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The relationship between serum levels of CA 125 and the degree of differentiation in ovarian neoplasms

A relação entre os níveis séricos de CA 125 e o grau de diferenciação em neoplasias ovarianas

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ABSTRACT

Introduction: Primary ovarian neoplasms exhibit a wide range of histopathological aspects, and tumors with epithelial differentiation are the most frequent. Among the malignant tumors, the most common histological type corresponds to serous adenocarcinoma, whose diagnosis is established in advanced stages of the disease in approximately 75% of the patients. Tumor marker CA 125 represents a glycoprotein synthesized mainly by neoplastic cells with epithelial differentiation, and its serum level seems to be associated with the biological potential of these lesions. **Objective:** To estimate the association between serum levels of CA 125 and the degree of differentiation in primary ovarian neoplasms. **Method:** Sixty distinct cases of primary ovarian tumors were selected, previously analyzed at the Laboratory of Pathology of the Hospital Complex of Universidade Luterana do Brasil (Ulbra), between 2005 and 2010, from patients undergoing concomitant analysis of CA 125. In each case, age, tumor size, histological type, degree of differentiation, presence of necrosis and tumor invasion of the albuginea or extraovarian tissues, pathological stage and serum CA 125 were determined. **Results:** A statistically significant relationship between CA 125 levels and histological grade ($p = 0.001$), age ($p = 0.009$), biological behavior of the tumor (malignant or benign – $p = 0.002$) and extraovarian invasion ($p = 0.005$) was found. No relationship between CA 125 levels and tumor size ($p = 0.1006$) and pathologic stage ($p = 0.1$) was determined. Histologic grade was associated with the presence of necrosis ($p = 0.001$), extraovarian invasion ($p = 0.009$) and tumor size ($p = 0.008$). **Conclusion:** In the present study, serum levels of CA 125 were associated with histological grade in primary ovarian neoplasms, especially in high-grade malignant tumors, suggesting that high levels of this glycoprotein are associated with lesions of more aggressive biological behavior.

Key words: ovarian neoplasm; tumor differentiation; carcinoma; CA 125 protein; chemiluminescence method.

INTRODUCTION

Primary neoplasms of the ovary comprise benign and malignant lesions, which may present superficial germinative epithelial differentiation of the stromal sexual cord. Malignant ovarian tumors are responsible for approximately 6% of all cancers affecting women, and correspond to the seventh most frequent cause of death, for around 80% of the cases are diagnosed in advanced stages[3, 4]. Survival associated with primary malignant neoplasms of the ovaries in five years is 95% in early stages (limited to ovaries), and 18 months in advanced stages. The degree of tumor differentiation is closely related to survival time, and may be related to tumor response to chemotherapy agents[4, 8, 9, 12].

The world prevalence of ovarian cancer is around half a million women in a period of five years, with 200,000 new cases happening each year. The incidence of ovarian carcinoma is higher in industrialized countries, although their concentration is greater in developing countries (96,700 *versus* 107,500). In Latin America, an incidence of 8/100,000 women is observed; in developed countries it is 10/100,000; and in developing countries,

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5/100,000[3, 4, 8, 21]. In Porto Alegre, an incidence of 13/100,000 women was estimated in 2010; in São Paulo, 11/100,000 women. The National Institute of Cancer (INCA) reports an increase in mortality from ovarian cancer of 6%-8%. It is one of the main death causes in women in Rio Grande do Sul[13].

Benign neoplasms encompass approximately 80% of the cases of primary ovarian tumors, predominantly in younger women (20 to 45 years old), when compared to malignant lesions. Conceptually, all benign neoplasms are classified as well-differentiated tumors due to their biological behavior. The most frequent ovarian neoplasms include serous and/or mucinous cystadenoma, serous or mucinous cystadenocarcinoma, granulosa cell tumors, fibromas/thecomas and teratomas (mature or immature)[1, 10, 11, 21].

In patients clinically investigated for pelvic lesions of probable ovarian origin, elevated serum levels of the tumor marker CA 125 complement the data obtained from physical and ultrasonographic examinations, suggesting, principally when in high levels, the presence of a primary ovarian cancer. Although it is the best-known and most widely used tumor marker in the clinical management of patients with ovarian tumors with epithelial differentiation, its specificity seems to be low in benign tumors with or without epithelial differentiation. The limitations of CA 125 testing include the presence of individual methodological and biological variations; the eventual presence of high serum levels in healthy patients (1% of the cases); and the possible rise of CA 125 levels in cases of pregnancy (up to 20%), cystic teratoma of the ovary, peritonitis, hepatic cirrhosis, metastases of breast carcinoma; and, eventually, in primary neoplasms of the liver, colon, lung and pancreas. CA 125 is a high molecular weight glycoprotein, also known as MUC16 (sialomucin), initially identified through antibodies produced by immunized animals with cells of human ovarian serous papillary cystadenocarcinoma. Serum CA 125 measurement may also be employed for the monitoring of patients submitted to adjuvant chemotherapy due to ovarian carcinomas, or for the early detection of tumor recurrence after initial treatment[2, 5-7, 14, 17, 20, 25].

