Prognostic Value of Serum and Ascites Levels of Estradiol, FSH, LH and Prolactin in Ovarian Cancer

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Abstract. *The aim of this study was to investigate the influence of sex hormone levels on tumor biology and patients' outcome in ovarian cancer. Patients and Methods: One hundred and six patients with ovarian cancer were enrolled into this prospective study. Serum and ascites samples were obtained intraoperatively. Concentrations of estradiol, FSH, LH and prolactin were measured and correlated with parameters of tumor biology, such as FIGO stage, tumor spread and postoperative tumor residual mass. Patients with primary ovarian cancer were compared to patients with recurrent disease. Influence factors on progression-free survival and overall survival were analyzed using the Kaplan-Meyer method. Results: Serum FSH concentrations were significantly higher and estradiol concentrations in ascites were significantly lower in patients with recurrent disease. According the multivariate analysis, only FSH level in ascites was seen to be an independent prognostic factor for patients' survival. Conclusion: High level of FSH in the ascites provides prognostic information in patients with ovarian cancer and is inversely correlated with patients' survival.*

Ovarian cancer is the leading cause of death in all gynecological malignancies. Despite achievements in their surgical and systemic treatment, the 5-year survival rate remains low (1). The aggressive behavior of the ovarian malignancies can be explained by the long symptom-free interval, the unspecific symptomatology and by the absence of appropriate screening tests. Accepted prognostic factors for overall survival are FIGO stage and postoperative tumor diameter (2). Many other factors like patient' s age, histological subtype, histological grading or lymph node involvement were not confirmed in larger series (3).

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The etiology of ovarian cancer remains largely unclear. Some evidence indicates an impact of endocrine factors on the tumorigenesis of ovarian cancer (4). Few pregnancies, but also a delayed menopause, increase the risk for ovarian cancer whereas multipe pregnancies, lactation and the use of oral contraceptives seem to have a protective effect (5). These observations support the "ovulation hypothesis" (6) postulating a negative effect of repeated ovulations and concomitant rupture of ovarian epithelium with a supposed increasing risk of spontaneous mutations. The gonadotropin hypothesis postulates a correlation of continuously increased serum levels of androgens, predominantly dehydroepiandrosterone (DHEA) induced by excessive stimulation from pituitary gonadotropins, and risk of ovarian cancer (4). In accordance with this hypothesis, ovarian cancer is more frequent in women suffering from polycystic ovarian syndrome where luteinizing hormone (LH) levels are constantly increased (7). *In vitro* studies suggest an important role of gonadotropin receptors in proliferation processes of benign and malignant ovarian epithelial cells (8-11). Similarly, obesity – from the endocrine perspective a state with persistently elevated estrogen levels – seems to provide favorable conditions for the development of ovarian cancer (12). This hypothesis assumes a tumor promoting effect of systemic estrogens by peripheral estrogen synthesis. There are therapeutic strategies based on endocrine treatment, mostly in patients with refractory or recurrent ovarian cancer at advanced tumor stages. Protocols with GnRH agonists, antiandrogens or antiestrogens like Tamoxifen or aromatase inhibitors have already been used in clinical trials (13, 14) with inconsistent data of efficacy. Recently, Smyth *et al*. reported the use of Letrozole in estrogen-receptor-positive patients with ovarian cancer, a step towards a specific and more individualized endocrine therapy in patients with ovarian cancer (15). Estrogens seem to interfere in tumorigenesis of ovarian cancer cells by regulating gene expression of tumor receptors and apoptosis (16-18). All of these findings suggest an influence of sex hormones on ovarian cancer or, *vice versa* that the female endocrine system is affected by ovarian cancer. Nevertheless, data about concentrations of sex hormones in

ovarian cancer patients are very limited. Due to the high prevalence of ascites in patients with advanced ovarian cancer and the fact that ascites presents the direct tumor environment, all hormones were analyzed in both ascites and serum samples.

The objective of this study was to determine the concentration of each hormone and to examine whether there is a prognostic value of serum or ascites sex hormone levels and if sex hormone levels depend on tumor biology (histologic subtype, tumor spread, grading). Therefore ascites and serum hormone levels were correlated with progression-free and overall survival, tumor histology, tumor spread with exact tumor rest and detailed tumor pattern, grading and FIGO stage.

