Circulating IGF system and treatment outcome in epithelial ovarian cancer

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Abstract

Aggressive epithelial ovarian cancers (EOCs) frequently progress and become fatal, even when cytoreduction surgery plus platinum-based chemotherapy are performed. Thus, the early detection of high-risk subgroups is important in order to provide opportunities for better treatment outcomes, using alternative therapeutic strategies. This study aimed to explore the expression of circulating IGF system components and their relationship with treatment outcome in EOC. We included 228 patients with a median follow-up time of 44 months at two tertiary centers. There were 68 cancer deaths and 108 cases of cancer progression in the cohort. Preoperative serum levels of total IGF1, IGF2, IGF-binding protein 2 (IGFBP2), and IGFBP3 were analyzed using an ELISA and were then converted into an IGF1:IGFBP3 molar ratio. The risks of mortality and progression were estimated using Cox regression models in univariate and multivariate analyses. Our results showed that high IGF1, IGF2, and IGFBP3 levels were significantly associated with an early cancer stage, non-serous histology, and optimal cytoreduction. High IGFBP2 levels were associated with an advanced stage and serous histology. Overall and progression-free survival durations were significantly better among patients with high IGF1 ($P=0.003$ and $P=0.001$), IGF2 ($P=0.003$ and $P=0.02$), or IGFBP3 levels ($P=0.02$ and $P=0.008$). In multivariate analysis, serum IGFBP2 levels were significantly associated with increased risk of mortality (hazard ratio = 1.84, 95% CI: 1.07–3.18, $P=0.03$), indicating that IGFBP2 could be used as an early predictor of EOC-related mortality. The combination of elevated IGFBP2 and reduced IGF1 levels at diagnosis could further facilitate the identification of a patient subgroup with the worst prognosis.

Key Words

- insulin-like growth factor
- insulin-like growth factor-binding protein
- epithelial ovarian cancer
- response to chemotherapy
- prognosis

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Introduction

Ovarian cancer is the leading cause of gynecological malignancy-related deaths. Annually, an estimated 22 280 cases are newly diagnosed and nearly 15 500 deaths are attributable to this disease in the USA (Siegel *et al.* 2012). Epithelial ovarian cancer (EOC) is usually diagnosed at an

advanced stage and accounts for the most cases and deaths (Cho & Shih 2009). Despite cytoreductive surgery followed by combination chemotherapy, many EOC patients eventually relapse with the development of chemoresistant tumors and subsequently die of their disease.

To date, there are no good clinical measures for predicting response to chemotherapy. Several groups have used gene expression profiling to generate prognostic signatures (Hartmann *et al.* 2005, Jazaeri *et al.* 2005, Tothill *et al.* 2008). While gene expression analysis can reflect an array of biologically and clinically important characteristics with which to predict cancer prognosis, these analyses require complex computational methods and subsequent acquisition and validation of data to connect an *in vitro* experimental result with an *in vivo* result of interest (Chang *et al.* 2011). Therefore, it is important to develop potential predictive markers to identify disease progression or chemotherapy sensitivity that can be more easily implemented into routine patient care.

The insulin-like growth factor (IGF) system comprises polypeptide ligands (IGF1 and IGF2), receptors, and binding proteins (IGFBPs). The ligands interact with IGF1 receptor (IGF1R), IGF2R, the insulin receptor, or hybrid receptors on cancer cells and thus activate downstream signaling to promote cell growth (Weroha & Haluska 2012). IGFBPs act as carrier proteins for IGFs to the receptors and modulate IGF transport and tissue distribution to enhance or restrain IGF signaling and bioactivity. IGFBP3 is the major serum carrier of IGFs, whereas IGFBP2 is less common. IGFs have been shown to stimulate invasion, metastasis, and angiogenesis, whereas IGFBPs inhibit IGF bioactivity (Beauchamp *et al.* 2010). Signaling through IGF1R includes stimulation of cellular proliferation through the RAS–RAF–MAPK pathway and mediation of cell survival through the phosphoinositide-3 kinase–Akt–mammalian target of rapamycin pathway (Scagliotti & Novello 2012).

Evidence has been accumulated which shows that the IGF system is relevant to the development (Renehan *et al.* 2004, Gunter *et al.* 2009, Rinaldi *et al.* 2010) and poor clinical outcomes (Fuchs *et al.* 2008, Rohrmann *et al.* 2012, Rowlands *et al.* 2012, Duggan *et al.* 2013) of prostate, breast, pancreatic, and colorectal cancers. According to the literature, a modest association has been shown between higher circulating IGF1 or IGF2 levels and an increased risk of various human cancers (Renehan *et al.* 2004, Rinaldi *et al.* 2010, Chi *et al.* 2013). However, other studies have not shown consistent results (Allen *et al.* 2007, Pham *et al.* 2007, Suzuki *et al.* 2009).

EOCs express high levels of IGF1, IGF2, or IGFBP2 relative to normal ovarian epithelium (Karasik *et al.* 1994, Sayer *et al.* 2005, Lancaster *et al.* 2006), and the increased expression tends to be associated with high-grade and advanced-stage disease (Sayer *et al.* 2005, Lancaster *et al.* 2006, Lu *et al.* 2006). Serum levels of IGFBP2 were shown

to be significantly higher and serum IGF1, IGFBP3, and IGFBP5 levels were lower in patients with EOC than in those with benign pathologies and normal controls (Flyvbjerg *et al.* 1997, Waksmański *et al.* 2001, Baron-Hay *et al.* 2004, Lancaster *et al.* 2006). To our knowledge, few studies have addressed the role of serum IGF2 in EOC. Furthermore, clinical studies have rarely focused on circulating IGF biomarkers and prognosis or response to chemotherapy in EOC, except for a study that showed the association of elevated serum IGFBP2 with early tumor relapse and poor prognosis in 99 patients (Baron-Hay *et al.* 2004).

