

Elevated Serum Insulin-Like Growth Factor Binding Protein-2 as a Prognostic Marker in Patients with Ovarian Cancer

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ABSTRACT

Purpose: The purpose of this research was to examine the diagnostic and prognostic significance of elevated serum insulin-like growth factor binding protein (IGFBP)-2 levels in women with ovarian cancer from diagnosis through treatment to relapse or remission.

Experimental Design: Serum collected pre- and postoperatively in women newly diagnosed with ovarian cancer, during adjuvant chemotherapy cycles, at 6 months follow-up and at relapse was analyzed for IGFBP-2. Control serum was from women undergoing pelvic or abdominal surgery for benign ovarian disease or nonovarian pathology.

Results: IGFBP-2 at diagnosis was significantly elevated ($P < 0.0001$) in women with ovarian cancer (887 ± 62 ng/ml) compared with benign controls (337 ± 25 ng/ml), and women undergoing nonovarian surgery (439 ± 49 ng/ml) and correlated positively with tumor stage and cellular differentiation but not with CA125. Unexpectedly, IGFBP-2 levels increased additionally 1-week postoperatively in ovarian cancer patients (1581 ± 90 ng/ml; $P = 0.0027$) as well as controls (977 ± 95 ng/ml; $P < 0.0001$) and was higher in women who had suboptimal debulking compared with optimal debulking of their tumor. IGFBP-2 levels returned to normal in women without evidence of progressive disease, but remained significantly elevated in women who later relapsed. Patients with IGFBP-2 levels in the highest tertile at diagnosis had a significantly shorter progression-free interval and overall survival.

Conclusion: In ovarian cancer IGFBP-2 is elevated at diagnosis, and corresponds to stage and histology with patients in the highest tertile of IGFBP-2 more likely to relapse and have a poorer outlook. Identification of these patients at diagnosis may allow more individualized, aggressive adjuvant treatment and follow-up, and IGFBP-2 may

therefore be an important additional prognostic marker in this disease.

INTRODUCTION

Epithelial ovarian cancer (EOC) remains the most lethal gynecological malignancy due to the absence of symptoms in the majority of women with early stage disease and a lack of effective screening tools. In contrast to most other solid tumors, 75% of EOC patients present at a late clinical stage, with a 14–30% overall survival rate (1). Despite being about one tenth as common as breast cancer, EOC is three times more lethal. Surgery and combination chemotherapy produce effective short-term results, although most tumors recur, and second line therapies are substantially less effective. Despite some improvement in the median and overall survival using combination chemotherapy in the last decade, long-term survival rates have improved only marginally (2).

In addition to the ongoing efforts to develop more effective primary therapy, there is a need to improve our understanding of the molecular biology of this disease, including the identification of new prognostic markers. This may allow better prediction of the biological behavior of these tumors and, hence, tailor patient treatment according to individual risk and potential benefit. The glycoprotein CA125 is the most widely used biomarker for ovarian cancer (3). It is elevated in ~80% of patients with advanced cancer; however, despite its high sensitivity, it lacks specificity and, therefore, has limited prognostic value (4). More reliable markers and potential therapeutic targets are, thus, required to assist with earlier diagnosis, prognosis, treatment, and subsequent follow-up.

The insulin-like growth factors (IGFs) I and II are structurally related polypeptides, which play an important role in cellular proliferation, differentiation, and apoptosis (5). They are regulated by a family of binding proteins (IGFBPs), of which six have been identified (6, 7). These can both attenuate and stimulate the mitogenic effect of the IGFs by controlling their access to the type I receptor (8, 9). In addition, some IGFBPs have been found to have direct effects on cellular growth and apoptosis independent of IGFs. Most members of the IGF family including the IGFs, IGF-I receptor, and acid labile subunit, as well as some of the IGFBPs and their proteases are found in the ovary (10). IGFBP-2 is the fourth most abundant binding protein (11, 12), exerting an inhibitory effect on cell growth and proliferation in the majority of tissues as a result of its ability to sequester IGFs. However, in a number of tumor cell lines, including adrenocortical carcinoma cells and prostate cancer cells, it has been shown to have a stimulatory effect (13, 14). Its major site of production and mechanism of action in both normal and malignant tissue remains unknown.

In the last decade, the IGFs and their binding proteins have been identified as potent mitogens of carcinogenesis (15), and in

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Table 1 Clinicopathological characteristics of the women with ovarian carcinoma, benign ovarian and nonovarian pathology undergoing surgery

	Ovarian cancer	Benign ovarian pathology	Nonovarian pathology
Number of patients	99	22	20
Median age years	63 (range 30–88)	63 (range 26–81)	47 (range 32–68)
Staging ^a (Patient no.)	I/II (16) III/IV (83)		
Diagnosis (Patient no.)		Cystadenoma (14) Benign teratoma (3) Fibrothecoma (1) Leiomyomata (1) Borderline tumors (1)	Endometriosis (17) Fibroids (2) Torted fallopian tube (1)
Cell histology (Patient no.)	Serous (51) Mucinous (19) Endometrioid (11) Clear cell (8) Mixed Mullerian (6) Poorly differentiated (4)		
Degree of differentiation ^b	Well (9) Moderate (26) Poor (64)		
Surgery ^c	Optimal (51) Suboptimal (27)		
Neoadjuvant chemotherapy	21		

^a Surgical stage (International Federation of Gynaecology and Obstetrics 1987).

^b Cellular differentiation (WHO Classification 1989).

^c Degree of debulking surgery (Substages in Advanced Ovarian Cancer 1992).

particular the serological levels of IGFBP-2 have repeatedly been shown to be elevated in patients with prostate cancer (16), adrenocortical tumors (17), small cell and non-small cell lung cancer (18), and Glioblastoma Multiforme (19). In contrast, the serological levels of IGFBP-3 and IGF-I are both reduced (16). The serological levels of IGF-I and IGFBP-3 have been shown to be predictors of cancer development in certain malignancies, particularly breast and lung cancer (20, 21). The significance of the serological alterations in IGFBP-2 as yet is unknown. A small study by Flyvberg *et al.* (22) found elevated serum levels of IGFBP-2 in 11 women with ovarian cancer. The levels were higher in the malignant ovarian cyst fluid compared with serum suggesting local production of the protein by the tumor cells. Thus, IGFBP-2 may have a physiological role in ovarian cancer development and clinical implications as a prognostic marker in ovarian cancer patients.