Patients and Methods

Between 1997 and 2000, 106 patients with primary or recurrent ovarian cancer were included into this study. All of them underwent surgical treatment. Inclusion criteria for enrollment were: at least 18 years of age, written informed consent, and no endocrine treatment within 6 months before surgery. Patients with other malignant or semimalignant ovarian tumors such as granulosa cell tumor, borderline tumor or germ cell tumor were excluded from the study.

Ascites samples were obtained for all 106 patients. This was done in 104 patients during operation and in 2 patients by percutaneous ascites puncture. After aspiration, ascites samples were centrifuged at 1650 rpm/min for 5 minutes. Blood samples were centrifuged at 3010 rpm/min. All samples were then aliquoted and stored at –80˚C until they were assayed.

Prolactin (P) concentrations were determined using the LIA-mat-Prolactin-Test (Byk-Sangtec Diagnostica, Dietzenbach, Germany), an immunoluminometric test. Estradiol (E) concentrations were determined using the RIA-mat Estradiol-*in vitro*-Test, a radioimmunoassay by Byk-Sangtec Diagnostica (Dietzenbach, Germany). Follicle stimulating hormone (FSH) and LH concentrations were determined by using ELISA (DRG FSH MTPL-EIA and DRG LH MTPL-EIA, respectively, DRG Instruments GmbH, Marburg, Germany).

Gynecological and surgical parameters were collected, data from the tumor bank and from a validated, self-developed, systematic surgical and histopathological documentation system (Intraoperative Mapping of Ovarian Cancer/IMO) including time of first diagnosis, tumor histology, tumor spread, grading and FIGO stage were added. If in a histological report two tumor grades were found to be present, the higher grade was taken (*i.e.* G2-G3 resulted in G3). Surgical methods, postoperative tumor residuals, pattern of metastasis and state of recurrence were further parameters. Any intra-abdominal organ was counted as affected if tumor invasion exceeded the serosa. Localization sites of metastases documented were peritoneum, bladder, omentum, mesenterium, bursa omentalis, stomach, small intestine, colon, spleen, diaphragm, abdominal wall, pleura, lung, liver, bone, brain, and skin. Postoperative tumor residuals were classified as being macroscopically tumor free, ≤2 cm, or >2 cm of the largest tumor diameter. Progression-free survival was defined as the interval between the time the samples were taken and the clinical diagnosis of tumor progression. Tumor progression was based on the results of radiological measurements (*e.g.* CT, MRI), palpation or histological diagnosis of recurrence. Overall survival was calculated from the time the sample was taken and the patient's death or last contact.

Statistics. To increase the validity of the subgroup analyses, the following FIGO stages and histological grading were summarized for statistical analysis: FIGO stages I + II and III + IV; histological grading: G1 + G2 and G3. For all statistical procedures, the statistical program SPSS for Windows 13.0 was used. No parameters showed a Gaussian distribution, so that medians and interquartile ranges are given instead of means. For statistical analysis of differences between subgroups, the Mann-Whitney *U*-test was used for continuous variables (two-tailed), the χ^2-test by Pearson and Fisher's Exact Test for discrete variables. Statistical significance was established at a two-tailed *p*-value <0.05. Survival rates were determined by Kaplan-Meier analysis. Overall survival probability was estimated using the Kaplan-Meier product limited method. Correlations between concentrations of the sex hormones and clinical parameters or survival rates were analyzed using univariate and multivariate Cox regression models. Odds ratios with 95% confidence interval (CI) were calculated.

Results

Altogether 106 patients with histologically proven malignant epithelial tumors of the ovaries were included. Sixty-one patients had the first diagnosis of ovarian cancer, 45 patients had an active relapse. Median age in patients with primary ovarian cancer was 61 years (range 33-88) and 58 years in patients with recurrent disease (range 35-83).