CA125, a well-known serum marker, acts as a tumor-associated marker, and its levels are elevated in ~50–60% of patients with early-stage disease. Although CA125 has no diagnostic specificity when screening patients with early-stage disease, it has been widely used in patients with EOC to assess treatment responses to chemotherapy or to detect disease progression (Vergote *et al.* 2000, Rustin *et al.* 2004). Preoperative CA125 levels could be of prognostic significance (Jhamb & Lambrou 2010). However, studies have rarely addressed the correlation between IGF system components and CA125 in ovarian cancer or their individual or adjusted prognostic effects.

To determine whether pretreatment serum levels of IGF1, IGF2, IGFBP2, IGFBP3, and the IGF1:IGFBP3 molar ratio could serve as prognostic markers in patients with EOC, we measured levels of these biomarkers in 228 patients with a diagnosis of EOC at two tertiary care centers. The secondary objective was to examine correlations between the IGF system and clinicopathological variables or their influence on prognosis in EOC patients.

Subjects and methods

Participant population

The research protocol and consent form were approved by the Institutional Review Boards of the two hospitals. We included consecutive patients who were diagnosed with EOCs between January 1992 and August 2008 at National Cheng Kung University Hospital (NCKUH) and National Taiwan University Hospital (NTUH), Taiwan. These patients underwent staging or cytoreductive surgery with or without adjuvant chemotherapy. Platinum-based chemotherapeutic agents were used as adjuvant chemotherapy regimens. Radiation therapy was permitted to relieve local discomfort resulting from metastases of the bone, brain, or other sites. Staging was performed according to the clinical staging criteria of the

International Federation of Gynecology and Obstetrics (FIGO). Histological classification was defined according to the World Health Organization classification (Lee *et al.* 2003). Data on levels of preoperative serum CA125 were also available. Patients who had undergone primary surgery elsewhere, whose preoperative blood samples were not available, or who did not provide written informed consent were excluded. Age-matched control participants were selected by random sampling among volunteer sets that comprised cancer-free women. The participants were followed up after treatment, and the date of the latest record retrieved was 31 August 2012. We reviewed the medical records and pathological slides, which provided information on the clinical characteristics, pathological diagnoses, treatments, and outcome. Complete treatment data were available for all included patients.

Clinical definitions

Preoperative BMI was calculated in units (kg/m^2). Optimal cytoreduction was defined as a maximal residual tumor nodule diameter of <1 cm or no gross residual disease, whereas suboptimal cytoreduction was defined as a tumor nodule diameter of ≥ 1 cm, according to the Gynecologic Oncology Group guidelines. Response to chemotherapy or progression was defined according to the objective Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1; Eisenhauer *et al.* 2009) or the Gynecologic Cancer Intergroup definition for CA125 progression (Vergote *et al.* 2000, Rustin *et al.* 2004). A complete response (CR) was achieved when all measurable lesions disappeared or when all nonmeasurable lesions disappeared, as reflected by a normalized CA125 level. Partial response (PR) was defined as a $\geq 30\%$ decrease in the sum of diameters (SOD) of the measurable lesions, compared with the baseline SOD. Progressive disease (PD) was defined as a $\geq 20\%$ increase in the SOD of the measurable lesions. Stable disease (SD) was defined as insufficient shrinkage to qualify as a PR or an insufficient increase to qualify as PD. PD (progression) also referred to the detection of a new lesion, a sufficient increase in the overall tumor burden in cases of SD, or discontinuation of therapy due to a PR. The term 'responsive' was used to designate either CR or PR, whereas the term 'poorly responsive' was used to designate either SD or PD. Patients who progressed without sustaining clinical benefits after two consecutive chemotherapy courses of platinum-based regimens were classified as refractory. Patients whose diseases recurred in <6 months were classified as having platinum-resistant disease,

whereas those whose diseases recurred in >6 months were defined as having platinum-sensitive disease. The study endpoints were progression-free survival (PFS), overall survival (OS), and the clinical response rate. Both the OS and PFS were calculated from the date of diagnosis. The OS duration was measured to the date of death from any cause; data on survivors were censored on the date at which they were last known to be alive. The PFS duration was measured to the date of first clinical progression or death from any cause, unless the patient was progression free at the time of last contact, in which case PFS duration was measured to the date of last contact.

Collection and storage of blood samples

Preoperative nonfasting blood samples were drawn from the participants at both institutes. All samples were stored at $\sim 15^\circ\text{C}$ and transferred to the central laboratory in each institute, where they were processed and aliquoted in the same manner. All samples were stored in the vapor phases of liquid nitrogen containers (-196°C) for an average period of ~ 5 years before processing. To avoid IGF and IGFBP degradation, none of the aliquoted samples had previously been defrosted.

Preoperative IGF and IGFBP serum levels

Commercially available ELISA Kits (DSL-10-2800, DSL-10-2600, DSL-10-6600, and DSL-10-7100) from Diagnostics Systems Laboratories (DSL), Inc. (Webster, TX, USA), with respective sensitivities of 0.01, 2.2, 0.017, and 0.04 ng/ml, were used to determine the preoperative concentrations of serum total IGF1, IGF2, IGFBP2, and IGFBP3 respectively. According to the manufacturer's instructions, all blood samples were acidified followed by ethanol precipitation of the IGFBPs fraction before measurement of free IGFs from their binding proteins. The peptide levels were measured in duplicate, and the average of the two measurements was used in data analysis. The measurements were repeated if the correlation coefficient between absorbance and the amount in the standards was <0.95 . Any test with an intra-assay coefficient of variation (CV) $>10\%$ was repeated. The respective average intra- and inter-assay CV were 2.8 and 3.6% for total IGF1, 2.3 and 1.4% for IGF2, 2.9 and 3.1% for IGFBP2, and 2.7 and 1.6% for IGFBP3. The IGF1:IGFBP3 molar ratio was also estimated according to the following conversion: $(0.13 \times \text{IGF1 concentration (ng/ml)}) / (0.036 \times \text{IGFBP3 concentration (ng/ml)})$ (Rohrmann *et al.* 2012).