To additionally examine this possible prognostic role we have prospectively measured IGFBP-2, IGFBP-3, IGFBP-5, IGF-I, and CA 125 in sequentially collected preoperative, postoperative, and serial serum samples during adjuvant chemotherapy in 99 women with newly diagnosed ovarian cancer. These samples were compared with serum collected from a group of age-matched controls undergoing similar surgery for either benign ovarian or nonovarian conditions, and a larger second cohort of age-matched healthy postmenopausal women not undergoing surgery. We hypothesized that if the tumor cells were producing IGFBP-2 then after debulking surgery and subsequent chemotherapy, the levels should return to normal. In keeping with our hypothesis, IGFBP-2 levels would again rise with disease recurrence; however, they would remain within the normal range for those patients in remission.

PATIENTS AND METHODS

The study was approved by the Human Research Ethic Committee of the Royal North Shore Hospital, North Shore Private Hospital, and the Mater Misericordiae Hospital, Sydney. From January 1999 to September 2001 serum samples were collected from women newly diagnosed with ovarian cancer ($n = 99$; median age, 64 years; range, 30–88) before their debulking surgery. Postoperative samples were collected in 30 of the patients 1 week after surgery.

Serum samples were additionally obtained in those women requiring adjuvant chemotherapy just before each cycle of treatment. Most patients received a minimum of six cycles of combination Carboplatin and Taxol chemotherapy. The patient characteristics are shown in Table 1. Twenty-one patients received neoadjuvant chemotherapy to chemically debulk their tumors before surgery. These patients had either stage IV disease or large volume ascites at diagnosis and were analyzed separately. Additional serum was collected at 6 months follow-up in those women remaining in remission ($n = 15$), or at relapse in patients with recurrent disease ($n = 45$). Pre- and 1 week postoperative serum was collected in a group of women with benign ovarian disease undergoing pelvic surgery ($n = 110$) during the study period, and a control group of women with nonovarian pathology ($n = 12$) who also underwent a similar laparotomy procedure. Benign and control patient characteristics are shown in Table 1. The control range for IGFBP-2 was established from two additional previous study sources, examining the serum collected from 10 women. The first of these came from serum collected from 50 postmenopausal control women who participated in an earlier trial and had their growth factor levels measured by the Kolling laboratory (23). These results were

pooled with the values measured from serum collected from a second control group of healthy postmenopausal women who participated in a different study during the same period and were also analyzed by the same laboratory methods ($n = 60$; median age, 64 years; range, 50–75; Ref. 24). These two groups of women had no prior history of malignancy, had not undergone a surgical procedures, and were matched for age and menopause status to the patients with ovarian cancer.

Clinicopathological Parameters. Tumor stage was determined radiologically, and after surgical and pathological evaluation. Surgical staging was in accordance with the Federation of Gynecology and Obstetrics staging system (25), and patients were divided into stage I/II ($n = 16$) and stage III/IV tumors ($n = 83$). Pathological evaluation for the degree of cellular differentiation (well, moderately well, and poor) was in accordance with the WHO classification, well differentiated tumors ($n = 9$), moderately well-differentiated tumors ($n = 26$), and poorly differentiated tumors ($n = 64$). The histological cell type was also recorded for each tumor as well as the extent of debulking surgery, defined as either optimal (<1 cm nodules of tumor remaining after surgery) or suboptimal (>2 cm of tumor nodules remaining; Ref. 26). Computerized tomographic scanning and transvaginal ultrasound were performed in most patients before surgery. All of the patients had CA 125 measurements at initial diagnosis, serially during chemotherapy, and at follow-up or relapse, collected at the same time as the IGFBP-2 serum sample. The majority of patients received postoperative adjuvant chemotherapy (96%). Treatment response was assessed in patients with measurable disease (≥ 1 cm) by computerized tomography and serum CA125 measurements. The patients have been followed for a median of 32 months (range, 15–44). Tumor recurrence was confirmed by consecutively elevated serum CA 125 measurements in 27 patients in combination with computed tomography scanning and subsequent second-look surgical resection in 6 of the women.

Assays. Serum concentrations of IGFBP-2 were measured by an established competitive RIA using a polyclonal rabbit antiserum raised against recombinant human IGFBP-2 (16). IGFBP-3 and IGF-I were measured by RIA, IGF-I after initial acid-ethanol extraction (27). Samples were processed in multiple assays to confirm that measures remained constant for each of the peptides. IGFBP-5 was measured by RIA using a polyclonal chicken antibody raised against purified IGFBP-5 (28).

Cancer Antigen 125 (CA 125). CA 125 was measured using the Abbott AxSYM CA 125 microparticle enzyme immunoassay technique by Pacific Laboratory Medicine Services.

Statistical Analysis. Measurements were compared between patients using unpaired Student's *t* test or by one-way ANOVA as appropriate. The IGFBP-2 levels in the three study groups were log transformed to stabilize the variance. Analysis of covariance was used to test for a difference in $\ln(\text{BP-2})$ levels between these groups after adjusting for age at diagnosis.

Analysis of the effects of the tumor characteristics, including stage, cellular differentiation, and degree of surgical debulking (and the interaction of these factors) was performed using two-way ANOVA. Comparisons between the nonparametric data in the different patients groups was tested by a Kruskal-Wallis test. The changes over time for the serial measurements

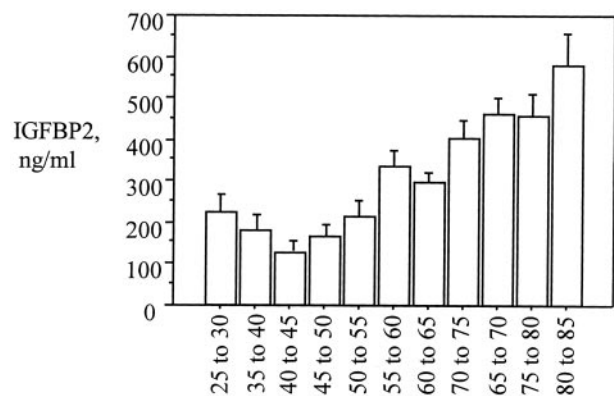


Fig. 1 Serum levels of insulin-like growth factor binding protein (IGFBP)-2 measured by RIA in the healthy control postmenopausal women categorized according to five-year age grouping. The mean is plotted for each group; bars, \pm SE.

(increments above the normal upper limit) were compared using repeated measures ANOVA. Correlations of IGFBP-2 with other numerical and ordinal variables were examined using the Spearman correlation coefficient. The overall survival was the time interval between surgery and death. IGFBP-2 was analyzed as a continuous variable in the Cox regression model, and after no significant association was identified a three-level ordinal variable of IGFBP-2 based on a tertile distribution was used in the survival analysis. The tertile distribution of IGFBP-2 was defined as normal to mild elevation 200–599 ng/ml, moderate elevation 600–999 ng/ml, and high elevation >1000 ng/ml. The IGFBP-2 level used for the analysis was that taken when the woman first presented and was diagnosed with ovarian cancer. All of the statistical analysis was performed using the computer software Statview 5.0. A $P < 0.05$ was considered as significant. Results are presented as mean \pm SE unless otherwise indicated.

