Serum Platelet-derived Growth Factor and Fibroblast Growth Factor in Patients with Benign and Malignant Ovarian Tumors

CHRISTINE VESTERGAARD MADSEN[1,4], KARINA DAHL STEFFENSEN[1,4], DORTE AALUND OLSEN[3], MARIANNE WALDSTRØM[2], CHARLOTTE HASSELHOLT SØGAARD[5], IVAN BRANDSLUND[3] and ANDERS JAKOBSEN[1,4]

Departments of [1]Oncology, [2]Pathology, and [3]Clinical Biochemistry, Vejle Hospital, Vejle, Denmark; [4]Institute for Regional Health Services Research, University of Southern Denmark, Odense, Denmark; [5]Department of Gynecology and Obstetrics, Aarhus University Hospital, Skejby, Denmark

Abstract. *Background: New biological markers with predictive or prognostic value are highly warranted in the treatment of ovarian cancer. The platelet-derived growth factor (PDGF) system and fibroblast growth factor (FGF) system are important components in tumor growth and angiogenesis. Materials and Methods: Pre-surgery peripheral blood samples were collected consecutively from 213 patients (42 with normal ovaries, 54 with benign, 21 with borderline, and 96 with malignant ovarian tumors) undergoing surgery for an untreated pelvic mass. Serum PDGF-AA, PDGF-BB, and FGF2 were quantified on a Luminex analyzer. Results: Median PDGF-AA, PDGF-BB, and FGF2 levels were higher in patients with ovarian cancer than in those with borderline tumors, and normal ovaries, and PDGF-BB and FGF2 were also higher as compared to patients with benign tumors. PDGF-AA and PDGF-BB were associated with FIGO stage and residual tumor and PDGF-BB was associated with histological subtype. Conclusion: PDGF-AA, PDGF-BB, and FGF2 are up-regulated in ovarian cancer and levels of serum PDGF-AA and PDGF-BB seem to be associated with stage and residual tumor in ovarian cancer.*

Angiogenesis and tumor microenvironment are known to play an important role during carcinogenesis (1, 2). In order to grow beyond the size of 1-2 mm^3, the tumor requires blood vessels that can promote the growth (3). The onset of a shift in the angiogenic balance allows the up-regulation of several pro-angiogenic factors (1, 2, 4, 5), such as vascular endothelial growth factor (VEGF), platelet-derived growth factor-BB (PDGF-BB), and fibroblast growth factor 2 (FGF2), which by ways of mutual interactions (6-8) stimulate tumor angiogenesis. Stromal cells may also contribute to neovascularization (9).

During the past decade, there has been an increased interest in the study of angiogenesis as a target for anticancer treatment (7). At the same time, there has been a growing awareness of the need for validated prognostic or predictive biomarkers to identify groups of patients that would benefit from such treatment (10-12). So far, there has been special focus on the VEGF system. However, it is presumed that other pro-angiogenic factors may be involved in tumor evasion of anti-VEGF treatment (13), and recently there has been an increased development of novel agents targeting multiple angiogenic pathways (*e.g.* VEGFR, PDGFR, and FGFR). This accentuates the need for an increased understanding of the mechanisms of action of the FGF and PDGF systems and their potential utility as cancer biomarkers.

FGF2 belongs to a large family of growth factors (14, 15) and acts as a potent pro-angiogenic factor, affecting endothelial cell migration and proliferation (16, 17). Increased levels of serum FGF2 in malignant tumors, as compared to healthy controls, have been demonstrated in studies of thyroid carcinoma (18), and metastatic colorectal (19), testicular (20), and breast cancer (21, 22). Serum FGF2 also seems to have a prognostic value in lung cancer (23, 24), as well as correlations with clinicopathological parameters in renal cell carcinoma (25) and hepatocellular carcinoma (26). Two studies reported higher levels of serum FGF2 in patients with epithelial ovarian cancer (27, 28) compared to healthy controls. High FGF2 levels were also found in ascites from those tumors (27).