Statistical analysis

Data analyses were performed using the Statistical Package of Social Science, version 17.0 for Windows Software (SPSS, Inc.). Because all IGF system components and CA125 were not normally distributed among the overall patient population or subgroups according to clinicopathological factors, statistical analyses were performed using nonparametric methods. Differences in the concentrations of IGF system components were analyzed using the Mann–Whitney *U* test between subgroups. Frequency distributions between categorical variables were compared using the χ^2 test and Fisher's exact method. Relationships among the IGF system components, CA125, BMI, and age were examined using Kendall's τ -*b* test. To study the relationships between the circulating IGF system components and the clinicopathological characteristics at diagnosis, we divided all recruited patients into two subgroups by categorizing each of the clinical or pathological variables. The receiver operating characteristic curve-determined cutoff value was optimized for diagnostic sensitivity and specificity for the prediction of progression or death. The cutoff CA125 value was then chosen with the maximal sensitivity that corresponded to the minimal false positive value for detecting survival. Because insufficient data were available to stratify by tertile, the median values of all IGF components were selected as the cutoff values. Subgroup analyses of IGF biomarker and CA125 expression among 180 patients with different responses to adjuvant chemotherapy were performed according to histology (serous or non-serous type), age (<50 and $\geq$ 50 years), and disease progression.

Survival was estimated according to the Kaplan–Meier method and compared using log-rank tests. Cox proportional hazards (PH) models were used to estimate hazard ratios (HR) and CI. The PH assumption for each selected factor was tested, and a log minus log plot of survival resulted in parallel lines, so that PH held. The following well-known ovarian cancer prognostic factors at the time of diagnosis (Jhamb & Lambrou 2010) were included in our study: age, stage, histology, residual tumor diameter, and preoperative CA125 level. Each IGF system component was analyzed singly in the univariate analysis and then adjusted for confounders in the multivariate analysis to examine the independent effects on survival and disease progression. A multivariable-adjusted model was adjusted as follows for the factors: age (<50 (referent) or $\geq$ 50 years), stage (early (referent) or advanced), histology (non-serous (referent) or serous type), residual tumor diameter (<1 (referent) or $\geq$ 1 cm), and hospital

(NCKUH (referent) or NTUH). Given the positive associations between the expression levels of IGF1, IGF2, and IGFBP3, IGF1 was selected and included in the full model. The full model included analyses of IGF1 (stratified as higher (referent) vs lower than the median value), IGFBP2 (stratified as higher vs lower (referent) than the median value), preoperative CA125 level (stratified as higher vs lower (referent) than the cutoff value), age, stage, histology, residual tumor diameter, and hospital. *P* values <0.05 (two-sided) were considered significant.

Results

Circulating IGF system components and clinicopathological characteristics

A total of 228 newly diagnosed EOC patients were recruited into our study. The patients ranged in age from 23 to 84 years (mean, 52 years) and in BMI from 15.8 to 43.4 (kg/m²) (mean, 22.8 kg/m²). One hundred and twenty-one (53.1%) patients were postmenopausal women. Eighty-four (36.8%) patients had FIGO stage I disease, 15 (6.6%) had stage II disease, 113 (49.6%) had stage III disease, and 16 (7.0%) had stage IV disease. Serous histology was found in 101 patients (44.3%), the endometrioid type in 49 (21.5%), the clear cell type in 40 (17.5%), and the mucinous type in 38 (16.7%). Of the 228 patients, 180 (78.9%) received adjuvant chemotherapy, which consisted of taxane plus platinum or platinum plus cyclophosphamide. Among the 180 patients who underwent first-line chemotherapy, overall response occurred in 133 (73.9%) patients. Furthermore, 136 (75.6%) had platinum-sensitive disease. The median follow-up time for all participants was 44 months (range, 1–219 months). During follow-up, 109 patients (47.8%) developed PD and 68 patients (29.8%) died.

The IGF biomarker expression levels between the age-matched control group and the EOC group are listed in [Supplementary Table 1](#), see section on [supplementary data](#) given at the end of this article. The levels of IGF2 and IGFBP3 were significantly higher, whereas IGF1:IGFBP3 molar ratios were significantly lower in the younger EOC patients than the controls ($\leq$ 29, 30–39, and 40–49 years). IGFBP2 levels were higher in EOC patients than in the controls, but the differences did not achieve statistical significance in most of the age subgroups. There were no difference in IGF1 levels between EOC and the age-matched control groups. In the control group, a decline in the IGF1 levels and IGF1:IGFBP3 ratio with advancing age was observed, whereas no similar trend was found for the other IGF biomarkers.

The relationships between the circulating IGF-system in EOC are listed in Table 1. Women who were younger (<50 years), premenopausal, or who had early-stage, non-serous histology, and a residual tumor diameter <1 cm, had significantly higher serum IGF1, IGF2, and IGFBP3 levels. In addition, the median serum IGF1 and IGFBP3 concentrations were significantly higher in patients without disease progression than in those with disease progression ($P=0.02$ and $P=0.02$ respectively). The median IGF1 levels were also significantly higher in patients with normalized preoperative CA125 levels. In contrast, the median serum IGFBP2 levels were significantly higher in patients with advanced-stage disease and serous histology. However, the IGF2 or IGFBP2 levels did not differ significantly between patients who did and those who did not show progression. Moreover, the IGF1:IGFBP3 molar ratio did not differ significantly between the categorical subgroups mentioned above (data not shown).

Preoperative serum markers and chemotherapy response

The relationship between serum markers and chemotherapy response is shown in Table 2. Among the 180 patients who received platinum-based chemotherapy, the levels of all IGF biomarkers did not differ significantly between the platinum-sensitive and platinum-resistant/-refractory groups. However, the IGF1 and IGF2 levels were

significantly higher in patients who had overall responses (CR/PR) to chemotherapy than in those who had poor responses (SD/PD) ($P=0.04$ and $P=0.04$ respectively). The IGFBP2 levels were significantly higher in those who had poor responses ($P=0.04$).

