterone levels were strongly and significantly associated with recurrences (3), adjustment for these variables (in tertiles) was also done in the present analysis. We also examined the effect of each growth factor on recurrences controlling for the other one, and we tested their interaction. The proportional hazards assumption was checked for each model by scaled Shoenfeld residual analysis. The analysis was carried out with the use of the STATA 8.0. statistical package.

Results

Out of 110 patients enrolled into the study, 32 patients developed recurrences, either local (4), distant (16), or contralateral breast cancer (12). Consistent with previous publications, we considered all these breast cancer events as recurrences (3-5). Table 1 reports the mean baseline values for all the variables under study in the patients who recurred and in the patients who did not. In a few cases, the available sera were not sufficient for analysis (see Table 1). The patients who recurred showed significantly greater baseline serum values of PDGF (11.9 ng/mL versus 9.4 ng/mL, $P = 0.01$). There was no difference between patients who developed local or distant recurrences and those who developed contralateral breast cancer. Serum IGF-I was higher in the recurrence group, but the differences were not significant.

For CRP and fructosamine, the average concentration was similar in the two patient groups.

Table 2 reports the HRs of recurrence for quartiles of the baseline serum levels of PDGF and IGF-I. The HR of recurrence for the upper quartile of PDGF with reference to the lower quartile was 3.3 (95% CI, 1.1-9.9) but decreased to 2.3 (95% CI, 0.7-7.1) after adjustment for body weight and serum testosterone. As for IGF-I, the risk of recurrence was higher above the first quartile, but the differences were not statistically significant and there was no concentration effect trend. When we examined the effect of each growth factor on recurrences controlling for the other, the results did not substantially change. In this analysis, the fully adjusted HR of recurrence for the upper quartile of PDGF with reference to the lower quartile was 2.8 (95% CI, 0.9-9.0); the fully adjusted HR of recurrence for the upper quartile of IGF-I versus the lower one was 3.7 (95% CI, 0.9-15.1). The interaction test between PDGF and IGF-I was not significant.

By combining PDGF and IGF-I, the fully adjusted HR of recurrence among the women with PDGF and IGF-I levels > median values (respectively, 9.3 and 174.4 ng/mL) was 6.4 (95% CI, 1.5-26.7) compared with the women with

Table 1. Means of baseline serum levels of IGF-I, PDGF, CRP, and fructosamine in patients who recurred and in patients who did not

Variables (n*)	Unit	Patients with recurrences	n	Patients without recurrences	n	P
		Mean ± SD		Mean ± SD		
IGF-1 (n = 107)	ng/mL	188.2 ± 55.3	30	172.1 ± 60.3	77	0.21
PDGF (n = 107)	ng/mL	11.9 ± 5.1	30	9.4 ± 4.0	77	0.01
CRP (n = 96)	mg/L	1.97 ± 2.3	28	1.95 ± 2.9	68	0.97
Fructosamine (n = 108)	μmol/L	329.1 ± 43.2	31	326.8 ± 48.7	77	0.82

*In a few cases, the available sera were not sufficient for analysis.

Table 2. HRs of recurrences according to baseline serum levels of PDGF and IGF-I

	Patients (N)	Adjusted HR*	Fully adjusted HR†
PDGF (quartiles; ng/mL)			
1° 2.2-6.6	27	1	1
2° 6.6-9.3	27	0.6 (0.2-2.1)	0.3 (0.1-1.2)
3° 9.5-12.6	27	1.5 (0.5-4.8)	1.1 (0.4-4.5)
4° 12.7-26.9	26	3.3 (1.1-9.9)	2.3 (0.7-7.1)
IGF-I (quartiles; ng/mL)			
1° 58.8-139.2	27	1	1
2° 139.3-174.4	27	3.3 (1.0-10.6)	2.6 (0.8-9.0)
3° 175.4-205.7	27	1.8 (0.5-6.1)	2.2 (0.6-8.1)
4° 212.5-420.1	26	2.2 (0.6-8.4)	2.0 (0.5-7.7)
Combined PDGF and IGF-I			
PDGF and IGF-I ≤ their median value	23	1	1
PDGF ≤ the median and IGF-I > the median value	31	1.1 (0.3-4.8)	0.7 (0.1-3.0)
PDGF > the median and IGF-I ≤ the median value	31	3.1 (0.9-11.3)	1.7 (0.4-7.0)
PDGF and IGF-I > their median value	22	4.0 (1.0-15.2)	6.4 (1.5-26.7)

*Adjusted for pathologic prognostic factors.
†Adjusted also for tertiles of body weight and serum testosterone.