RESULTS

IGFBP-2 Levels. The levels of circulating IGFBP-2 in the 110 postmenopausal control women were found to increase with age, a trend not reported previously in women (Fig. 1). The IGFBP-2 range from birth to puberty is the only currently published literature on the normal range (29). This trend toward increasing levels with age has, however, been observed in males (16).

The serum levels of IGFBP-2 at diagnosis were significantly elevated in the patients with ovarian cancer (902 ± 58 ng/ml) compared with patients with benign ovarian disease (416 ± 50 ng/ml; $P < 0.0001$) and the women who underwent surgery for nonovarian pathology (344 ± 30 ng/ml; $P < 0.001$; Fig. 2A). Given that IGFBP-2 levels change with age the IGFBP-2 values were log transformed for each group, and the mean and median for the IGFBP-2, $\ln(\text{IGFBP-2})$, and age are shown in Table 2. The trend of the log-transformed IGFBP-2 levels at diagnosis in each of the 3 patient groups adjusting for age is demonstrated in Fig. 2B.

After adjusting for age, there was still a statistically significant difference in \ln particular, the IGF BP-2 levels between

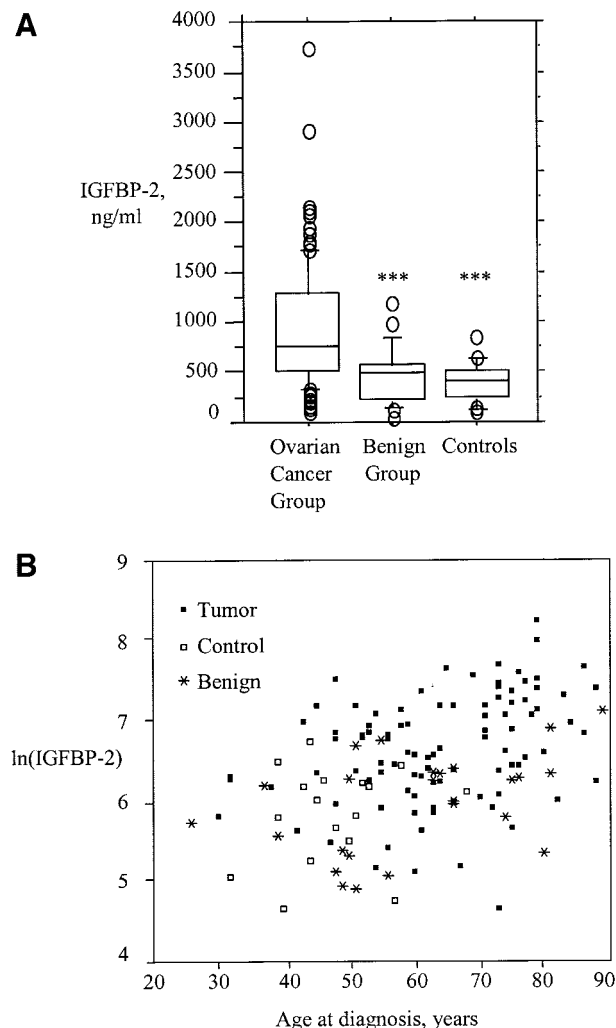


Fig. 2 A, serum levels of insulin-like growth factor binding protein (IGFBP)-2 at diagnosis in the women with ovarian cancer (902 ± 58 ng/ml) compared with patients with benign ovarian disease (416 ± 50 ng/ml; $P < 0.0001$) and the women who underwent surgery for non-ovarian pathology (344 ± 30 ng/ml; $P < 0.001$); bars, \pm SE. B, scattergram of the log-transformed serum levels of IGFBP-2 at diagnosis adjusting for age. Analysis of covariance of the log-transformed values, after this adjustment for age identified a statistically significant difference between the three patient groups ($P < 0.001$).

patient groups ($P < 0.001$; Table 3). In particular, the IGFBP-2 levels among the tumor patients were 1.68 times higher than those in the control group (95% confidence interval, 1.18–2.40; $P = 0.004$) and 1.82 times higher than those in the benign group (95% confidence interval, 1.38–2.42; $P < 0.001$) after adjusting for age (Table 3).

The highest IGFBP-2 values were in the patients with the more advanced tumor stage ($P < 0.0001$) and poorly differentiated cellular architecture (poorly differentiated *versus* moderately well differentiated, $P = 0.0004$; poorly differentiated *versus* well differentiated, $P = 0.0002$; Fig. 3). The IGFBP-2 levels varied between the histological cell types with the poorly differentiated and mucinous cell types having the highest levels. However the difference did not reach statistical significance.

The IGFBP-2 level 1-week postoperatively was significantly elevated compared with the preoperative level in all three of the patient groups (Fig. 4, A–C).

The preoperative IGFBP-2 level was higher in the patients who subsequently had a suboptimal debulking procedure compared with those that were optimally debulked, but it did not reach statistical significance (suboptimal debulking preoperative IGFBP-2 968 ± 93 ng/ml, optimal debulking 819 ± 74 ng/ml; $P = 0.2$). The postoperative IGFBP-2 level was also higher in those patients who had suboptimal tumor debulking compared with those with optimal removal; however, again this level did not reach significance (suboptimal 1852 ± 149 ng/ml, optimal 1331 ± 130 ng/ml; $P = 0.18$).

The IGFBP-2 level was significantly lower preoperatively in those women who had received neoadjuvant chemotherapy before their debulking surgery (neoadjuvant chemotherapy 624 ± 81 ng/ml) compared with the women who had primary surgery (primary surgery 1203 ± 89 ng/ml, $P = 0.0004$). With each cycle of neoadjuvant chemotherapy there was a fall in the IGFBP-2 level (Fig. 5).

The women remaining in remission had a normal IGFBP-2 level at 6 months after treatment, compared with those women who had relapsed and again had a significantly elevated IGFBP-2 level (Table 4). There was a significant difference in the initial IGFBP-2 level taken before treatment between those women remaining in remission and those who relapsed ($P = 0.037$). There was no difference in the pretreatment serum for the other measured parameters, CA 125, IGFBP-3, or IGF-I. During the follow-up period 45 patients have relapsed (45%) and 31 patients have died (31%), 28 from ovarian cancer and the other 3 from nonrelated causes including a cerebrovascular accident, pulmonary embolus 2 days postoperatively, and pneumonia, unrelated to chemotherapy. There was no statistically significant difference in IGFBP-2 level at diagnosis between those women who had died of the disease and those who remain in remission (IGFBP-2 at diagnosis and alive 714 ± 55 ng/ml; IGFBP-2 at diagnosis and deceased 810 ± 73 ng/ml, $P = 0.3$).