PDGF-AA and PDGF-BB belong to the PDGF family, which is known to play an important role in cell growth (29), chemotaxis (29, 30), and in the regulation of the tumor stroma (31-34). PDGF-BB promotes pericyte recruitment and the

Correspondence to: Christine Vestergaard Madsen, Department of Oncology, Vejle Hospital, Kabbeltoft 25, DK-7100 Vejle, Denmark, Tel: +45 79405446, e-mail: christine.vestergaard.madsen@slb.regionsyddanmark.dk

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stabilization of microvasculature (35, 36), which makes it an important component in angiogenesis. PDGF-AA may affect the recruitment of tumor-associated stroma that produces angiogenic factors (9). Elevated levels of serum PDGF-AA and/or PDGF-BB have been found in breast (22), head and neck (37), colorectal (38), and esophageal cancer (39), as well as pleural mesothelioma (40), compared with normal or benign tumors, but the findings reported in the literature are not consistent (19, 41). Serum PDGF-AA and/or PDGF-BB also seem to have a prognostic value, as well as some relation to clinicopathological parameters (39-42), but again the literature is not consistent.

Expression of the PDGF system has been demonstrated in ovarian cancer (43-50) and PDGF-AA and PDGF-BB have been found in ascites from patients with ovarian cancer (46, 51) with a correlation between the levels of PDGF-BB and VEGF (51). However, studies of serum PDGF-AA and PDGF-BB are very sparse for this cancer type, as they are for FGF2. Therefore, the aim of the present study was to investigate the levels of serum PDGF-AA, PDGF-BB, and FGF2 in patients with normal ovaries, and in those with benign, borderline, or malignant ovarian tumors. Furthermore, we investigated whether these markers were associated with clinicopathological parameters or clinical outcome in ovarian cancer.

Materials and Methods

Materials. This study consisted of blood samples from 213 patients who underwent surgery for an untreated pelvic mass at two gynecological departments in the period from March 2005 to April 2011. The patients were enrolled in a prospective Danish translational research protocol, approved by the Danish Biomedical Research Ethics Committee and the Danish Data Protection Agency. Written informed consent was obtained from all patients. Pre-surgery peripheral blood samples were collected consecutively, centrifuged at 2000 ×*g* for 10 min at room temperature and serum was subsequently stored at minus 80˚C until use.

Malignant ovarian cancer, including primary ovarian, peritoneal and fallopian cancer, were found in 96 of the patients; borderline tumors of serous and mucinous types were seen in 21 of the patients; and benign ovarian tumors, which included serous cystadenofibroma, serous cystadenomas, mucinous cystadenomas, fibrothecoma, fibroma, and dermoid cysts, were found in 54 of the patients. Forty-two of the patients had either normal ovaries or functional cysts, such as of the corpus luteum, follicle, endometrioid cysts, or paraovarial in benign cysts.

Patients who were diagnosed as having a non-ovarian malignant disease, synchronic endometrial cancer, or who were already known to suffer from another type of disseminated cancer were excluded from the current study. The malignant ovarian tumors were classified using the World Health Organization (WHO) histological classification of 2003 (52), graded according to Shimizu and Silverberg (53), and further classified according to Kurman and Shih (54).

Quantification of PDGF-AA, PDGF-BB, and FGF2 in serum. PDGF-AA, PDGF-BB, and FGF2 were quantified simultaneously using commercial Fluorokine MAP multiplex kits (cat#LAN000; R&D systems, Minneapolis, Minnesota, USA) on a Luminex analyzer (Luminex Corporation, Texas, Austin, USA). The serum samples were diluted five-fold in sample diluent provided with the kit, then 100 μl of standard, control, and diluted samples were added to the plate together with 50 μl of the antibody capture bead mixture, and the plate was incubated for 2 h. Washing was then carried out three times using assay buffer and vacuum filtration. Fifty microliters of diluted biotin-coupled antibody cocktail were added to each well and the plate was incubated for 1 h followed by washing. Fifty microliters of diluted Streptavidin conjugated with phycoerythrin were added to the plate and incubated for 30 min in

