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ORIGINAL ARTICLE

Usefulness of beta hCG as tumor marker in the diagnosis and follow up of patients with ovarian cancer

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Summary

Purpose: *To test the possibility of using beta human chorionic gonadotropin (β-hCG) as a tumor marker in ovarian cancer by determining its diagnostic and prognostic value, and see for any relationship between disease stage, histological tumor types and serum and ascitic fluid β-hCG levels, as well as to identify false positive and false negative results.*

Methods: *This was a prospective study in 60 surgically treated patients with ovarian cancer in the period 2006-2010. The diagnosis was confirmed postoperatively based on the histopathological findings and the continuous determination of β-hCG serum levels, during the 2 postoperative years at regular quarterly intervals. The obtained results were statistically processed using multivariate analysis.*

Results: *β-hCG showed no reliable diagnostic value in ovarian cancer. A statistically significant difference between serum β-hCG levels and different FIGO stages was noted, but not between β-hCG levels and different histological groups of tumors. There were 10.2% of false positive and 18.9% of false negative results in all measurements.*

Conclusion: *The use of β-hCG as a tumor marker for ovarian cancer is justified only in patients with preoperatively high levels in advanced FIGO stages (III and IV), regardless of histological type of tumor.*

Key words: beta hCG, follow up, ovarian cancer, tumor marker

Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein with molecular weight of 38-40000 daltons consisting of 2 unequal chains, alpha and beta subunits. Alpha subunit is nearly identical in all glycoprotein hormones (LH, FSH, TSH and hCG) while the beta subunit is hormone-specific. Reference values of serum β-hCG are up to 5 IU/ml. Elevated levels of serum β-hCG may be caused by different bacterial and gastrointestinal diseases, the interaction of LH and FSH in radioimmunoassays and ectopic production of substances similar to hCG in normal tissues [1]. Besides its application in the diagnosis of pregnancy, elevated levels of β-hCG may indicate the presence of malignancy. In gynecologic oncology, extremely high values indicate gestational trophoblastic disease, germ-cell tumors or ovarian epithelial tumors [2,3].

The main aim of this study was to examine the reliability of β-hCG as a tumor marker in ovarian cancer by determining its diagnostic and prognostic value, and see for any relationship between disease stage, histological tumor types and its serum and ascitic fluid levels, as well as to identify false positive and false negative results.

Methods

The study was conducted prospectively at the Department of Obstetrics and Gynecology, Clinical Center of Vojvodina, in Novi Sad in the period 2006-2010. The study group (group A) included 60 patients who had undergone surgery for ovarian cancer. The preoperative diagnosis was based on pelvic and abdominal computed tomography (CT) and postoperatively on the histological results of the surgically removed material. FIGO stage determination was performed after intraoperative inspection and palpation of the pelvic and abdominal

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organs and peritoneal diaphragm. Patient distribution according to FIGO disease stage was as follows: stage I 20 (33.3%), II 2 (11.7%), III 21 (35%) and IV 12 (20%) patients. Histological types of malignant ovarian tumors were distributed as follows: 48 (80%) epithelial tumors, 6 (10%) germ-cell tumors and 6 (10%) sex-cord tumors. Two control groups: group B - 50 clinically healthy women who had undergone corrective surgery for static disorders of genital organs; and group C - 50 patients with benign cystic ovarian tumors (simple cysts, serous and mucinous cystadenoma, dermoid cysts, fibrothecoma, tubo-ovarian abscess, pyoovarium). The final diagnosis was confirmed by postoperative histopathological examination. The research objectives and guidelines for subsequent determinations of β-hCG and clinical examinations were explained to each participant. All participants signed informed consent for this research.