Subgroup analyses were performed to examine the expression levels of the circulating biomarkers between patients who were responsive and those who were poorly responsive to platinum-based chemotherapy. Among the serous and older patient subgroups, the IGFBP2 levels were significantly higher in those with poor responses ($P=0.03$ and $P=0.006$ respectively). No significant difference in the IGFBP2 levels was found between the responsive and poorly responsive patients among the non-serous and young patient subgroups. The IGF2 levels were significantly lower in the poorly responsive patients than in the responsive patients in the subgroup of patients with disease progression ($P=0.02$). However, the IGF1, IGFBP2, and IGFBP3 levels did not differ in this clinical setting. Furthermore, the preoperative CA125 levels and IGF1:IGFBP3 molar ratios did not differ significantly between patients with overall responses and those with poor responses in the subgroup analyses (data not shown).

CA125

One hundred and ninety-two (84.2%) patients had elevated preoperative serum CA125 levels, and this

Table 1 Circulating IGF biomarkers and clinicopathological factors in epithelial ovarian cancer ($n=228$)

Variable	Group	n	IGF1 (ng/ml)		IGF2 (ng/ml)		IGFBP2 (ng/ml)		IGFBP3 (ng/ml)	
			Median (IQR)	P	Median (IQR)	P	Median (IQR)	P	Median (IQR)	P
Age	<50	97	138.7 (174.3)	<0.001	2246.8 (1374.4)	<0.001	521.8 (1121.2)	0.44	3844.5 (3164.2)	0.02
	≥50	131	71.3 (101.4)		1324.9 (1268.6)		643.2 (2047.7)		3238.0 (3376.2)	
BMI	<25	173	97.1 (160.9)	0.53	1828.4 (1343.4)	0.59	645.4 (1595.6)	0.46	3546.7 (3541.6)	0.41
	≥25	55	105.7 (142.1)		1822.8 (1511.6)		414.0 (1792.6)		3777.8 (2690.6)	
Menopause	No	107	122.9 (194.6)	0.002	2246.8 (1450.0)	<0.001	466.5 (1083.1)	0.05	3844.5 (3365.3)	0.004
	Yes	121	72.9 (101.2)		1380.3 (1239.1)		761.3 (2101.0)		3169.5 (3120.7)	
FIGO stage	Early	99	158.4 (179.5)	<0.001	2248.1 (1424.8)	<0.001	397.0 (774.6)	<0.001	4678.0 (3164.7)	<0.001
	Advanced	129	71.3 (82.3)		1486.8 (1319.0)		1160.5 (2539.2)		2956.4 (2682.3)	
Cell type	Serous	101	72.3 (90.6)	<0.001	1537.0 (1159.8)	0.001	947.6 (2384.5)	0.001	3160.0 (2695.9)	0.004
	Non-serous	127	123.9 (182.1)		2124.5 (1495.1)		448.8 (1047.6)		4196.3 (3379.4)	
Preoperative CA125 level	Normalized	36	153.8 (163.5)	0.009	2350.3 (1541.2)	0.06	399.4 (644.7)	0.07	4345.0 (2536.8)	0.06
	Elevated	192	88.2 (135.0)		1672.0 (1349.1)		646.2 (1812.2)		3437.0 (3419.8)	
Residual tumor diameter (cm)	<1	169	117.9 (164.8)	0.002	1947.0 (1435.3)	0.002	586.0 (1351.9)	0.59	3874.2 (3393.2)	<0.001
	≥1	59	54.1 (78.6)		1338.3 (1422.5)		564.3 (2809.8)		2707.3 (2582.4)	
Progression	No	119	121.8 (180.2)	0.02	2035.4 (1670.5)	0.14	564.8 (1278.1)	0.91	3796.3 (3429.1)	0.02
	Yes	109	81.3 (107.8)		1605.9 (1165.2)		565.4 (1816.2)		3178.9 (3055.6)	

Data were analyzed by Mann–Whitney *U* test. IGF1, insulin-like growth factor 1; IGFBP2, IGF-binding protein 2; IQR, interquartile range.

Table 2 Circulating IGF biomarkers, CA125, and response to first-line chemotherapy in epithelial ovarian cancer ($n=180$)