PDGF and IGF-I levels ≤ their median values (Table 2). High IGF-I levels did not show any association with recurrence risk (HR, 0.7; 95% CI, 0.1-3.0) in the patients with PDGF levels under the median value. In the presence of high serum levels of PDGF, the risk associated with low IGF-I levels was 1.7 (95% CI, 0.4-7.0).

There was no association of fructosamine and CRP with breast cancer events (data not shown).

Discussion

The data from the present study on the risk of breast cancer recurrences during 5.5 years of follow-up suggest that baseline serum levels of PDGF are associated with breast cancer prognosis. Furthermore, these data suggest that high IGF-I levels may increase the risk of breast cancer recurrences only in the presence of high PDGF levels. Contrary to our a priori hypothesis, we did not find any relationship with the baseline serum levels of fructosamine, a marker of insulin resistance and abnormal glucose metabolism, or with the baseline serum levels of CRP, a marker of inflammation.

IGF-I and PDGF are two essential growth factors that synergistically promote cell proliferation and the transformation of several types of cells. Several *in vitro* studies have pointed to interactions between PDGF and IGF-I receptor (13-15). The results from our study suggest that *in vivo* IGF-I and PDGF may also interact to increase the risk of breast cancer progression. We found that high IGF-I levels were associated with recurrence risk only when PDGF levels were also high above the median value.

The mechanism(s) responsible for the higher breast cancer recurrence rate in the women with high serum levels of PDGF is not known, but a PDGF ''priming'' effect on IGF-I receptors may be involved. *In vitro* studies have shown that PDGF stimulated IGF-I receptor mRNA expression and increased the number of IGF-I binding sites at the cell surface through the activation of the IGF-I receptor gene promoter, thus enhancing IGF-I responsiveness and cell proliferation (13-15).

The present study deals with small numbers of patients, and its results require confirmation by larger studies; however, they were not unexpected because two recent prospective cohort studies reported a positive association of serum IGF-I and breast cancer risk in postmenopausal women (16, 17). Previous smaller studies, however, did not find any association (18). A few small studies suggested that serum PDGF may be associated with breast cancer progression (19, 20). We previously showed that metabolic syndrome, especially if associated with high testosterone, an anabolic hormone, is a major predictor of breast cancer recurrence (3, 5). High levels of PDGF and IGF-I may belong to the same pattern of metabolic risk factors associated with western lifestyle that increase breast cancer risk and relapse.

A limitation of the present study is the lack of information about adjuvant chemotherapy. No patient, however, was under chemotherapy at the time of recruitment. Because chemotherapy regimen is strongly associated with the stage of disease defined by T and N status and with the hormonal receptor status, we do not expect that controlling for chemotherapy regimen would have modified the stage and hormonal adjusted odds ratio estimates.

The Diet and Androgens intervention trials showed that a sustainable dietary modification aimed at lowering circulating insulin levels reduces serum insulin and testosterone levels; increases serum sex hormones binding globulin, IGF binding protein 1, and IGF binding protein 2 levels, thus reducing the bioavailability of both IGF-I and sex hormones; and favorably modifies anthropometric and metabolic parameters (3, 10, 21, 22). Studies by Fontana et al. on individuals who voluntarily restricted calorie and protein intake also showed decreased levels of circulating PDGF and IGF-I (23, 24).

Our previous analysis suggested that the reduction of body weight and serum testosterone induced by such diet may favorably influence breast cancer prognosis (3). Unfortunately, for the present study the available sera were not sufficient to analyze the prognostic effect of the change in PDGF levels.

If the predictive value of serum PDGF and IGF-I will be confirmed in larger studies, dietary or other medical intervention to reduce their serum levels should be considered.

Acknowledgments

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.