Inclusion / exclusion criteria

The main inclusion criterion onto the study was the postoperative histopathological confirmation of the presence of ovarian cancer in the study group A, the absence of pathological conditions in the control group B and the benign nature of disease in the control group C. Exclusion criteria from the study in group A were: histologically unconfirmed ovarian cancer and stoppage of follow-up estimations of β-hCG and clinical examination for two consecutive times. In group B, the criterion for study exclusion was confirmation of pathological processes of the uterus (leiomyoma, adenomyosis, chronic endometritis, cervical or endometrial cancer), and in group C histological confirmation of malignant or borderline ovarian tumors.

Follow up

All group A patients followed the protocol plan that included 4 sets of data about: (1) the patient; (2) the current disease status (FIGO stage, histological tumor type); (3) therapeutic modality (type of surgery, chemotherapy); and (4) recurrence of disease and overall survival. Control clinical examinations and serum determination of β-hCG levels were performed on the 30th day postsurgery and then every 3 months during the next 2 postoperative years. Control abdominal and pelvic CT scans were performed at 12 and 24 months postsurgery, as well as in case of suspicion of disease recurrence or activation of residual tumor after chemotherapy. When necessary, additional actions that included biopsy of suspicious sites in the vagina, colonoscopy, cystoscopy or second-look surgery, were carried out. Recurrence or residual tumor activation were proven only after histopathological confirmation of bioptic or intraoperative specimens of tumor tissue. Serum upper limit of normal of β-hCG was set at 5 IU/ml. Values above these concentrations were considered "positive" and those below "negative". All higher concentrations of β-hCG before surgery and postoperatively in patients with proven recurrence or confirmed progression of malignant disease were considered as true positive. Elevated concentrations in patients without proof of any recurrence or tumor activity in the postoperative period were considered false positive. β-hCG levels <5 IU/ml in patients without clinically proven relapse or progression of the disease were marked as true negative. Values <5 IU/ml determined before surgery and postoperatively in patients with relapsed or disease progression were considered false negative.

Plan of the research and sampling

The serum levels of β-hCG were determined from samples taken the day before the planned operation in the study group A and control groups B and C. Subsequent determination on the 7th and 30th postoperative days and at regular quarterly intervals thereafter over the next 2 postoperative years were performed in the study group A. Analysis of ascitic samples was performed in patients in advanced disease stages (III and IV) and in group C in patients with cystic formations. After coagulation of 5 ml of venous blood at room temperature the blood was centrifuged 10 min at 3000 rpm. Serum was separated and put in a clean dry test tube and frozen at -20° C until assayed. β-hCG levels in ascitic fluid and benign cystic tumors were determined by the same principle of intraoperatively obtained specimens. Determination of β-hCG was performed using commercial "Serozyme-sets" for β-hCG with the Serozyme-1 (Serono) device. The principle of the test is enzyme immunometric reaction in which specific antiserum containing 3 monoclonal antibodies to β-hCG is applied.

Statistical methods

Statistical methods included paired t-test for two independent groups with different number of cases, and x^2 test. For determination of the diagnostic significance of certain parameters, sensitivity, specificity, positive and negative predictive value and test accuracy were used. In the multivariate analysis of the obtained data, and analysing their quantitative traits we applied the following procedures shown in Figure 1: The profile analysis, MANOVA, MANOCOVA, ANOVA, ANOCOVA, Student's t-test, Hotelling's T^2 test and discriminant analysis. MANOVA: T^2 test tests the hypothesis Ho that there are no significant differences between the

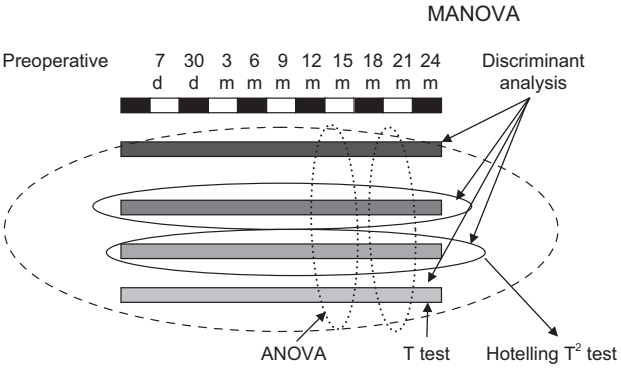

Figure 1. Sequence of steps during the application of multivariate statistical analysis (MANOVA and ANOVA). d: day, m: month (postoperatively).