Table I. *Patients' characteristics.*

	N. (%)
Ovarian cancer	96 (45)
Borderline ovarian tumors	21 (10)
Benign ovarian tumors	54 (25)
Normal ovaries including functional cysts	42 (20)
Total	213 (100)
Ovarian cancer	96 (100)
Age, years	
<60	30 (31)
≥60	66 (69)
FIGO stage	
I	20 (21)
II	8 (8)
III	58 (61)
IV	10 (10)
Histological tumor grade	
I	19 (20)
II	18 (19)
III	47 (49)
Not graded	12 (12)
Histological cell type	
Serous	71 (73)
Mucinous	7 (7)
Endometrioid	5 (5)
Clear cell	6 (6)
Mixed	3 (3)
Carcinosarcoma	5 (5)
Residual tumor*	
0 cm	44 (46)
<1 cm	10 (10)
>1 cm	42 (44)
Type I tumors	
Low-grade serous, low-grade endometrioid, clear cell, and mucinous	26 (28)
Type II tumors	
High-grade serous, high-grade endometrioid, undifferentiated. carcinoma.	68 (72)
First line chemotherapy	
Platinum based	77 (80)
Other agents	5 (5)
No systemic therapy	14 (15)

*From primary debulking surgery.

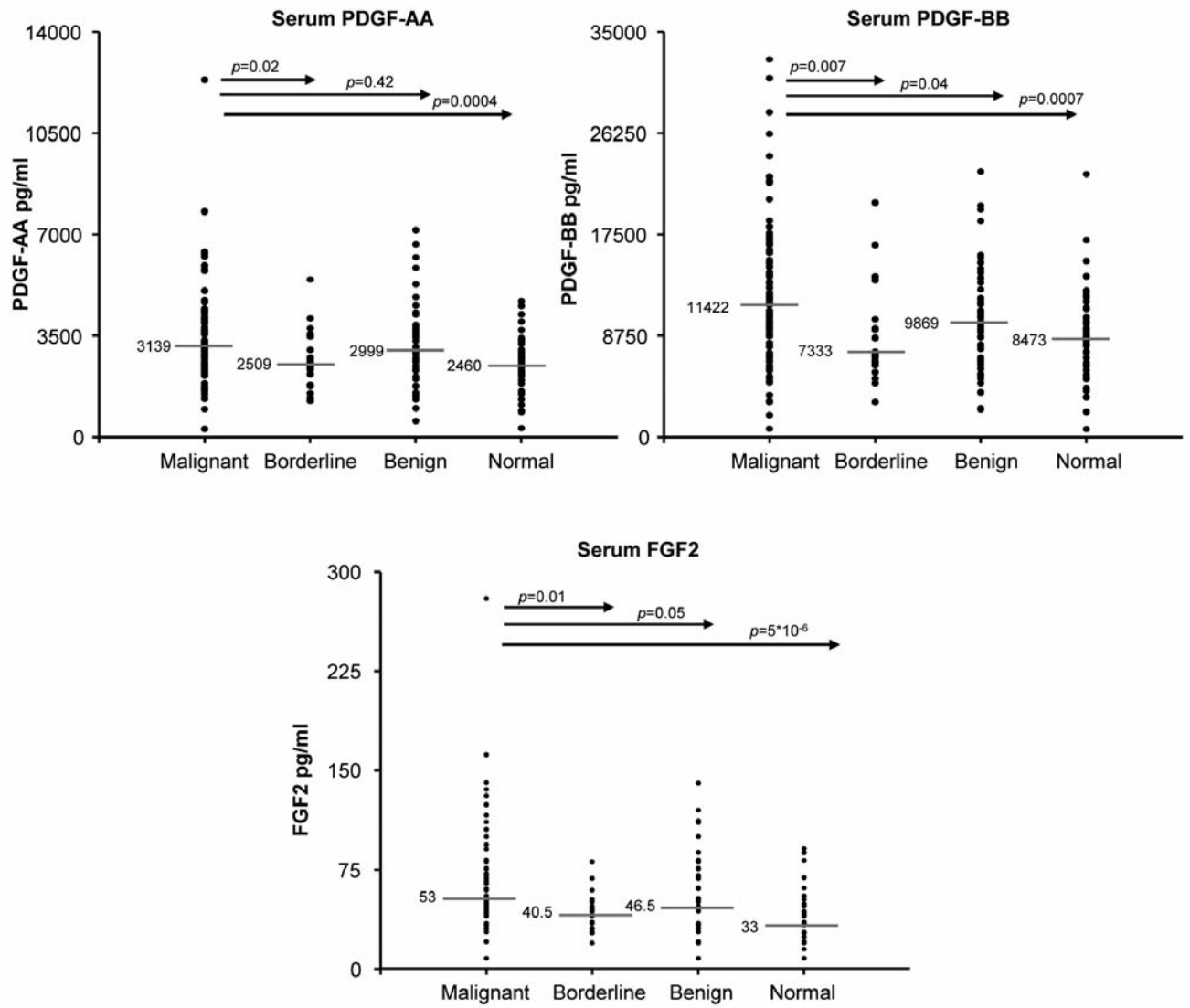

Figure 1. *Dot plots of serum Platelet-Derived Growth Factor (PDGF)-AA, PDGF-BB and Fibroblast Growth Factor 2 (FGF2) between groups. Median values are marked by horizontal bars.*

the dark. Finally after washing, 100 μl of assay buffer were added and the plate was incubated for 2 min, after which the analysis was carried out on the Luminex analyzer. All incubations were performed on a plate shaker at room temperature. Standard curves were used for the determination of PDGF-AA, PDGF-BB, and FGF2 concentrations. The total coefficient of variation determined from an in house serum pool was 5.7% for PDGF-AA, 7.9% for PDGF-BB, and 19.9% for FGF2.