Subgroup	<i>n</i>	IGF1 (ng/ml), median (IQR)	IGF2 (ng/ml), median (IQR)	IGFBP2 (ng/ml), median (IQR)	IGFBP3 (ng/ml), median (IQR)	CA125 (IU/ml), median (IQR)
All undergoing chemotherapy						
Platinum-sensitive	136	91.5 (121.5)	1672.0 (1312.5)	709.1 (1689.6)	3494.7 (3297.9)	331.5 (820.4)
Platinum-resistant/-refractory	44	62.2 (94.1)	1290.4 (1293.1)	1237.7 (2237.6)	3160.0 (3105.0)	449.8 (761.5)
<i>P</i>		0.15	0.13	0.33	0.33	0.17
CR/PR	133	94.4 (120.4)	1776.8 (1306.0)	695.3 (1471.6)	3497.8 (3088.1)	340.0 (951.2)
SD/PD	47	58.2 (93.5)	1238.0 (1247.1)	1254.4 (3485.2)	2725.5 (3207.3)	407.0 (818.6)
<i>P</i>		0.04	0.04	0.04	0.22	0.69
Serous histology						
CR/PR	67	83.7 (90.9)	1537.0 (1162.2)	817.0 (1864.2)	3160.0 (2600.5)	599.0 (1384.0)
SD/PD	29	56.9 (66.5)	1223.2 (1169.0)	1874.8 (3660.1)	2478.2 (2881.1)	593.3 (1102.5)
<i>P</i>		0.07	0.27	0.03	0.65	0.74
Non-serous histology						
CR/PR	66	117.6 (147.0)	1993.0 (1518.4)	588.2 (1062.2)	4061.8 (3117.7)	190.1 (343.0)
SD/PD	18	85.5 (184.9)	1307.0 (1588.8)	473.6 (1783.7)	3835.3 (3849.1)	90.4 (299.2)
<i>P</i>		0.38	0.12	0.64	0.40	0.24
Older age (≥ 50 years)						
CR/PR	81	71.7 (90.2)	1320.8 (1135.3)	695.3 (1611.7)	3238.0 (3401.9)	348.1 (1187.4)
SD/PD	31	47.1 (86.2)	1056.2 (964.7)	1874.8 (3707.1)	2485.4 (3356.9)	466.7 (1146.5)
<i>P</i>		0.11	0.28	0.006	0.58	0.53
Young age (<50 years)						
CR/PR	52	118.8 (185.2)	2226.5 (1307.9)	692.1 (1277.7)	3769.1 (2675.3)	321.6 (645.4)
SD/PD	16	82.8 (96.3)	2106.9 (1271.5)	542.1 (1405.1)	2979.9 (2777.5)	348.7 (553.8)
<i>P</i>		0.12	0.44	0.65	0.19	0.77
Progressor						
CR/PR	62	84.7 (105.1)	1890.5 (1227.8)	489.1 (1760.6)	3347.4 (2614.5)	524.0 (1093.4)
SD/PD	38	60.2 (85.9)	1206.0 (1088.6)	716.5 (2981.0)	2481.8 (3201.5)	397.8 (661.8)
<i>P</i>		0.30	0.02	0.22	0.47	0.09

Data were analyzed by Mann–Whitney *U* test. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; IQR, interquartile range.

elevation was significantly more frequent in patients with advanced age (patients ≥ 50 years old, $P=0.02$), menopause ($P=0.007$), advanced stage ($P<0.001$), serous histology ($P<0.001$), suboptimal cytoreduction ($P<0.001$), and disease progression ($P<0.001$) (data not shown in Table 1). However, preoperative CA125 levels were not significantly different between the responders and poorly responsive patients in the subgroup analyses (Table 2). Of the 228 EOC patients, those with high preoperative CA125 levels exhibited shorter OS and PFS (Supplementary Fig. 1I and J, see section on supplementary data given at the end of this article; $P=0.04$ and $P<0.001$ respectively). In addition, among the 180 patients who received adjuvant chemotherapy, patients with high CA125 levels had poorer OS and PFS ($P=0.03$ and $P=0.001$ respectively) than those with low CA125 levels (Supplementary Fig. 2E and F).

Correlations between serum markers, BMI, and age

The relationships between circulating CA125, IGF markers, BMI, and age were examined (Table 3). Although

moderately positive correlations were found among IGF1, IGF2, and IGFBP3, weak correlations were observed between these three markers and either CA125, BMI, age, or the other IGF biomarkers. Notably, only weak correlations were found with IGFBP2 and the other IGF biomarkers.

IGF system components and clinical outcomes

The OS and PFS curves for all patients are illustrated in Supplementary Fig. 1. Patients with low IGF1, IGF2, or IGFBP3 levels had significantly poorer OS and PFS than patients with high IGF1 ($P=0.002$ and $P=0.001$ respectively), IGF2 ($P=0.002$ and $P=0.02$ respectively), or IGFBP3 ($P=0.02$ and $P=0.04$ respectively) levels. In contrast, the OS time was longer in patients with low IGFBP2 levels ($P=0.005$) than in patients with high levels, although PFS did not differ between the groups.

The survival curves for 180 patients who received adjuvant chemotherapy are illustrated in Supplementary Fig. 2. The patients with low IGF1 levels had shorter OS and PFS than those with high levels (Supplementary

Table 3 Correlation between IGF biomarkers, CA125, BMI, and age in epithelial ovarian cancer ($n=228$)

	Level, median (IQR)	Correlation between factors						
		IGF1 (r)	IGF2 (r)	IGFBP3 (r)	IGFBP2 (r)	CA125 (r)	BMI (r)	Age (r)
IGF1 (ng/ml)	98.1 (149.7)	–	0.42*	0.43*	–0.04	–0.12*	0.00	–0.20*
IGF2 (ng/ml)	1822.8 (1473.3)	0.42*	–	0.44*	–0.31*	–0.11	0.05	–0.31*
IGFBP3 (ng/ml)	3558.5 (3478.6)	0.43*	0.44*	–	–0.04	–0.17*	0.08	–0.14*
IGFBP2 (ng/ml)	565.1 (1615.8)	–0.04	–0.31*	–0.04	–	–0.13*	–0.01	0.07
CA125 (IU/ml)	308.6 (674.0)	–0.12	–0.11	–0.17*	0.13*	–	–0.03	0.12*
BMI (kg/m ²)	22.2 (4.4)	0.00	0.05	0.08	–0.01	–0.03	–	–0.03
Age (years)	51.0 (19.0)	–0.20*	–0.31*	–0.14*	0.07	0.12*	–0.03	–

* $P<0.05$. IGF1, insulin-like growth factor 1; IGFBP2, IGF-binding protein 2; IQR, interquartile range. Data were analyzed by Kendall's τ -b test.

Fig. 2A and B), but this difference was not statistically significant ($P=0.06$ and $P=0.08$ respectively). OS time, as shown in Supplementary Fig. 2C and D, was longer in patients with high IGF2 levels and low IGFBP2 levels ($P=0.004$ and $P=0.002$ respectively), but PFS did not differ between the groups (figure not shown).