Baseline characteristics. The median patients' age was 59 years. In 59 of the 61 patients with primary ovarian cancer, a laparotomy was performed with the aim of maximal tumor debulking. Among these, complete tumor resection was achieved in about one third of patients (Table I). Among 45 patients with recurrent ovarian cancer a therapeutic laparotomy was performed in 37 cases (82%). Among these, complete or nearly complete tumor resection was achieved in about two third of cases.

In 95 patients (90%), a peritoneal carcinomatosis was observed during laparotomy. Seventy patients (66%) underwent lymph node dissection. In 53 cases, lymph node metastases were detected by histological examination. Among all cases, ascites volume was ≤500 mL in 34% (n=36), and in all other cases >500 mL (n=70).

Sex hormone concentrations in serum and ascites. Overall, median serum levels of FSH and LH were 22.6 U/L (interquartile range) and 18.6 U/L, respectively. Median E concentration was 7.0 pg/mL, median P level was 74.0 ng/mL. Median ascites levels of FSH and LH were 21.4 U/L and 12.0 U/L, respectively, and concentrations of E and P were 9.7 pg/mL and 18.0 ng/mL, respectively.

Primary versus recurrent ovarian cancer. Serum FSH concentrations were significantly higher and E concentrations in ascites were significantly lower in patients with recurrent disease than in patients with primary ovarian cancer (median concentrations of 29.4 [interquartile range of 15.7-41.1] U/L

Table I. *Patients' characteristics*.

	Primary ovarian cancer	Recurrent ovarian cancer	*P*
N	61 (35.3%)	45 (26.0%)	
Median age (years. range)	61 (33-88)	58 (35-83)	**0.0001**
Histology			
Serous-papillary	45 (73.8%)	36 (80.0%)	0.33
mucinous	5 (8.2%)	2 (4.4%)	0.26
endometrioid	5 (8.2%)	1 (2.2%)	**0.034**
Undifferentiated	2 (3.2%)	4 (8.9%)	0.41
Unclassified	1 (1.6%)	0	
other	1 (1.6%)	2 (4.4%)	
Grading			
I	2 (3.3%)	1 (2.2%)	0.56
II	22 (36.1%)	9 (20.0%)	**0.020**
III	35 (57.4%)	30 (66.7%)	0.62
IV	2 (3.3%)	5 (11.1%)	0.26
Postoperative tumor residuals			
No residual mass	21 (34.4%)	10 (22.2%)	**0.024**
<2 cm	28 (45.9%)	19 (42.2%)	0.89
>2 cm	10 (16.4%)	8 (17.8%)	0.64
Ascites volume			
<500 mL	15 (24.6%)	21 (46.7%)	0.32
>500 mL	46 (75.4%)	24 (53.3%)	**0.009**

vs. 14.2 [7.1-25.8] U/L and 5.5 [5.0-17.5] pg/mL *vs*. 12.9 [5.0-29.1] pg/mL, respectively, $p<0.05$ for both). Apart from these findings, there were no significant differences in serum or ascites hormone levels between patients with primary or recurrent ovarian cancer.

Correlation with clinical parameters. There were no significant differences in hormone levels of patients with different tumor grades or different FIGO stages. Hormone levels, either in serum or in ascites, were not found to depend on ascites volumes. Hormone levels in serum showed a positive correlation between serum and ascites for FSH (correlation coefficient of 0.95) and LH (0.83), whereas there was no correlation seen for E (r=0.13) and P (r=0.07). Median survival time was 37.5 months. Overall, 38 patients had died during the study period. Median progression-free survival time for all patients was 35 months.

According the multivariate analysis with Cox regression, FSH level in ascites was an independent prognostic predictor for the time of survival with worse outcome associated with higher FSH levels ($p<0.05$). With a FSH level <10 U/L, median overall survival time was 36 months, whereas median overall survival time was 29 months with a FSH level >10 U/L (Figure 1). The only other independent predictor was postoperative tumor residuals (0.001). Amount of ascites, age, tumor grade, histology, serum E, P or LH levels or ascites FSH, LH or P levels were no independent prognostic factors for patients' survival.