mean values at different levels for one feature. ANOVA confirms or rejects the Ho hypothesis that there are no statistically significant differences between the mean values for one feature at different levels. Depending on the size of k1 and k2 from tables of Fisher's distribution one can find a Ft (F-table). If F is less than F-table (F<Ft), the Ho hypothesis is accepted or rejected in the opposite case with the risks of 5% or 1%. In each table, the p-value is shown next to the F value, which indicates the degree of risk to a conclusion in the case of rejection of the Ho hypothesis [4,5].

Results

Table 1 shows the average and range of β-hCG values in the serum of the examined groups and the range of values in the specimens from ascites and benign cystic formations.

Table 2 shows the differences between the average values of preoperative concentration of β-hCG in serum, ascites and benign cystic contents.

Table 3 shows the diagnostic value of preoperative β-hCG (upper limit of normal=5 IU/ml) determination in serum.

Continuous determination of β-hCG levels before surgery and during the 2 postoperative years according to the disease stages is shown in Figure 2.

The 2-year period of serum β-hCG determina-

Table 1. The average and range of values of β-hCG in serum, ascites, and benign cystic formation

Statistical tests	*Test groups*				
	A	*B*	*C*	*Ascites*	*Cyst content*
Range of values (IU/ml)	0.2 - 92.5	0.8 - 192.6	0.5 - 257.2	0.5 - 111.2	1.5 - 105.4
Average values (x)	9.73	21.87	32.24	21.41	32.59
Standard deviation	14.23	42.5	64.87	15.11	32.17
Coefficient of variation	146.31	194.3	195.13	117.26	98.74

A: study group, B: control group, C: control group

Table 2. Statistical analysis of differences of preoperatively determined average values of β-hCG in the respective groups

Statistical significance	*Test groups*				
	A/B	*A/C*	*B/C*	*A - serum/ ascites*	*Ascites/ cyst content*
Student's t-test	0.77	1.96	0.38	0.28	8.35
p-value	0.44	0.05	0.70	0.78	0.00

A: study group, B: control group, C: control group

Table 3. The diagnostic value of β-hCG in serum and ascitic fluid

Diagnostic tests (%)	*Test groups*		
	A	*C*	*Ascites*
Sensitivity	48.3	56	62.8
Specificity	66	66	19.3
Positive predictive value	63	62.2	46.8
Negative predictive value	53.2	60	31.6
Prevalence	54.5	50	53
Test accuracy	56.3	55.4	42.4

A: study group, C: control group

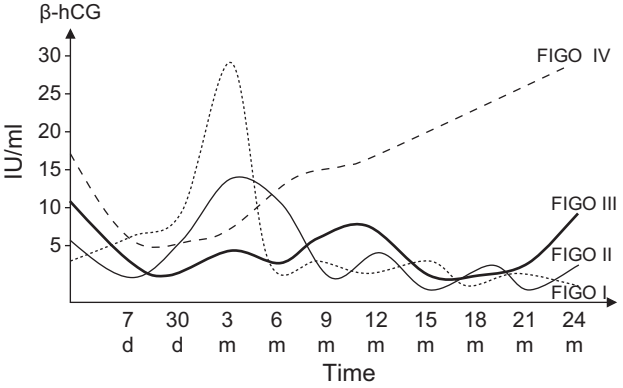

Figure 2. Serum β-hCG levels in relation with FIGO stages of ovarian cancer over time (d: day, m: month).

tion was divided into 3 time periods, each considered separately: period I (preoperatively-6th postoperative month), period II (7-12 postoperative month) and period III (13-24 postoperative months). Within these 3 time periods, the serum concentration of β-hCG was determined at certain intervals: preoperatively, on the 7th and 30th postoperative day and then every 3 months (3-24 months) during the 2 postoperative years. Statistical evaluation of the results, especially in different time periods of determination, was related to the presence of residual tumor, recurrence and mortality from the disease with the influence on the levels of β-hCG concentration in certain periods. Tables 4-6 show the statistical assessment of differences between β-hCG concentrations and FIGO stages (1-4) in different time periods and intervals of measurement.