References

1. Berrino F, Villarini A, De Petris M, Raimondi M, Pasanisi P. Adjuvant diet to improve hormonal and metabolic factors affecting breast cancer prognosis. Ann N Y Acad Sci 2006;1089:110–8: 110–8.
2. Platek M, Krogh V, Micheli A, et al. Serum fructosamine and subsequent breast cancer risk: a nested case-control study in the ORDET prospective cohort study. Cancer Epidemiol Biomarkers Prev 2005;14:271–4.
3. Berrino F, Pasanisi P, Bellati C, et al. Serum testosterone levels and breast cancer recurrence. Int J Cancer 2005;113:499–502.
4. Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. J Clin Oncol 2002;20:42–51.
5. Pasanisi P, Berrino F, De Petris M, Venturelli E, Mastroianni A, Panico S. Metabolic syndrome as a prognostic factor for breast cancer recurrences. Int J Cancer 2006;119:236–8.
6. Ferrante AW, Jr. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. J Intern Med 2007;262:408–14.
7. Fontana L, Klein S. Aging, adiposity, and calorie restriction. JAMA 2007;297:986–94.
8. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–45.
9. Il'yasova D, Colbert LH, Harris TB, et al. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiol Biomarkers Prev 2005;14: 2413–8.
10. Berrino F, Bellati C, Ooldani S, et al. DIANA trials on diet and endogenous hormones. IARC Sci Publ 2002;156:439–44: 439–44.
11. Seifert RA, van Koppen A, Bowen-Pope DF. PDGF-AB requires PDGF receptor α-subunits for high-affinity, but not for low-affinity, binding and signal transduction. J Biol Chem 1993;268:4473–80.
12. Decensi A, Gandini S, Serrano D, et al. Randomized dose-ranging trial of tamoxifen at low doses in hormone replacement therapy users. J Clin Oncol 2007;25:4201–9.
13. Carlberg M, Larsson O. Stimulatory effect of PDGF on HMG-CoA reductase activity and N-linked glycosylation contributes to increased expression of IGF-1 receptors in human fibroblasts. Exp Cell Res 1996;223:142–8.
14. Rubini M, Werner H, Gandini E, Roberts CT, Jr., LeRoith D, Baserga R. Platelet-derived growth factor increases the activity of the promoter of the insulin-like growth factor-1 (IGF-1) receptor gene. Exp Cell Res 1994;211:374–9.
15. Yee D, Rosen N, Favoni RE, Cullen KJ. The insulin-like growth factors, their receptors, and their binding proteins in human breast cancer. Cancer Treat Res 1991;53:93–106.
16. Baglietto L, English DR, Hopper JL, Morris HA, Tilley WD, Giles GG. Circulating insulin-like growth factor-I and binding protein-3 and the risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2007;16: 763–8.
17. Rinaldi S, Toniolo P, Muti P, et al. IGF-I, IGFBP-3 and breast cancer in young women: a pooled re-analysis of three prospective studies. Eur J Cancer Prev 2005;14:493–6.
18. Campagnoli C, Pasanisi P, Peris C, Berrino F. Insulin-like growth factor-I and breast cancer: epidemiological and clinical data. In: Pasqualini JR, editor. Breast cancer: prognosis, treatment and prevention. 2nd ed. Paris: INFORMA-Healthcare, New York, London, Boca Raton, Singapore; 2008.
19. Ariad S, Seymour L, Bezwoda WR. Platelet–derived growth factor (PDGF) in plasma of breast cancer patients: correlation with stage and rate of progression. Breast Cancer Res Treat 1991;20:11–7.
20. Seymour L, Dajee D, Bezwoda WR. Tissue platelet derived-growth factor (PDGF) predicts for shortened survival and treatment failure in advanced breast cancer. Breast Cancer Res Treat 1993;26:247–52.
21. Berrino F, Bellati C, Secreto G, et al. Reducing bioavailable sex hormones through a comprehensive change in diet: the diet and androgens (DIANA) randomized trial. Cancer Epidemiol Biomarkers Prev 2001;10:25–33.
22. Kaaks R, Bellati C, Venturelli E, et al. Effects of dietary intervention on IGF-I and IGF-binding proteins, and related alterations in sex steroid metabolism: the diet and androgens (DIANA) randomised trial. Eur J Clin Nutr 2003;57:1079–88.
23. Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proc Natl Acad Sci U S A 2004;101:6659–63.
24. Fontana L, Klein S, Holloszy JO. Long-term low-protein, low-calorie diet and endurance exercise modulate metabolic factors associated with cancer risk. Am J Clin Nutr 2006;84:1456–62.

Serum Insulin-Like Growth Factor-I and Platelet-Derived Growth Factor as Biomarkers of Breast Cancer Prognosis

Patrizia Pasanisi, Elisabetta Venturelli, Daniele Morelli, et al.

Cancer Epidemiol Biomarkers Prev 2008;17:1719-1722.