The limit of detection (LOD) for FGF2 was 3.36 pg/ml when uncorrected for dilution, and 16.8 pg/ml when corrected for dilution. LOD for PDGF-AA was 0.086 pg/ml when uncorrected for dilution, and 0.43 pg/ml when corrected for dilution. LOD for PDGF-BB was 0.0483 pg/ml when uncorrected for dilution, and 0.24 pg/ml when corrected for dilution.

The limit of quantification (LOQ) for FGF2 was 6.45 pg/ml when uncorrected for dilution, and 32.25 pg/ml when corrected for dilution. LOQ was PDGF-AA 0.2 pg/ml when uncorrected for dilution, and 1.0 pg/ml when corrected for dilution. LOQ for PDGF-BB was 0.1 pg/ml when uncorrected for dilution, and 0.5 pg/ml when corrected for dilution.

In 22 of the samples (four malignant, nine benign, and nine normal), FGF2 was below the detection limit, and in these cases 8.0 pg/ml (corrected for dilution) was used as the appropriate value.

Statistics. The concentrations of PDGF-AA, PDGF-BB, and FGF2 did not fit a Gaussian-Distribution and the Mann-Whitney *U*-test was used for comparing the medians between the patients groups. Correlations between continuous data were described by Spearman rank correlation coefficient (r). Kaplan-Meier estimates were used for univariate overall survival (OS) analysis, illustrated by survival plots, and the log-rank statistic was used for comparing the survival between two groups. OS was calculated as the interval from the time