IGFBP-3. The IGFBP-3 levels were significantly lower in women with ovarian cancer (2.7 ± 0.1 μ g/ml) before surgery compared with the women with benign pathology (3.7 ± 0.2 μ g/ml; $P = 0.0003$) and control groups (4.0 ± 0.3 μ g/ml; $P < 0.0001$). This level was not significantly different between the tumor stages or pathological grades. There was no significant change in the levels with neoadjuvant chemotherapy or with the degree of debulking surgery. However, in contrast to the IGFBP-2 measurements the postoperative IGFBP-3 levels were additionally significantly reduced in all three of the groups (Fig. 4B). The IGFBP-3 levels returned to within the normal range in women who remained in remission (IGFBP-3 at diagnosis 2.6 ± 0.1 μ g/ml, IGFBP-3 at follow-up 4.3 ± 0.2 μ g/ml; $P < 0.0001$). In the women whose disease recurred after their primary therapy, the IGFBP-3 level at relapse was again reduced (IGFBP-3 at diagnosis 3.2 ± 0.3 μ g/ml, IGFBP-3 at relapse 2.9 ± 0.3 μ g/ml; $P = 0.5$).

IGFBP-5. The changes in the IGFBP-5 levels also paralleled the changes in IGFBP-3 with values significantly lower in the patients with ovarian cancer (91 ± 3 ng/ml), benign (133 ± 7 ng/ml; $P < 0.0001$), or control (128 ± 6 ng/ml; $P = 0.0002$). Again there was no difference in the levels between the

Table 2 Mean and median of the IGFBP-2,^a ln(IGFBP-2), and age at diagnosis in the three study groups

			Mean	SD	Median	Percentile 25	Percentile 75
BP-2	Status	Benign	458.5	275.2	487.5	209.0	563.9
		Control	387.1	200.7	410.0	219.4	505.7
		Tumor	927.5	620.6	756.2	494.6	1285.4
Age	Status	Benign	62.1	16.3	63.0	49.9	76.5
		Control	47.6	8.6	46.5	41.9	52.9
		Tumor	63.7	12.9	63.2	55.0	73.6
ln(BP-2)	Status	Benign	5.95	.63	6.19	5.34	6.33
		Control	5.81	.61	6.02	5.38	6.23
		Tumor	6.62	.69	6.63	6.20	7.16

^a IGFBP, insulin-like growth factor binding protein.

Table 3 Parameter estimates^a

Parameter	Coefficient	SE	P	95% Confidence interval	
				Lower bound	Upper bound
Intercept	5.484	0.273	0.000	4.944	6.023
Age	1.769E-02	0.004	0.000	9.501E-03	2.589E-02
Benign	-0.601	0.142	0.000	-0.882	-0.320
Control	-0.520	0.179	0.004	-0.874	-0.166
Tumor	0				

^a Dependent variable: ln(BP-2).

various tumor characteristics; however, the immediate postoperative level fell in parallel with IGFBP-3 (EOC preoperatively 99 ± 5 ng/ml, postoperatively 73 ± 5 ng/ml, $P = 0.0004$; benign preoperatively 132 ± 7 ng/ml, postoperatively 84 ± 10 ng/ml, $P = 0.0012$; control preoperatively 126 ± 5 ng/ml, postoperatively 87 ± 5 ng/ml, $P = 0.0001$; Fig. 4C). The IGFBP-5 level taken just before surgery in those women who received neoadjuvant chemotherapy was higher than those women who underwent up-front debulking surgery, although this did not reach statistical significance (up-front surgery IGFBP-5 69 ± 6 ng/ml, postneoadjuvant chemotherapy IGFBP-5 86 ± 8 ng/ml; $P = 0.15$). In parallel with IGFBP-3 and IGF-I, the IGFBP-5 level in women remaining in remission had returned to within the normal range (IGFBP-5 at diagnosis 79 ± 5 ng/ml, at 6 months in remission 140 ± 9 ng/ml; $P < 0.0001$). However, unlike IGFBP-3 and IGF-I, IGFBP-5 was not reduced in those women who relapsed (IGFBP-5 at diagnosis 79 ± 4 ng/ml, at relapse 121 ± 8 ng/ml; $P < 0.0001$).

IGF-I. In parallel with IGFBP-3 the initial IGF-I levels were significantly lower in the patients with ovarian cancer (EOC 9 ± 0.45 nmol/liter; benign 14 ± 1.4 nmol/liter; control 13.9 ± 1.5 nmol/liter; $P < 0.0001$, $P = 0.0004$, respectively). There was no significant difference between the levels analyzed for tumor stage, histological grade, degree of surgical debulking, or neoadjuvant chemotherapy. The postoperative IGF-I levels fell significantly 1 week postoperatively in parallel with the IGFBP-3 levels in all three of the groups (Fig. 4D). The IGF-I had returned to within the normal range in those women in remission (IGF-I at diagnosis 7.5 ± 1 nmol/liter, IGF-I at follow-up 15.4 ± 1.3 nmol/liter; $P < 0.0001$). Similar to IGFBP-3 the IGF-I level at relapse was again reduced compared with the normal reference range for IGF-I (IGF-I at diagnosis 8.8 ± 0.673 nmol/liter, IGF-I at relapse 11.4 ± 1.1 nmol/liter; not significant).

CA 125. The CA 125 levels were significantly elevated in the women with ovarian cancer (1464 ± 306 units/ml) compared with the benign (53 ± 24 units/ml; $P = 0.02$) and control groups (103 ± 58 units/ml; $P = 0.04$). Within the tumor group the CA 125 did not distinguish between the stage of disease or histological grade. In contrast to a previous small study with 11 patients (22) there was no correlation between the IGFBP-2 and CA 125 levels either at diagnosis ($R = 0.085$; $P = 0.36$) or at any of the other time points measured during the treatment period. There was also no correlation between the IGFBP-2 levels at relapse and corresponding CA 125 values ($R = 0.348$; $P = 0.17$). The CA 125 value was not predictive of those women likely to relapse from their disease.