Univariate analysis

The influences of circulating IGF biomarkers at diagnosis or possible prognostic features on the risks of time to death or to disease progression were also estimated using the Cox PH model (Table 4). Low IGF1, IGF2, or IGFBP3 levels and higher IGFBP2 levels were significantly associated with the risk of death. Low IGF1, IGF2, and IGFBP3 but not IGFBP2 levels were significantly associated with an increased risk of disease progression. The IGF1:IGFBP3 molar ratio had no influence on prognosis. Patients with low IGF1 and high IGFBP2 expression levels (subgroup B) had significantly higher risks of death and disease progression than those with high IGF1 and low IGFBP2 expression levels (subgroup A) or those with both low or both high IGF1 and IGFBP2 levels (subgroup C, $n=73$; Fig. 1A and B). When compared with 53 subgroup A patients, 54 subgroup B patients were found to have the most unfavorable outcomes (hazard ratio for death: 4.7, $P<0.001$ and for progression: 2.1, $P=0.015$). The 5-year OS for subgroup B was 52.0% (87.5% for early stage EOC and 47.0% for advanced stage), which was in contrast to the rate of 83.3% in subgroup A (90.0% for early stage EOC and 66.8% for advanced stage). The 5-year PFS for subgroup B was 39.7% (66.7% for early stage EOC and 34.8% for advanced stage), which was in contrast to the rate of 56.3% in subgroup A (72.9% for early stage EOC and 32.6% for advanced stage). It is worthy of note that among the 48 patients who did not receive chemotherapy the 5-year OS were 95.5 and 33.3% for subgroups A and B

respectively ($P=0.001$). Of the 107 premenopausal patients, the 5-year OS were 83.6 and 58.0% for subgroups A and B respectively ($P=0.02$).

Multivariate analysis

Table 5 shows the prognostic influence of circulating IGF biomarkers based on age-adjusted and multivariable-adjusted Cox PH models. In the age-adjusted model, total IGF1 levels were significantly associated with poor outcomes when the lower and higher levels were compared ($HR=1.71$, $P=0.04$ for cancer death and $HR=1.81$, $P=0.004$ for progression). Higher IGFBP2 levels were significantly associated with an increased risk of cancer death ($HR=1.80$, $P=0.02$), but not of cancer progression. Lower IGFBP3 levels were significantly associated with the risk of progression ($HR=1.60$, $P=0.02$) but not of cancer death. There was no significant association between IGF2 or free IGF1 levels and cancer mortality and progression.

In the multivariable-adjusted model, neither the circulating IGF biomarkers nor the CA125 levels were found to be the predictors of death or disease progression after adjustment. In the full models (Table 6), however, higher IGFBP2 levels ($HR=1.79$, $P=0.04$) and older age ($HR=1.84$, $P=0.03$) were significantly associated with an elevated risk of cancer death. Moreover, advanced-stage disease was an independent predictor of death ($HR=2.35$, $P=0.02$) and disease progression ($HR=2.31$, $P=0.003$). There were no associations between total IGF1 or CA125 levels and cancer death after adjusting for the other confounders. In addition, no confounding effect by hospital was found (data not shown). When the platinum-resistant/-refractory status was included as a prognostic factor during primary therapy or post-therapy surveillance in the full model, it was the strongest predictor of death ($HR=2.81$, 95% CI: 1.62–4.86; $P<0.001$) and progression ($HR=2.25$, 95% CI: 1.47–3.46, $P<0.001$).

Table 4 Univariate analysis of prognostic factors and serum markers in epithelial ovarian cancer (n=228)

Variable	Death events	Median time to death (months)	HR for death (95% CI)	P	Progression events	Median time to progression (months)	HR for progression (95% CI)	P
Serum IGF1 (ng/ml)								
Low	42	84	2.10 (1.28–3.44)	0.003	63	35	1.95 (1.32–2.88)	0.001
High	26	–	1.00		45	61	1.00	
Serum IGF2 (ng/ml)								
Low	43	92	2.58 (1.38–4.79)	0.003	56	41	1.80 (1.09–2.97)	0.02
High	25	–	1.00		53	112	1.00	
Serum IGFBP2 (ng/ml)								
High	44	92	2.00 (1.22–3.30)	0.006	53	44	1.02 (0.69–1.49)	0.94
Low	24	168	1.00		56	54	1.00	
Serum IGFBP3 (ng/ml)								
Low	40	92	1.78 (1.10–2.89)	0.02	59	43	1.68 (1.14–2.46)	0.008
High	28	–	1.00		50	59	1.00	
Serum IGF1:IGFBP3 molar ratio								
Low	37	107	1.21 (0.75–1.95)	0.44	57	47	1.09 (0.74–1.61)	0.66
High	31	98	1.00		51	54	1.00	
Serum CA125 (U/ml)								
High	41	77	1.68 (1.02–2.77)	0.07	66	29	2.14 (1.42–3.15)	<0.001
Low	25	112	1.00		39	93	1.00	
Age								
≥ 50	48	77	2.51 (1.49–4.23)	0.001	65	44	1.55 (1.06–2.29)	0.03
< 50	20	–	1.00		44	57	1.00	
BMI								
≥ 25	16	84	1.10 (0.63–1.92)	0.75	30	47	1.21 (0.79–1.86)	0.39
< 25	52	98	1.00		79	53	1.00	
FIGO stage								
Late	53	68	3.15 (1.77–5.59)	<0.001	82	29	2.98 (1.93–4.62)	<0.001
Early	15	168	1.00		27	137	1.00	
Histology								
Serous	40	84	1.60 (0.99–2.61)	0.06	67	28	2.20 (1.49–3.24)	<0.001
Non-serous	28	–	1.00		42	158	1.00	
Residual tumor nodule diameter (cm)								
≥ 1	27	56	1.96 (1.20–3.20)	0.007	40	29	1.95 (1.31–2.89)	0.001
< 1	41	112	1.00		69	58	1.00	
Platinum sensitivity								
Resistant/refractory	30	54	3.71 (2.22–6.18)	<0.001	44	17	3.02 (20.2–4.50)	<0.001
Sensitive	30	168	1.00		56	56	1	

Data were analyzed by Cox proportional hazard regression model. IGF1, insulin-like growth factor 1; IGFBP2, IGF-binding protein 2.