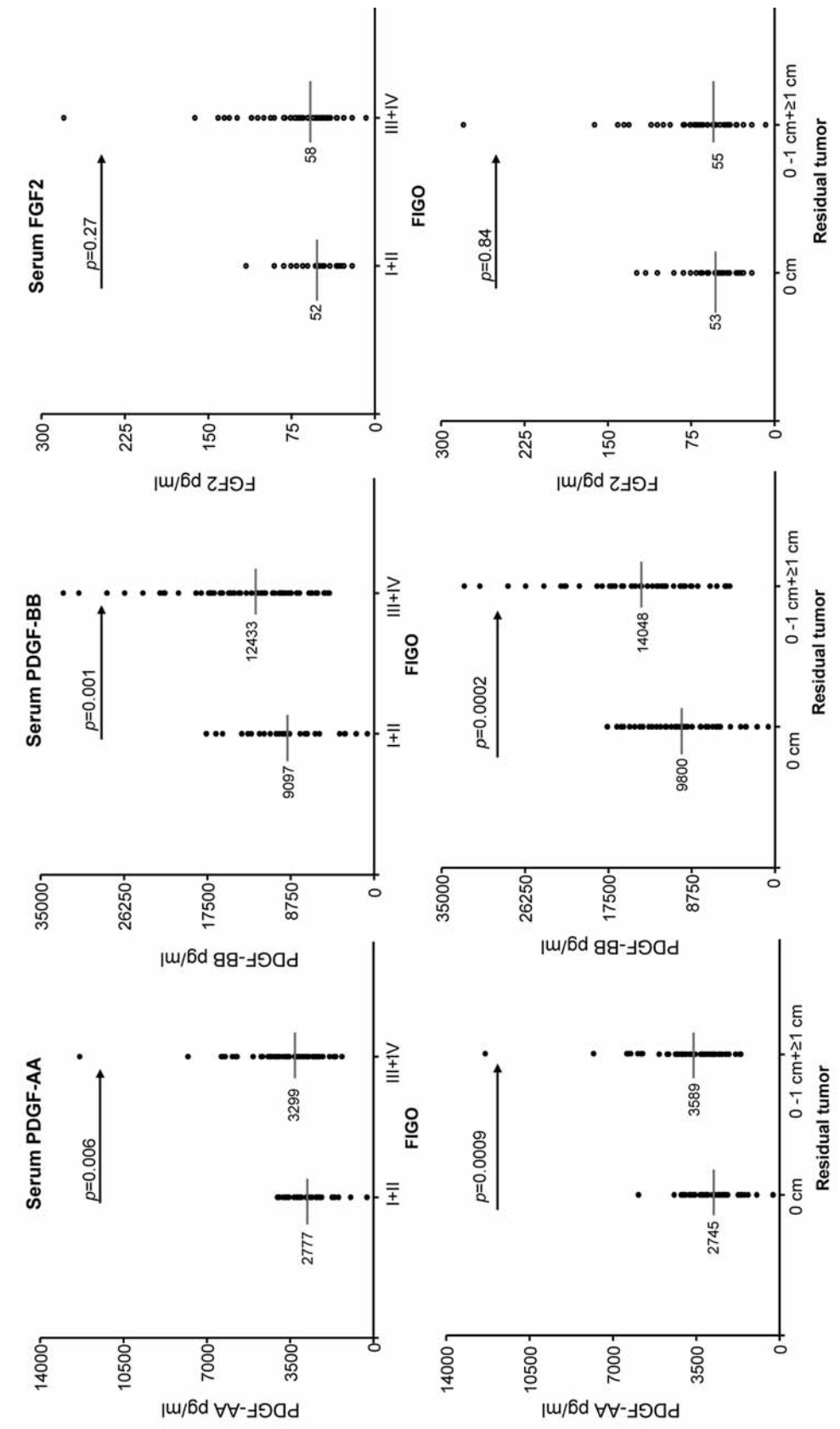

Figure 2. *Dot plots of serum markers in relation to FIGO stage and residual tumor in patients with ovarian cancer. Median values are indicated by horizontal bars.*

Table II. *Serum Platelet-Derived Growth Factor (PDGF)-AA, PDGF-BB, and Fibroblast Growth Factor2 (FGF2) in ovarian cancer patients and their relation to clinicopathological variables.*