Serial IGFBP-2, IGFBP-3, IGF-I, and CA 125. Serial serum samples were collected from the patients undergoing adjuvant chemotherapy before each administered cycle of treatment. Most of the women received six cycles of standard combination Carboplatin and Taxol chemotherapy (30). Those

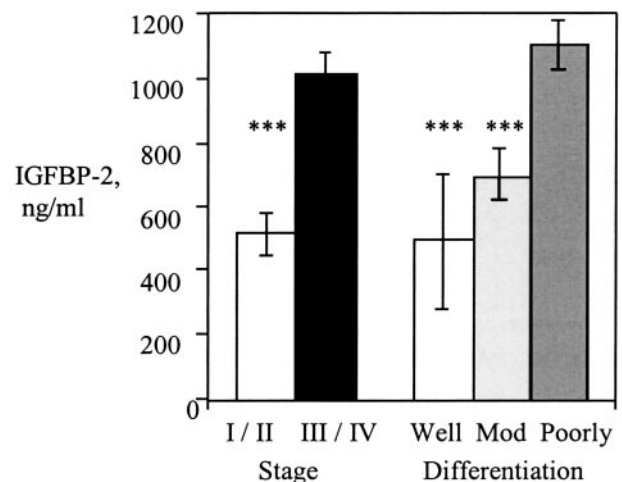


Fig. 3 Serum insulin-like growth factor binding protein (IGFBP)-2 level at diagnosis in the women with ovarian cancer analyzed according to tumor stage and degree of cellular differentiation. The highest IGFBP-2 values correlated with more advanced tumor stage (stage I/II 516 ± 67 ng/ml, stage III/IV 1014 ± 67 ng/ml; $P < 0.0001$), Kruskal Wallis test $P = 0.002$ and poorly differentiated cellular architecture (well differentiated 495 ± 212 ng/ml, $P = 0.0002$; moderately well differentiated 707 ± 80 ng/ml, $P = 0.0004$; poorly differentiated 1103 ± 78 ng/ml, $P = 0.0004$), Kruskal Wallis test $P < 0.0001$; bars, \pm SE.

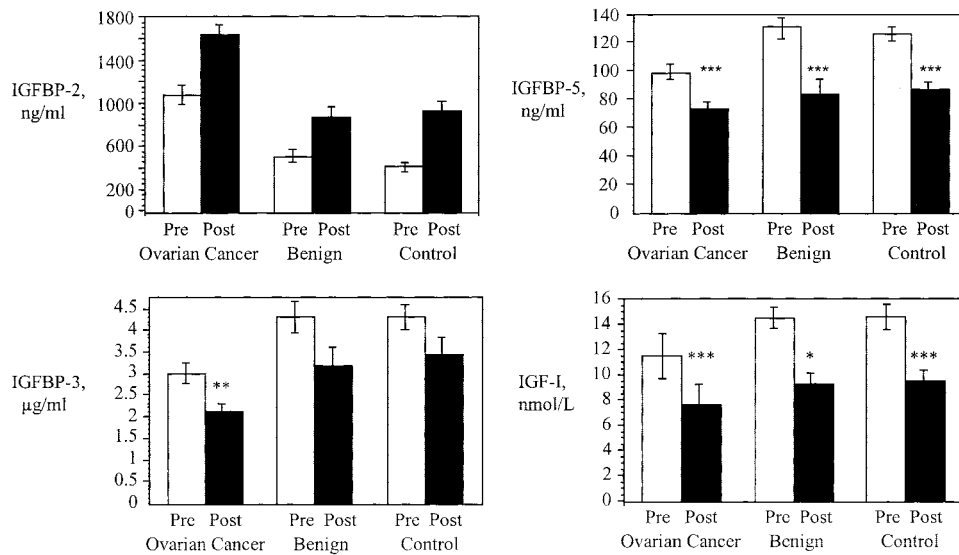


Fig. 4 Serum pre- and postoperative levels of insulin-like growth factor binding protein (IGFBP)-2, IGFBP-3, IGFBP-5, and insulin-like growth factor (IGF)-I in the three groups of women undergoing surgery. **A**, IGFBP-2 levels in the women with ovarian cancer [$n = 26$, pre-operation (op) 1119 ± 92 ng/ml; post-op 1717 ± 123 ng/ml, $P = 0.0003$], benign ovarian disease ($n = 13$, pre-op 530 ± 58 ng/ml; post-op 912 ± 94 ng/ml, $P = 0.002$), and surgical controls ($n = 12$, pre-op 444 ± 47 ng/ml; post-op 977 ± 95 ng/ml, $P < 0.0001$). **B**, IGFBP-3 levels pre- and post-op in the women having surgery for ovarian cancer (pre-op 3.0 ± 0.2 µg/ml; post-op 2.1 ± 0.2 µg/ml, $P = 0.003$), benign ovarian disease (pre-op 4.3 ± 0.4 µg/ml; post-op 3.4 ± 0.4 µg/ml, not significant) and surgical controls (pre-op 4.3 ± 0.3 µg/ml; post-op 3.4 ± 0.4 µg/ml, not significant). **C**, IGFBP-5 levels pre- and post-op in the women having surgery for ovarian cancer (pre-op 99 ± 5 ng/ml; post-op 73 ± 5 ng/ml, $P = 0.0004$), benign ovarian disease (pre-op 132 ± 7 ng/ml; post-op 84 ± 10 ng/ml, $P = 0.0012$) and surgical controls (pre-op 126 ± 5 ng/ml; post-op 87 ± 5 ng/ml, $P = 0.0001$). **D**, IGF-I levels pre- and post-op in the women having surgery for ovarian cancer (pre-op 11.4 ± 0.9 nmol/l; post-op 7.6 ± 0.8 nmol/l, $P = 0.0025$), benign ovarian disease (pre-op 14.5 ± 1.8 nmol/l; post-op 9.2 ± 1.6 nmol/l, $P = 0.04$), and surgical controls (pre-op 14.2 ± 1.1 nmol/l; post-op 9.5 ± 0.9 nmol/l, $P = 0.0016$); bars, \pm SE.

women who had a persistently elevated CA 125 at the completion of six cycles received additional treatment at the discretion of the treating oncologist. The mean of the serial IGFBP-2 measures during each cycle of chemotherapy is shown in Fig.

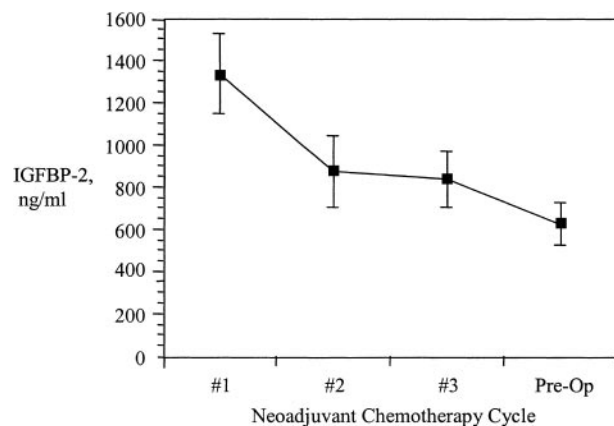


Fig. 5 Serum insulin-like growth factor binding protein (IGFBP)-2 levels in the patients who received neoadjuvant chemotherapy before each cycle of treatment and pre-operatively ($n = 21$) demonstrating a fall in the level with each treatment. IGFBP-2 level at diagnosis, before cycle 1 of chemotherapy (1337 ± 191 ng/ml), before cycle 2 (915 ± 171 ng/ml), before cycle 3 (839 ± 134 ng/ml), and pre-operatively (624 ± 101 ng/ml); bars, \pm SD.