T test showed that before surgery, there was statistically significant difference in the concentration of β-hCG between stage I and III of ovarian cancer. On the 30th postoperative day, significant differences were present in stages II and III, in the 6th postoperative month between stages I and IV and between stages III and IV, in the 9th postoperative month between stages I and IV, and in the 12th postoperative month between stages I and III. The overall assessment of the relations between serum levels of β-hCG and the different FIGO stages of ovarian cancer was as follows: there were statistically significant differences ($p<0.05$) between all stages, but only in the second period of determination. In the first period statistically significant differences ($p<0.05$) were registered between stages I and IV, and III and IV, which, observed together in terms of mutual relations of all stages in all time intervals, did not have a significant impact on overall statistical analysis. β-hCG concentration in all FIGO stages of ovarian cancer showed a statistically significant difference ($p<0.05$) while the parallel flow was present in all 3 periods of measurements.

In order to determine the relationship between different histological types of ovarian cancer, patients were divided into 3 groups: epithelial cancers, germ-cell cancers and sex-cord tumors. Distribution of β-hCG concentration in various periods of determination in relation to histological types of ovarian cancer is shown in Figure 3.

Tables 7-9 show the statistical assessment of differences between the concentration of β-hCG and the histological types of ovarian cancer in different time

Table 4. Statistical evaluation of differences in the concentration of β-hCG to all FIGO stages of ovarian cancer

Statistical analysis	*Time period*					
	Period I		*Period II*		*Period III*	
	F	*p-value*	*F*	*p-value*	*F*	*p-value*
MANOVA	1.32	0.19	2.20	0.04	0.92	0.51
Parallelism	1.38	0.18	1.58	0.20	1.10	0.37
Direction	1.04	0.38	2.87	0.04	0.36	0.70

Table 5. Assessment of statistical differences between concentration of β-hCG in different FIGO stages (1-4) and in different time periods (Hotelling T^2 test)

Hotelling T^2 test			*Time period*			
	Period I		*Period II*		*Period III*	
FIGO stages	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*
1:2	0.78	0.57	0.24	0.78	0.25	0.90
1:3	1.65	0.17	2.57	0.08	1.29	0.29
1:4	3.90	0.00	8.13	0.00	0.00	0.00
2:3	2.31	0.07	0.66	0.52	2.94	0.06
2:4	0.68	0.64	2.72	0.11	0.00	0.00
3:4	4.74	0.00	1.64	0.21	0.00	0.00

Table 6. Assessment of differences between β-hCG concentration in all FIGO stages according to certain periods of determination

ANOVA	*Time intervals*										
	Preop	*7 d*	*30 d*	*3 m*	*6 m*	*9 m*	*12 m*	*15 m*	*18 m*	*21 m*	*24 m*
F	1.96	0.03	0.37	0.93	3.99	2.53	4.22	0.40	0.76	0.52	2.81
p-value	0.13	0.99	0.77	0.42	0.01	0.06	0.00	0.66	0.47	0.59	0.07

Preop: preoperative, d: day, m: month

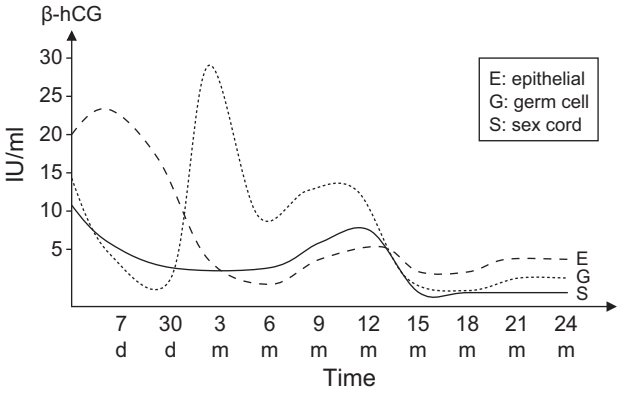

Figure 3. The relation between concentration of β-hCG and histological type of ovarian cancer (d: day, m: month).

periods of determination: period I (preoperatively-6th postoperative month), period II (7-12 postoperative months) and period III (13-24 postoperative months).