Variable	Median PDGF-AA, pg/ml	*p*-Value	Median PDGF-BB, pg/ml	*p*-Value	Median FGF2, pg/ml	*p*-Value
Age, years		0.75		0.87		0.31
<60	3242		10571		60	
≥60	3105		11754		53	
FIGO stage		0.006		0.001		0.27
I+II	2777		9097		52	
III+IV	3299		12433		58	
Tumor grade		0.18		0.39		0.30
I	3025		11399		53	
II+III	3290		11862		60	
Histological cell type		0.06		0.02		0.89
Serous	3290		12418		54	
Non-serous	2766		9950		53	
Residual tumor**		0.0009		0.0002		0.84
0 cm	2745		9800		53	
≤1cm + >1cm	3589		14048		55	
Kurman's		0.07		0.12		0.35
Type 1 tumors	2873		10462		53	
Type 2 tumors	3127		11862		58	

**From primary debulking surgery including a few patients never operated.

of diagnosis until death from any cause; progression-free survival (PFS) was calculated from the time of diagnosis until disease recurrence or death from any cause. The Cox regression model was used for multivariate analysis of prognostic parameters. A value of $p \leq 0.05$ was considered statistically significant.

The number Cruncher Statistical System (NCSS), version 2007, (Kaysville, UT, USA) software package was used for the statistical analyses.

Results

Patients' characteristics. Table I summarizes the patients´ characteristics. The median age at the time of diagnosis was 67 years for patients with ovarian cancer, 63 years for those with borderline tumors, 60 years for those with benign tumors, and 50 years for patients with normal ovaries and/or functional cysts.

The majority of patients with ovarian cancer were diagnosed with disease in advanced stage (71%) and classified as type II tumors (73%) using the criteria suggested by Kurman *et al*. Serous adenocarcinoma was the most frequent histological subtype (73%). The median duration of follow-up was 4.4 years (95% Confidence Interval (CI)=4.1 to 4.8 years) for those still alive (December 2011).

Serum concentrations of PDGF-AA, PDGF-BB and FGF2. There was a highly positive correlation between serum PDGF-AA and PDGF-BB (r=0.72, $p<0.001$), and a weaker correlation between PDGF-AA and FGF2 (r=0.29, $p<0.001$) and between PDGF-BB and FGF2 (r=0.31, $p<0.001$) (data not shown).

As shown in Figure 1, the median serum levels of PDGF-AA were significantly higher in patients with ovarian cancer than in patients with borderline tumors and normal ovaries, but not higher than in patients with benign tumors. Median serum levels of PDGF-BB and FGF2 were the highest in patients with ovarian cancer, when compared to patients with borderline tumors, benign tumors, and normal ovaries. There was a certain degree of overlaping for each serum marker among the different patient groups, also illustrated in Figure 1.

Serum PDGF-AA, PDGF-BB, and FGF2 and their relation to clinicopathological parameters in patients with ovarian cancer. As demonstrated in Table II and Figure 2, significantly higher preoperative median PDGF-AA and PDGF-BB levels were seen in patients with residual disease after primary surgery than in patients with no residual disease. The median PDGF-AA and PDGF-BB levels were also higher in patients with FIGO III and IV stage compared to patients with FIGO I and II. Furthermore, a relation to histological subtype was seen, with median PDGF-BB being higher in serous adenocarcinoma than in non-serous adenocarcinoma, whereas PDGF-AA showed the same tendency without being significant. Serum FGF2 was not clearly related to clinicopathological parameters.

Prognostic value of serum PDGF-AA, PDGF-BB, and FGF2 in patients with ovarian cancer. As can be seen in Figure 3, serum PDGF-AA, PDGF-BB, and FGF2 levels were divided

into quartiles and investigated in relation to PFS and OS. Patients with PDGF-AA and PDGF-BB levels above the 75th percentile seemed to fare worst. The difference became statistically significant for PDGF-AA and PDGF-BB when the patients were divided into two groups, one with a level above the 75th percentile and the other with a level below the 75th percentile. Patients with low serum PDGF-AA had higher PFS (p=0.04) and overall survival (p=0.04) than patients with high levels of PDGF-AA. The same result was seen for PDGF-BB regarding PFS (p=0.04) and OS (p=0.05). However, the prognostic value of serum PDGF-AA and PDGF-BB was not confirmed, making them independent prognostic markers in multivariate analysis which included the classic prognostic markers such as age, FIGO stage, grade, histological subtype, and residual tumor (data not shown). There was no clear relationship between serum FGF2 and survival in the studied material.