6A, demonstrating an elevated level throughout treatment with a return to within the normal range at follow-up.

In comparison the IGFBP-3 and IGF-I levels increased with subsequent chemotherapy treatment and had returned to normal by the fourth cycle (Fig. 6, B and C), and CA 125 levels fell with each cycle (Fig. 6D).

Five of the women receiving adjuvant treatment progressed on chemotherapy. The CA 125 failed to fall with each chemotherapy cycle, and their chemotherapy treatment was changed, with the assumption that their disease was platinum resistant. In each of these patients the IGFBP-2 levels also rose with each chemotherapy cycle. Three of the patients responded to second-line therapy with a drop in their CA 125, but in none did the IGFBP-2 or CA 125 levels return to within the normal range. Four of these patients have subsequently died of progressive disease, and the fifth remains on treatment.

Multivariate Analysis. Multivariate analysis showed that the IGFBP-2 levels were positively interrelated with age ($R = 0.443$, $P = 0.003$; Fig. 7A) and stage ($R = 0.42$, $P = 0.005$), and to a lesser extent IGFBP-5 levels ($R = 0.35$, $P = 0.02$). IGFBP-3 and IGF-I levels were strongly positively interrelated ($R = 0.64$, $P < 0.0001$; Fig. 7B), which is to be expected given that IGFBP-3 is the major binding protein for IGF-I in the circulation. Both IGFBP-3 and IGF-I were positively correlated with IGFBP-5 ($R = 0.54$, $P = 0.0002$ and $R = 0.51$, $P = 0.0004$, respectively; Fig. 7, C and D). IGF-I was also positively interrelated with stage ($R = 0.33$, $P = 0.03$) and histology ($R = 0.33$, $P = 0.03$).

Table 4 Serum IGFs,^a IGFBPs, and CA125 levels in the women with recurrent disease compared to the women remaining in remission

		Relapse Mean ± SE	Remission Mean ± SE	<i>P</i> ^b
Total number		45	13	
Median age		62 years	60 years	
	Range	(32–79 years)	(47–70 years)	
Median time between diagnosis and relapse, months	Range	10 (3–26)		
Stage ^c	I/II	1	10	
	III/IV	45	3	
Degree of differentiation ^d	Well	1	4	
	Mod well	15	8	
	Poorly	29	1	
Surgery ^e	Optimal	13	13	
	Suboptimal	32		
IGFBP-2 level at diagnosis (ng/ml)		900 ± 89	565 ± 82	<i>P</i> = 0.037
IGFBP-2 level at relapse		1194 ± 139		<i>P</i> = 0.01
IGFBP-2 level at follow-up			324 ± 60	<i>P</i> = 0.027
IGFBP-3 level at diagnosis (µg/ml)		3.25 ± 0.32	2.6 ± 0.15	NS
IGFBP-3 level at relapse		2.96 ± 0.3		NS
IGFBP-3 level at follow-up			4.3 ± 0.19	<i>P</i> < 0.0001
IGF-I level at diagnosis (nmol/L)		8.8 ± 0.67	7.5 ± 1	
IGF-I level at relapse		11.4 ± 1.2		NS
IGF-I level at follow-up			15.4 ± 1.3	<i>P</i> < 0.0001
IGFBP-5 level at diagnosis (ng/ml)		79.4 ± 3.9	78.8 ± 4.6	NS
IGFBP-5 level at relapse		121.8 ± 8.5		<i>P</i> < 0.0001
IGFBP-5 level at follow-up			139.6 ± 9	<i>P</i> < 0.0001
CA 125 level at diagnosis (U/ml)		2025 ± 809	461 ± 170	NS
CA 125 level at relapse		959 ± 302		NS
CA 125 level at follow-up			7.6 ± 1.2	<i>P</i> = 0.06

^a IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; NS, non-significant (*P* > 0.05).

^b *P*, probability of a difference between the growth factor level at diagnosis and follow-up or relapse, via ANOVA and Kruskal-Wallis test as appropriate.

^c Surgical stage (International Federation of Gynaecology and Obstetrics 1987).

^d Cellular differentiation (WHO Classification 1989).

^e Degree of debulking surgery (Substages in Advanced Ovarian Cancer 1992).

IGFBP-2 and Ovarian Cancer Survival. The Kaplan-Meier curve was used to illustrate the progression-free and overall survival for the three IGFBP-2 tertiles (Figs. 8 and 9 respectively). The log rank test for homogeneity was highly significant for both (*P* = 0.001). Patients with the highest tertile of IGFBP-2 at diagnosis had a significantly greater risk of relapse earlier and overall poorer survival rate. A Cox proportional hazards model was used to quantify the increased risk associated with increasing levels of IGFBP-2. The hazard ratios for survival are shown in Table 5. The hazard ratio increased by a factor of 1.97 (95% confidence interval, 1.35–2.88; *P* < 0.0001) per tertile of IGFBP-2. Univariate survival analysis with the Cox regression model confirmed that stage, histological grade, residual tumor, and debulking surgery were all associated with risks for both disease progression and death as reported previously (data not shown; Ref. 31).

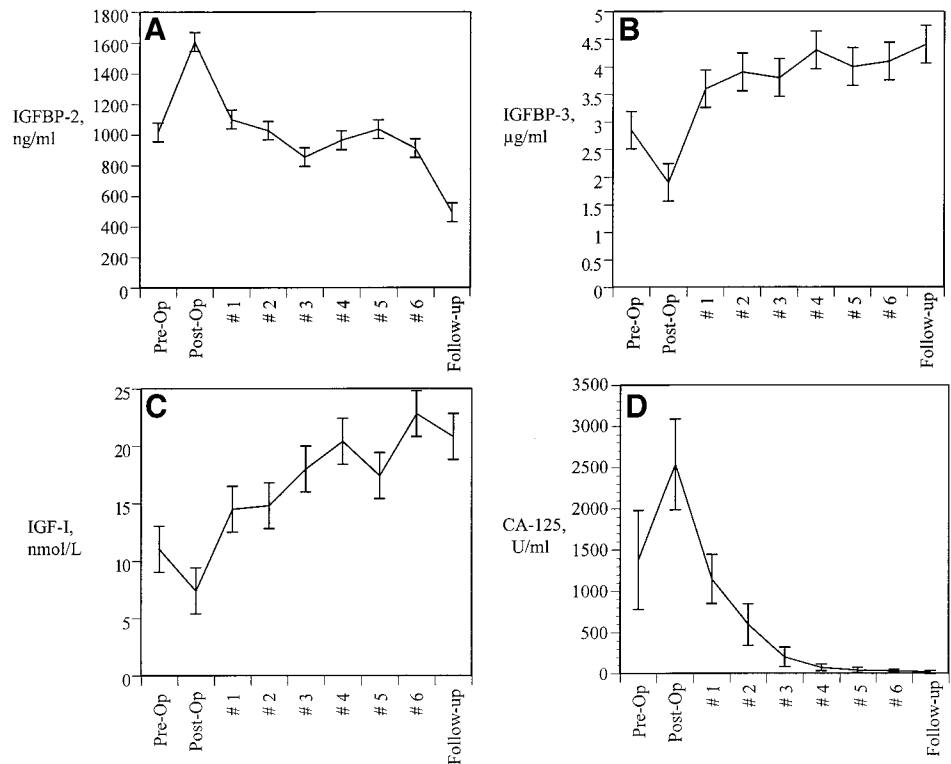
Cox multivariate analysis including stage, histological grade, age, preoperative CA125, debulking surgery, and IGFBP-2 revealed the only independent predictor of survival was stage (hazard ratio, 11.1; 95% confidence interval, 3.2–40.1; *P* < 0.0001). After adjusting for the effect of tumor stage at diagnosis IGFBP-2 was no longer statistically significant (Table 6). This is explained by the moderate correlation between stage and IGFBP-2 (Spearman rank correlation, 0.6; *P* = 0.06) and additionally confirmed in the cross-tabulation count.