Discussion

This study is a comprehensive analysis of EOC patients whose pretreatment IGF serum marker levels correlated with the clinicopathological features, response to chemotherapy, and clinical outcomes. Our conclusions are supported by the following findings: i) high levels of circulating total IGF1, IGF2, and IGFBP3 are associated with favorable clinicopathological characteristics and better outcomes, whereas high IGFBP2 levels are associated with unfavorable prognostic features and prognosis. ii) Low levels of IGF1 or IGF2 and high levels of IGFBP2 correlate with a poor response to chemotherapy. This correlation with IGFBP2 is specifically significant in

patients with serous histology and of older age. iii) Serum IGFBP2 levels, age ≥ 50 years, and advanced-stage disease might serve as independent predictors of EOC-related death after adjusting for clinicopathological variables during the clinical diagnostic phase.

Preclinical findings implicated IGF1 in the induction of cell proliferation and invasion (Cao *et al.* 2007, Lau & Leung 2012). Furthermore, the positive effect of IGF1 on ovarian cancer progression was supported by the correlation of increased IGF1 expression in cancer tissues with disease progression (Brokaw *et al.* 2007) or poor prognosis (Spentzos *et al.* 2007). Similarly, IGF2 gene expression was found to be increased in tumors with poor prognosis (Sayer *et al.* 2005, Lu *et al.* 2006) and to be an independent

Figure 1

(A) Overall survival (OS) and (B) progression-free survival (PFS) curves of 180 ovarian cancer patients who underwent adjuvant chemotherapy, stratified according to IGF1 expression in combination with IGFBP2. High IGFBP2 levels combined with low IGF1 levels significantly correlated with the poorest 5-year OS and PFS.

predictor of poor survival (Sayer *et al.* 2005). Regarding chemotherapy resistance, the IGF2 levels were found to be increased in a paclitaxel-resistant cell line, indicating that IGF2 inhibition might facilitate increased paclitaxel sensitivity (Huang *et al.* 2010).

Recent studies have shown that, in EOC tissues, decreased concentrations of IGFBP3 were significantly associated with advanced stage, suboptimal cytoreduction, and an elevated risk of progression (Katsaros *et al.* 2001, Lu *et al.* 2006, Torng *et al.* 2008). In contrast, one study demonstrated the opposite findings (Walker *et al.* 2007). Accumulating evidence supports the idea that IGFBP2 might act as a promoter, rather than a tumor suppressor, in EOC. This evidence includes the

overexpression of both the *IGFBP2* gene and protein in malignant ovarian cyst fluid (Kanety *et al.* 1996), activated cell invasion in response to IGFBP2 induction (Lee *et al.* 2005), and the concurrent elevation of IGFBP2 levels in EOC tumor tissues and serum (Lancaster *et al.* 2006).

The significance of some circulating IGF biomarkers in our cohorts and those of other investigators seems to be distinct from that in the cellular microenvironment as reported in the literature. In contrast to the positive effects of IGF1 and IGF2 on ovarian cancer cell growth and spread, circulating IGF1 and IGF2 are associated with favorable clinicopathological characteristics and better outcomes. Our results differ substantially from other reports that showed no significant correlations between serum IGF1 levels and clinically important prognostic factors (Baron-Hay *et al.* 2004).

Our results regarding serum IGFBP3 agree to some extent with the previous studies that analyzed tumor samples (Katsaros *et al.* 2001, Lu *et al.* 2006, Torng *et al.* 2008). However, in opposition to our present findings, those studies did not find a significant relationship between IGFBP3 expression and responses to chemotherapy (Katsaros *et al.* 2001) or OS (Katsaros *et al.* 2001, Lu *et al.* 2006) in EOC. Our data agree with the findings (Baron-Hay *et al.* 2004, Serin *et al.* 2008) that changes in the serum IGFBP3 levels parallel the changes in IGF1 levels, but differ in that their study did not show correlations between serum IGFBP3 levels and clinicopathological variables (Baron-Hay *et al.* 2004). In addition, Baron-Hay *et al.* (2004) showed that the highest tertile of serum IGFBP2 levels in EOC patients was related to advanced stage, serous histology, and an unfavorable outcome. Our results are consistent with their findings and indicate the predictive role of IGFBP2 in prognosis. Future studies might investigate the applications of IGFBP2 for predicting the likelihood of optimal cytoreduction or to monitor chemotherapy response in clinical practice. Furthermore, the development of potential antibodies to target IGFBP2 in the serum or tumor tissues of EOC patients is justified on the basis of these compatible findings in circulation and cancer cells.

Evidence currently exists to support IGF1R targeting in ovarian cancer with an IGF1R kinase inhibitor (e.g. NVP-AEW541, BMS-536924, or BMS-554417), antibodies against IGF1R and hybrid receptors (e.g. 486/STOP or EM164), or AMPK activators (e.g. metformin, C93, or compound C) (Beauchamp *et al.* 2010). Nevertheless, ligand-targeted strategies (e.g. somatostatin analogues) have had limited success in clinical trials for nonovarian cancers (Pollak 2008a,b, 2012). Our results indicate that

Table 5 Variable-adjusted hazard ratio (95% CIs) for the association of pretreatment levels of total IGF1, IGF2, IGFBP2, IGFBP3, and IGF1:IGFBP3 molar ratio with the risk of death or disease progression