The overall assessment of correlation between serum β-hCG levels of the various histological types of cancer was as follows: there was no statistically significant difference between different histological types of ovarian cancer in relation to the periods of β-hCG analysis ($p>0.05$). There was a statistically significant difference between subgroups of epithelial ovarian cancers and germ-cell tumors only in the 2nd period in the 3rd month, but this did not affect the overall statistical results observed in all 3 periods. The concentration of β-hCG differed significantly only in the 2nd period ($p<0.05$) and was observed for all histological types together and showed a parallel course in all periods.

Reliability test based on determination of false positive and false negative results in serum β-hCG concentration assessment is shown in Figure 4.

Discussion

The role of β-hCG in the diagnosis and management of gestational trophoblastic disease approaches the definition of the "ideal tumor marker" and can be used as hall mark for comparisons for other tumor markers in oncology. It is known that high preoperative β-hCG levels are recorded in > 50% of all cases of germ-cell ovarian tumors and that a progressive increase in postoperative values correlates with poor patient outcome [6-8]. However, when it comes to other histologies of ovarian cancer (epithelial carcinoma, sex cord/stromal tumors

Table 7. Statistical evaluation of differences in the concentration of β-hCG in relation to all histological types of ovarian cancer

Statistical analysis	*Time period*					
	Period I		*Period II*		*Period III*	
	F	*p-value*	*F*	*p-value*	*F*	*p-value*
MANOVA	1.25	0.26	1.74	0.14	0.44	0.89
Parallelism	1.08	0.38	0.18	0.83	0.23	0.96
Course	1.95	0.15	3.40	0.04	1.07	0.35

Table 8. Differences between the concentrations of β-hCG of different histological types of ovarian cancer in the examined periods

Hotelling T^2 test	*Time period*					
	Period I		*Period II*		*Period III*	
Subgroups	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*
E:G	2.20	0.06	3.97	0.02	0.24	0.90
E:O	0.72	0.60	2.10	0.13	0.67	0.61
G:O	0.56	0.72	0.14	0.86	0.29	0.86

E: epithelial, G: germ cell, O: sex cord

Table 9. Differences between concentration of β-hCG of the various histological types of ovarian cancer in individual time intervals

ANOVA	*Time intervals*										
	Preop	*7 d*	*30 d*	*3 m*	*6 m*	*9 m*	*12 m*	*15 m*	*18 m*	*21 m*	*24 m*
F	1.45	0.75	0.06	3.10	0.24	3.34	2.93	0.47	0.91	0.44	0.59
p-value	0.24	0.47	0.93	0.05	0.78	0.04	0.06	0.62	0.40	0.63	0.55