Patients with the highest IGFBP-2 tertile at diagnosis are more likely to have advanced stage disease (Table 7).

DISCUSSION

Ovarian cancers are characterized by a broad spectrum of biological behaviors ranging from tumors with excellent prognosis and a high likelihood of cure to those that progress rapidly and have a poor prognosis. This wide spectrum of clinical behavior is reflected by a number of identifiable clinicopathological prognostic variables which include Federation of Gynecology and Obstetrics staging, histological subtype and grade, volume of residual tumor postsurgical resection, performance status, and age (31, 32). Despite having these prognostic variables, all of the patients are treated with the same standard adjuvant chemotherapy, and, hence, some patients do well, whereas others do very badly. As well as incorporating these individual variables into multivariate models to predict outcome, effort continues to be directed toward identifying new prognostic variables with biological rationale. Our study has concentrated on the value of serological IGFBP-2 to identify different tumor characteristics in patients with EOC. Serum IGFBP-2 levels strongly correlated with tumor stage and histological differentiation, with the more aggressive tumors having higher IGFBP-2 levels. Those patients with highest preoperative

Fig. 6 Serial measures of insulin-like growth factor binding protein (IGFBP)-2, IGFBP-3, insulin-like growth factor (IGF)-I and CA 125 in the three groups of women undergoing surgery at diagnosis, during the six cycles of adjuvant chemotherapy and follow-up ($n = 36$). **A**, serial serum level of IGFBP-2 demonstrating a delayed fall to within normal levels during the six cycles of chemotherapy. **B**, serial levels of IGFBP-3. **C**, serial levels of IGF-I. **D**, serial levels of CA 125. #: cycle of chemotherapy; bars, \pm SE.



IGFBP-2 levels were more likely to have a recurrence of their tumor and poorer overall survival. Thus, IGFBP-2 may be able to identify those patients with aggressive disease requiring individualized management. Its ultimate value may be in combination with CA 125 to identify women at risk of early relapse who may require either additional chemotherapy or maintenance therapy and vigilant follow-up.

Because EOCs have been shown to express endogenous IGFBP-2 (33), we initially hypothesized that after the standard treatment of debulking surgery the elevated preoperative levels should drop. We found the converse, with values rising significantly further postoperatively. The postoperative levels were also markedly raised in both the patients with benign ovarian disease and those with nonovarian pathology, both groups hav-

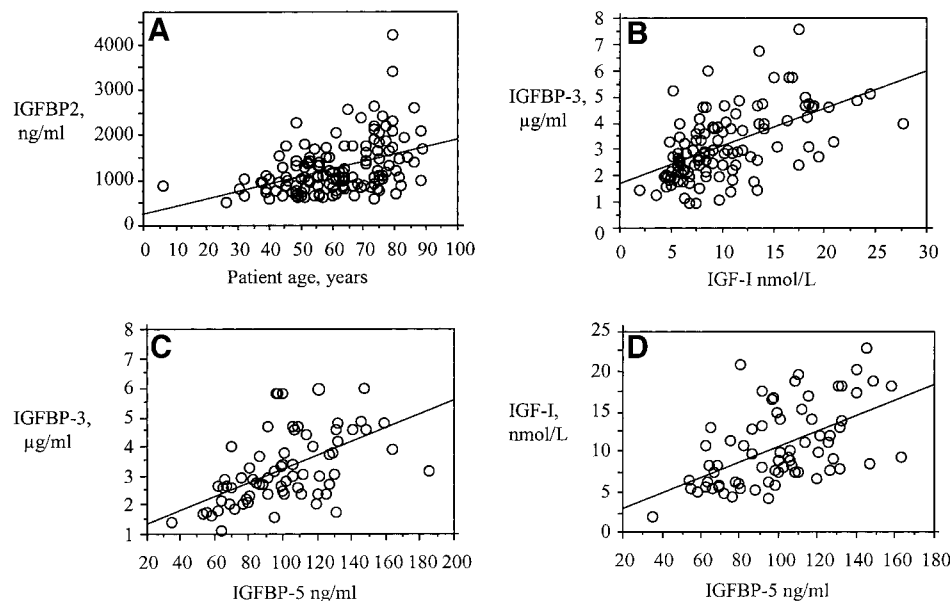


Fig. 7 Serum insulin-like growth factor binding protein (IGFBP)-2 levels at diagnosis in the women with ovarian cancer correlated positively with the patients age (**A**, $R = 0.443$; $P = 0.003$). IGFBP-3 was positively interrelated with IGF-I (**B**, $R = 0.64$; $P < 0.0001$) and IGFBP-5 (**C**, $R = 0.54$; $P = 0.0002$) IGF-I was also positively interrelated to IGFBP-5 (**D**, $R = 0.51$; $P = 0.0004$).

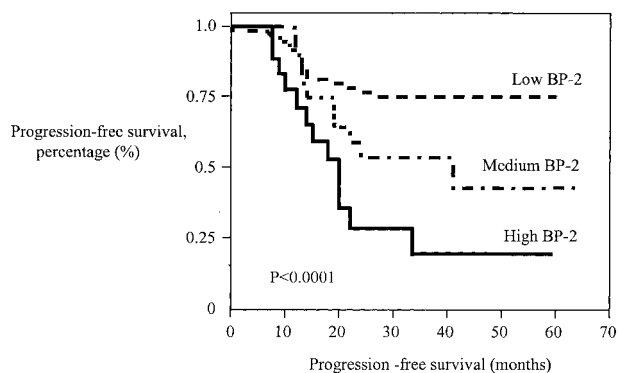


Fig. 8 The Kaplan-Meier survival curve for progression-free survival among patients with high (third tertile), medium (second tertile), and low (first tertile) insulin-like growth factor binding protein-2 levels.