Factor	Death			Progression		
	Low	High	P	Low	High	P
IGF1, median cutoff (ng/ml)	<98.1	≥98.1		<98.1	≥98.1	
No. of events/no. of patients	42/114	26/114		63/114	45/114	
Age-adjusted HR (95% CI)	1.71 (1.03–2.85)	1.00	0.04	1.81 (1.21–2.71)	1.00	0.004
Multivariable-adjusted HR (95% CI)	1.37 (0.81–2.31)	1.00	0.24	1.27 (0.82–1.94)	1.00	0.28
IGF2, median cutoff (ng/ml)	<1822.8	≥1822.8		<1822.8	≥1822.8	
No. of events/no. of patients	43/114	25/114		56/114	53/114	
Age-adjusted HR (95% CI)	1.85 (0.96–3.54)	1.00	0.06	1.39 (0.82–2.33)	1.00	0.22
Multivariable-adjusted HR (95% CI)	1.74 (0.89–3.41)	1.00	0.11	1.18 (0.69–2.02)	1.00	0.54
IGFBP2, median cut-off (ng/ml)	<565.1	≥565.1		<565.1	≥565.1	
No. of events/no. of patients	24/114	44/114		53/114	56/114	
Age-adjusted HR (95% CI)	1.00	1.80 (1.09–2.95)	0.02	1.00	0.96 (0.66–1.41)	0.84
Multivariable-adjusted HR (95% CI)	1.00	1.59 (0.94–2.70)	0.08	1.00	0.83 (0.56–1.26)	0.39
IGFBP3, median cut-off (ng/ml)	<3558.5	≥3558.5		<3558.5	≥3558.5	
No. of events/no. of patients	40/114	28/114		59/114	50/114	
Age-adjusted HR (95% CI)	1.55 (0.95–2.53)	1.00	0.08	1.60 (1.08–2.35)	1.00	0.02
Multivariable-adjusted HR (95% CI)	1.28 (0.77–2.14)	1.00	0.35	1.15 (0.76–1.73)	1.00	0.50

Data were analyzed by Cox proportional hazard regression model. Multivariable model adjusted for factors at diagnosis: age, stage, histology, residual tumor diameter, and hospital.

the circulating levels of IGF1 or IGF2 differ from those reported for cancer cells in the literature and clarify the protective effects of circulating IGF1 or IGF2 on EOC prognosis. These findings are similar to our previous findings on cervical cancer, which showed an association between reduced total IGF1 levels and an increased risk of cancer death (Huang *et al.* 2008). This should alert clinicians and might provide an explanation for the failure of ligand-targeted-method implementation in cancer therapy. In addition, our evidence does not support the hypothesis that the unfavorable effects of serum IGF1 or IGF2 are limited to sex-hormone-related cancers (Schaffer *et al.* 2007). The discrepancies between circulating IGF1 levels and those in the cellular micro-environment might be explained by the following theories: i) racial differences in socioeconomic and dietary influences and ii) paracrine or autocrine influences of

IGFs on cancer cells (Schips *et al.* 2004, Schillaci *et al.* 2005, Serin *et al.* 2008).

The clinical significance of CA125 for cancer screening is limited, and thus, it is recommended as an adjunct in this clinical setting (Sturgeon *et al.* 2008). Nevertheless, determination of CA125 levels is recommended for treatment monitoring and surveillance for ovarian cancer recurrence. In our cohorts, increased serum CA125 levels at diagnosis were not significantly related to clinically defined platinum sensitivity and chemo-response in the subgroup analysis. IGFBP2 might serve as a better predictor of chemo-responses in serous histology or older patients.

Our study is limited by a lack of consecutive data with regard to the circulating IGF system components during adjuvant chemotherapy. Further investigation is warranted to determine not only IGF system alterations

Table 6 Full model included multivariate analysis of prognostic factors and serum markers at diagnosis in ovarian cancer (*n*=228)

Variable	HR (95% CI) for death	P	HR (95% CI) for progression	P
Serum IGF1 level (low vs high)	1.49 (0.87–2.55)	0.15	1.33 (0.86–2.06)	0.20
Serum IGFBP2 level (high vs low)	1.79 (1.04–3.10)	0.04	0.85 (0.55–1.30)	0.44
Serum CA125 level (high vs low)	1.10 (0.62–1.95)	0.76	1.28 (0.81–2.03)	0.30
Age (≥50 vs <50)	1.84 (1.03–3.23)	0.03	1.26 (0.83–1.91)	0.28
FIGO stage (advanced vs early)	2.35 (1.14–4.83)	0.02	2.31 (1.32–4.06)	0.003
Histology (serous vs non-serous)	0.74 (0.39–1.40)	0.35	1.31 (0.80–2.12)	0.28
Residual tumor diameter (≥1 vs <1 cm)	1.53 (0.84–2.76)	0.16	1.01 (0.62–1.63)	0.98

Data were analyzed by Cox proportional hazard regression model. Full model adjusted for IGF1, IGFBP2, CA125, age, stage, histology, residual tumor diameter, and hospital.

during treatment but also the usefulness of the IGF system in monitoring treatment responses, compared with serial serum CA125 levels. Furthermore, more investigative efforts are needed in order to answer certain unresolved questions by determining the expression of IGF system components in clinical tumor specimens from our cohorts. Another issue is the result of inflation of the type I error rate due to multiple testing in different subgroups. Adjustments for multiple testing are required in a future confirmatory study.

Notwithstanding the limitations, the present large-sample study aims to analyze the relationships between preoperative circulating IGF system levels and prognosis for the same EOC patients who were followed for a maximum of 18 years at two tertiary centers. Many essential clinicopathological factors that were included in our analysis and the long-term surveillance periods facilitated the determination of the significance of the influence of target biomarkers on prognosis. We provide evidence that indicates certain roles for circulating IGF biomarkers in EOC clinical outcomes. In our cohorts, the integration of the IGFBP2 and IGF1 levels could help to identify the patients with worse predicted outcomes. The redirection of these patients to alternate therapies such as dose-dense chemotherapy (Katsumata *et al.* 2009) or the combination of chemotherapy with targeted therapeutic agents (Penson *et al.* 2010, Heitz *et al.* 2012) could potentially provide more efficacious therapies to patients with the most aggressive EOCs.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-13-0274>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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