Preop: preoperative, d: day, m: month

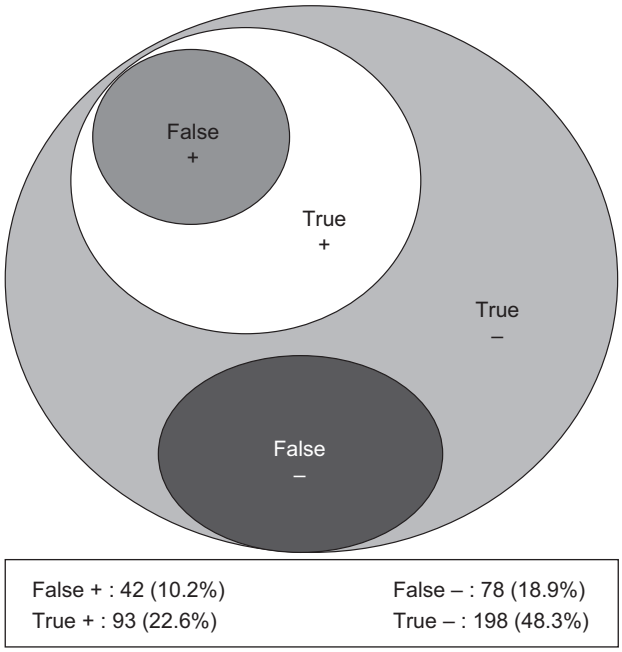

Figure 4. False positive/false negative and true positive/true negative results for the serum β-hCG levels.

and metastatic tumors), there is still no clearly defined attitude considering the use of β-hCG as a tumor marker in monitoring the progress of the disease, and therefore the aim of this study was defined in that context [9]. According to different authors, preoperatively elevated serum concentrations of β-hCG were observed in the range of 38-51% in different histological types of ovarian cancer and about 5% of patients with benign ovarian tumors and uterine myomas [10,11]. Several authors have recorded elevated serum values of β-hCG in 41-45% of epithelial ovarian cancer but have not determined the correlation between the histological subtypes of epithelial ovarian cancer and serum concentration of β-hCG, while other studies have shown that preoperatively high values of β-hCG in the ascitic fluid (> 42.5 IU/ml) are seen in 88.8% of the tested samples in patients with epithelial ovarian cancer [12-15]. Literature data show that β-hCG is a reliable tumor marker in the differentiation of certain histological types of ovarian cancer and that the level of serum β-hCG is related to the tumor volume only in the group of epithelial cancers [16-18]. Other authors have not confirmed this correlation [19,20]. According to our results, preoperative quantification of serum β-hCG levels has no reliable diagnostic value in ovarian cancer. The same applies for the determination of the concentration in the ascitic fluid in advanced disease stages. In our study there was statistically significant difference ($p<0.05$) between serum β-hCG levels in relation with the different FIGO stages of all groups of ovarian cancer. We found no statistically significant difference ($p>0.05$) between serum β-hCG concentration and the different histological groups of ovarian cancer (epithelial, germ cell, sex cord). Incorrect results for β-hCG were noted in 29.1% of all analyses (10.2% false positive and 18.9% false negative). β-hCG, which is produced by epithelial ovarian carcinomas, induces the production of steroid hormones with inhibitory effects on the production of LH and FSH from the pituitary gland. It was found that a number of different tissues in healthy humans contains substances of similar molecular weight and structure as hCG that react with specific hCG/LH receptors, or RIA assays for hCG, which may lead to false positive results. Up to now, it is not clear whether these "hCG-like substances" are identical with the hCG originating from the trophoblast [21,22]. However, it should be noted that in all cases where commercial sets are used there is a risk that a test interference may occur, which will cause a change in the results. In the presence of high concentrations of antigen in samples of undiluted test material, sandwich assays can give falsely low results because the immune reactions are prevented by the analyte in the sample content, which may partially explain the occurrence of false negative results. On the other hand, some people have serum heterotropic or human-mice antibodies (HAMA-s) in the blood that act as barriers between the two antibodies, so in the absence of specific antigen false positive results for the markers can be encountered. All the above-mentioned suggest that the values of tumor markers obtained by using different commercial sets can sometimes fluctuate and the differences are related to the application of antibodies that react against different epitopes of certain substances. However, even when using identical antibodies different results can be obtained, a phenomenon related to the "matrix" effect, or the pronounced heterogeneity of antibodies that should be considered when evaluating the flow curves of tumor markers in affected individuals [23,24].

Conclusion

We conclude that the use of β-hCG as a tumor marker for ovarian cancer is justified only in patients with preoperatively elevated levels in the advanced FIGO stages III and IV, regardless of histological type of the tumor.

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