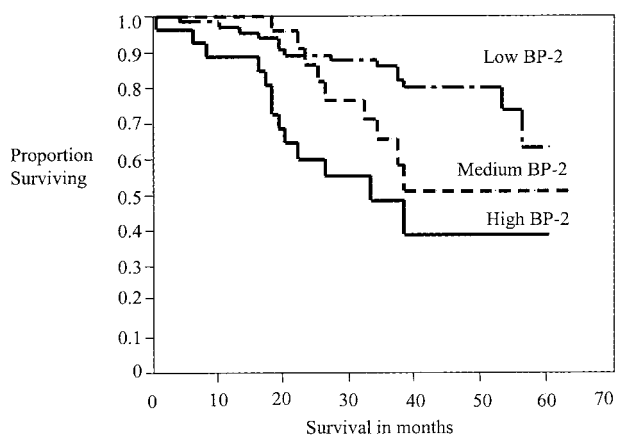


Fig. 9 The Kaplan-Meier survival curve for overall survival among patients with high (third tertile), medium (second tertile), and low (first tertile) insulin-like growth factor binding protein (IGFBP)-2 levels.

ing an initial serological IGFBP-2 level within the normal range. IGFBP-2 may play a role in the normal tissue response to injury and inflammation, which warrants additional investigation. The postoperative elevation in IGFBP-2 in the patients with ovarian cancer could be explained by the process of tumor cell repopulation after surgical debulking, a process that is known to occur in tumors when a substantial volume is removed. This does not explain the rise in the benign and control groups, however. The higher postoperative IGFBP-2 levels in patients who had sub-optimal debulking surgery compared with optimal debulking also remains unexplained, but again is possibly due to the excess residual ovarian tumor secreting the peptide.

Cytoreductive surgery has been the initial management of EOC, confirming the diagnosis and removing the major tumor bulk. However, a number of patients who undergo cytoreductive surgery do not achieve optimal resection, especially if they are too ill to undergo aggressive surgery, have tumor nodules in close proximity to vital structures, or have liver parenchymal disease. Patients with gross ascites or who are physically unfit for initial surgery are offered neoadjuvant chemotherapy to chemically debulk the tumor before surgical resection. In our

group of 21 women receiving neoadjuvant chemotherapy the IGFBP-2 levels progressively dropped with each of the three cycles, and the preoperative level was significantly lower compared with levels in women having upfront surgery. This finding possibly supports our hypothesis that the ovarian cancer cells are producing the protein and that chemotherapy, by destroying the bulk of disease, lowers the level.

Serial serum samples from the patients undergoing adjuvant chemotherapy demonstrated a persistent elevation in IGFBP-2 levels during the six cycles compared with the IGFBP-3, IGFBP-5, and IGF-I levels, which returned to within the normal range by the second cycle. Without a second look laparotomy, which is rarely performed, because its morbidity outweighs its clinical benefit, there is no way of determining which patients have residual disease at the completion of chemotherapy (34). If the CA 125 level is in the normal range at the end of the six cycles of adjuvant chemotherapy, treatment is ceased, and patients are entered onto a surveillance program. Markers to identify patients requiring ongoing therapy, beyond the standard six cycles, such as a persistently elevated IGFBP-2 and lowered IGFBP-3 and IGF-I levels, would be of immense clinical value.

The tumor marker measured routinely in patients with EOC is CA 125, a tumor-associated glycoprotein antigen, which is frequently elevated (35, 36). However, although CA 125 is a sensitive marker, it is not tumor specific and can be elevated in

Table 5 Univariate survival analysis with the Cox regression model

	P	HR ^a	95% CI for HR
IGFBP-2			
Normal to mild elevation	0.002		
Moderate elevation	0.083	2.1	0.9–4.9
High elevation	0.001	3.9	1.8–8.3

^a HR, hazard ratio; CI, confidence interval.

Table 6 Multivariate analysis with the Cox regression model

This is explained by the moderate correlation between stage and BP-2, (Spearman rank correlation = 0.6, $P = 0.06$).

	P	HR ^a	95.0% CI for HR ^b
IGFBP-2 level at diagnosis	0.900	1.026	0.7–1.5
Stage	0.0001	11.102	3.1–40.1

^a HR, hazard ratio; CI, confidence interval; IGFBP, insulin-like growth factor binding protein.

^b Adjusted for age, stage, tumor debulking surgery and histological grade.

Table 7 IGFBP-2^a tertiles and stage cross-tabulation count

IGFBP-2 tertiles	IGFBP-2 ng/ml	Stage			Total
		Benign	I/II	III/IV	
Normal to mild elevation	0 – 599	17	12	19	65
Moderate elevation	600 – 999	5	3	24	25
High elevation	>= 1000		1	40	27
Total		22	16	83	121

^a IGFBP, insulin-like growth factor binding protein.

a number of benign conditions such as endometriosis, menstruation, and pregnancy, as well as other malignancies, including breast, lung, and gastrointestinal. The CA 125 level can be discordant with tumor response, both as a false-positive and false-negative. Its main value is to monitor the course of disease both in terms of treatment response and relapse (35). Persistently rising levels may be associated with disease progression, whereas decreasing values may indicate a favorable response to treatment (36). A complimentary marker such as IGFBP-2, which is not elevated in benign disease and can reflect tumor load, may assist in management decisions.

In summary, we have demonstrated changes in the IGF/IGFBP axis in women with newly diagnosed ovarian cancer. Patients with ovarian cancer have significantly elevated serological levels of IGFBP-2 directly proportional to the disease stage, grade of the tumors, and residual tumor size. The patients with a higher IGFBP-2 level at diagnosis were more likely to relapse, and those with levels >1000 ng/ml had a significantly worse overall survival. It is these women with a high IGFBP-2 level at diagnosis who may require either tailored chemotherapy or more intensive follow-up at the completion of standard treatment. In contrast, IGFBP-3 and IGF-I were both significantly lower in the malignant group as reported previously (22, 37).

In summary the findings of this study support the role of IGFBP-2 as a possible prognostic marker in ovarian cancer, although greater patient numbers are required. Additional patients are being accrued to the study, whereas those already enrolled continue to be followed. Additional work is continuing to determine how IGFBP-2 may regulate ovarian cancer cell growth and progression. The elevated levels postoperatively in all of the patient groups also warrant additional investigation.

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