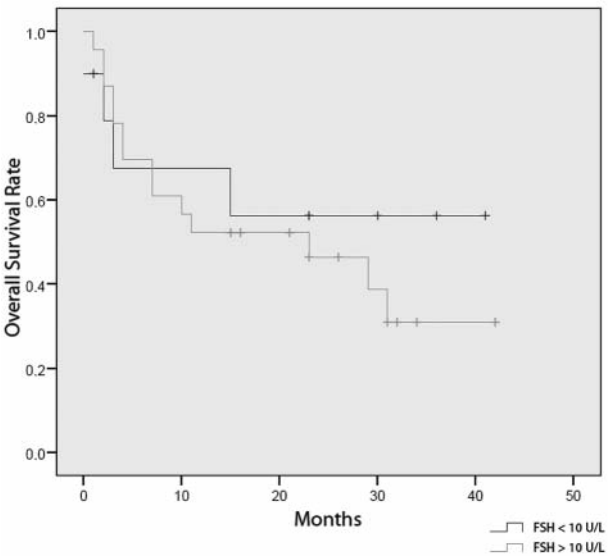

Figure 1. *Overall survival rate and FSH in ascites*.

Discussion

In this prospective study the association of serum and ascites levels of FSH, LH, estradiol and prolactin with classical prognostic factors were investigated along with the effect of these hormones on the patients' outcome. For this study samples from serum and ascites in 106 patients with ovarian cancer were obtained during surgical procedures. Concerning epidemiological and basic tumor parameters, the patients' cohort largely reflects a representative sample of patients with ovarian cancer as to age, histology and tumor stage. A positive correlation between serum and ascites levels of FSH and LH was observed, and thus ascites gonadotropin levels seem to be more an ultrafiltrate of serum levels rather than the result of an endogenous intraperitoneal production of FSH and LH. In addition, ascites levels do not exceed serum levels, median concentrations are about the same (FSH) or somewhat lower (LH), making the ultrafiltrate hypothesis more probable. On the contrary, ascites concentrations of estradiol did not correlate to serum levels and even exceeded them on a generally low level, making an intraperitoneal origin of ascites estradiol probable. On the other hand, ascites estradiol levels did not influence the patients' outcome (survival parameters) nor was any correlation found with the ovarian cancer tumor biology (histological subtype, grading, metastases). In an unpublished study on 67 patients with benign gynecological diseases, Douglas samples were obtained and a median E concentration of 72.6 pg/mL with a range of 5.0-8000 pg/mL was observed, therefore indicating higher E levels than in patients with malignant disease. These observations suggest an intraperitoneal, ovarian origin of estradiol and that an intact ovary is a better source of E

production than is an ovary which is altered by malignant transformation. It is of interest that E concentrations were significantly lower in patients with recurrent ovarian cancer. Therefore it can be stated that even after primary malignant transformation, ovarian cells are capable of keeping up a basal estradiol production and that this is altered by advancing selection of malignant cell lines in recurrent cancer.

Systemic prolactin concentrations were about four times higher than intraperitoneal concentrations and were not different to patients with benign diseases. Thus, malignant disease does not seem to affect prolactin synthesis nor its levels in the abdominal cavity, although there is evidence that prolactin has a negative impact on apoptosis in ovarian cancer cells (19).

The existence and mass of postoperative tumor residuals were found to be independent parameters of patients' overall survival, and this is in accordance with clinical knowledge (2). FSH ascites levels corresponded to a worse outcome in patients with ovarian cancer. As ascites levels did have a strong correlation to serum FSH levels, an indirect influence of patients' age and menopause status rather than a biological background of this result cannot be excluded, even though age was not an independent parameter. These results may highlight the role of the endocrine system in tumor proliferation. This assumption is supported by the fact that novel therapeutic concepts in the management of ovarian cancer include endocrine therapy (13). Gonadotropins and ovarian hormones are essentially involved in basic developmental processes of the ovary, and thus they are considered to be involved in defective developments and in tumorigenesis. Up to know, this central role was regarded as limited to the time before a macroscopic tumor arises and becomes symptomatic. Now, it is stated that high levels of FSH in the ascites are associated with an unfavorable outcome of patients with ovarian cancer.

In summary, a prognostic value of ascites concentrations of FSH for the outcome of patients with ovarian cancer and thereby an direct proof for the involvement of the endocrine system in tumor biology is hereby shown.

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