Discussion

The emergence of new agents designed to target the PDGF and FGF systems accentuates the need for a better biological understanding of these systems. The discovery of new prognostic or predictive markers may prove useful in the stratification of patients in the direction of more individualized treatment strategies

In our study, serum median PDGF-AA, PDGF-BB, and FGF2 levels were found to be the highest in patients with ovarian cancer, showing that these systems are up-regulated in malignant tumors and may contribute to tumor growth. Regarding FGF2, these results are in agreement with the results from Barton *et al*. (27) and Le Page *et al*. (28). In tissue analysis, Henriksen *et al*. (48) demonstrated higher immunohistochemical expression of PDGF in tumor cells from ovarian carcinoma compared to normal and benign tumors. Nevertheless, we do not consider serum PDGF-AA, PDGF-BB, and FGF2 to be useful for diagnostic purposes, since there was a considerable degree of overlaping among the different patient groups. Regarding FGF2, we found a relatively high value of limit of quantification (LOQ), but this seems to be of minor importance in the present study as our investigation did not aim to monitor patients or to serve diagnostic purposes, but only to compare the serum levels between the groups.

In our study, higher levels of PDGF-AA and PDGF-BB were seen in patients with advanced-stage (FIGO III and IV) ovarian cancer compared to patients with early-stage (FIGO I and II) disease, indicating that the serum levels are correlated to tumor stage. This study also revealed a positive association between serum PDGF-AA and PDGF-BB and the presence of residual tumor after surgery. It is well-known that primary surgery has an impact on survival in ovarian cancer (55, 56) and the goal is cytoreduction to microscopic disease (57). The fact that the levels of PDGF-AA and PDGF-BB were significantly higher in patients in whom macroscopic complete resection was not possible, raises the question of whether preoperative serum PDGF-AA and PDGF-BB could be useful in selecting patients for neoadjuvant chemotherapy. This hypothesis seems worth pursuing.

High serum levels of PDGF-AA or PDGF-BB greater than the 75th percentile were related to an unfavorable prognosis in our study, but neither of them were confirmed as independent prognostic markers in multivariate analysis. Previous studies have demonstrated a possible relation between the expression of PDGFR-α, measured by means of immunohistochemistry, which appeared to have an influence on clinical outcome and overall survival in ovarian cancer (48, 47) as well as a relation to stage and residual tumor (47). To the best of our knowledge, there are no published studies about serum PDGF-AA and PDGF-BB in ovarian cancer and their relation to survival. Regarding other cancer types, a study of PDGF-AB in pleural mesothelioma (40) demonstrated a relation between high serum PDGF-AB and low survival in univariate analysis. In a study of pancreatic cancer (41), high levels of serum PDGF-AA were a predictor of poor prognosis, whereas high levels of PDGF-BB were associated with a favorable prognosis. In a study of patients with breast cancer (42), high serum levels of PDGF in combination with high insulin-like growth factor-1 (IGF-1) were associated with the risk of breast cancer recurrence.

The advantages and disadvantages of measuring PDGF in serum *vs*. plasma are well-discussed in three studies (39, 58, 59) and the serum levels of growth factors may be an indicator of both their cellular and soluble concentrations (58, 59). Being less invasive, the measurement of biomarkers in blood is preferable to the measurement in the tumor tissue (10, 12). However, PDGF and FGF are not cancer-specific markers, and more knowledge regarding their expression in tumors and their biological variation in the circulation is needed before a possible clinical role can be decided.

In conclusion, our results suggest that PDGF-AA, PDGF-BB, and FGF2 are up-regulated in ovarian cancer. Preoperative serum PDGF-AA and PDGF-BB were, in the present study, associated with FIGO stage and residual tumor after surgery in patients with ovarian cancer and PDGF-BB was associated with histological subtype.

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Conflicts of Interest

The Authors declare that there are no conflicts of interest.

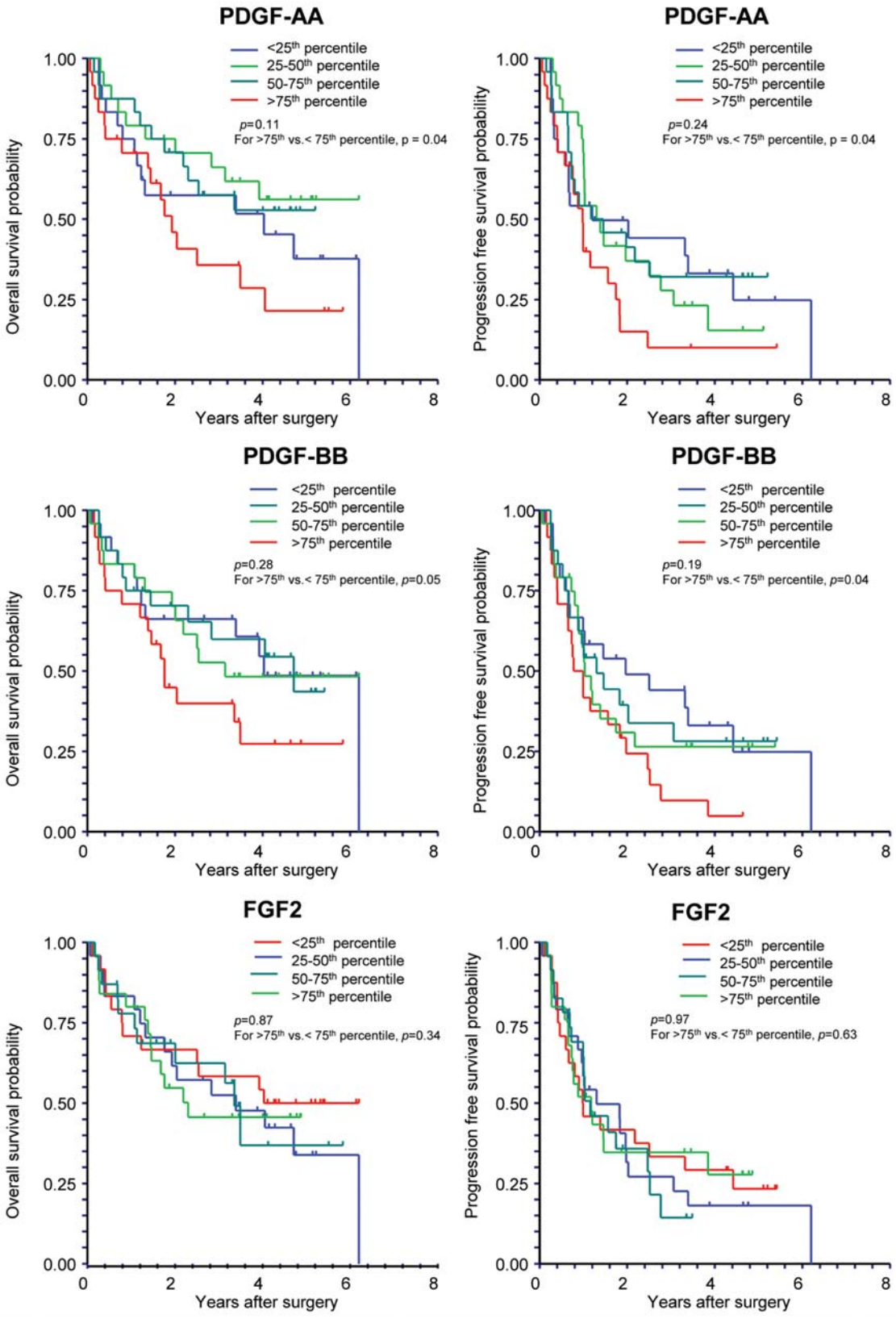

Figure 3. *Kaplan Meier survival curves according to serum Platelet-Derived Growth Factor (PDGF)-AA, PDGF-BB, and Fibroblast Growth Factor 2 (FGF2) in patients with ovarian cancer.*

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