In this study, the authors evaluated 60 distinct cases of benign and malignant primary ovarian tumors, of different cell lines and degrees of differentiation, aimed at assessing the relationship between serum levels of CA125 and the biological behavior and/or the histological degree of these neoplasms.

METHOD

Patient group

The current cross-sectional, analytical and retrospective study evaluated 60 cases of primary ovarian neoplasms previously analyzed at the pathology laboratory of Universidade Luterana do Brasil (Ulbra), between January 2005 and October 2010, over a study period of 58 months. The sample cases encompassed surgical specimens from oophorectomy (sometimes performed in conjunction with hysterectomy and pelvic peritoneal biopsy) of patients who also had their serum CA 125 levels measured at the clinical laboratory of the same institution. All surgical specimens were initially fixed in 10% formalin and were paraffin-embedded. The research was approved by the ethics committee of Ulbra.

All cases were histologically reevaluated: three-micrometer-thick tissue sections stained with hematoxylin and eosin were prepared by two pathologists, individually and together, with an agreement of 100% between the first diagnosis and the current study (kappa test 1+). The cases corresponding to only ovary biopsy, ovarian lesions of infectious and inflammatory etiology, solid or cystic ovarian lesions of non-neoplastic origin, metastatic neoplasms and patients whose serum CA 125 was not measured were excluded of the sample. No cases of malignant neoplasms showed clinical evidence of distance metastases or lymph node metastases (classified as N0, and M0 according to the tumor-node-metastasis [TNM] system) at the moment of the surgical procedure (oophorectomy). The specimens encompassed only cystic lesions, with nodular solid parts just in the malignant lesions. In each specimen the following anatomopathological characteristics were determined:

- size of the lesion – in centimeters (at the longest axis);
- histologic type/cell line;
- degree of differentiation – poorly, moderately and well differentiated;
- presence of necrosis;
- albuginea invasion;
- extraovarian invasion;
- pathological staging – by TNM classification.

Measurement of serum CA 125

For measurement of serum CA 125, after a four-hour fast patients' sera were used as biological samples, with no hemolysis, collected in red-top tubes, minimum volume: 2 ml, without anticoagulant, centrifuged, refrigerated and sent to laboratory, where 0.5 ml was added to cuvettes for the use of chemiluminescence in an immunology system (Elecsys – Roche Diagnostics, Basel, Switzerland). The method is based on the detection of light emitted by a chemical reaction between the glycoprotein antigen molecule and the chemiluminescent substrate, that is, the emission of

visible light is proportional to the investigated reagent. Results are expressed in U/ml, with values below 35 U/ml considered negative, between 35 and 65 U/ml considered high, and those above 65 U/ml considered positive.

Statistical tests

The statistical analysis of this study was done by means of tables and descriptive statistics (average and standard deviation), with the chi-square test being used to verify the association among variables. In order to examine specific associations, such as that among degree of differentiation, size and pathological staging, Fisher's exact test was also used. Results were considered significant at a maximum significance level of 5%. For data processing and analysis, the statistical software SPSS version 16.0 was used.

RESULTS

In the 60 sample cases, patients' age ranged from 20 and 80 years, with a mean age of 50.24 years (standard deviation of ± 11.12 years). **Table 1** presents the analyzed histologic types. The tumor average size was 6.84 cm (ranging from 3.5 to 20 cm). The serum CA 125 levels ranged from 5 U/ml to 408 U/ml.

TABLE 1 – Ovarian neoplasms: assessed histological types

Histological type	*n*-%
Mucinous cystadenoma	5-8%
Serous adenocarcinoma	5-8%
Mucinous adenocarcinoma	9-15%
Serous cystadenofibroma	14-23%
Serous cystadenoma	14-23%
Mature cystic teratoma	14-23%

Table 2 determines the data obtained in relation to the analyzed variables. The values obtained for benign neoplasms are lower than 35 U/ml. **Table 3** exhibits the serum CA 125 levels found in malignant neoplasms, with results between 83 U/ml and 408 U/ml. Considering the significance level of 5%, a statistically significant relation was observed between serum CA 125 and the biological behavior of the neoplasm (malignant and/or benign – $p = 0.002$), differentiation degree of malignant neoplasms ($p = 0.001$), age ($p = 0.009$), invasion of the albuginea ($p < 0.005$) and tumor extension to pelvic structures ($p = 0.005$). No relation was observed between serum CA 125 level and tumor size ($p = 0.1006$) and pathological staging ($p = 0.1$). An average of CA 125 values of 279.8 U/ml ($p = 0.001$) was observed in patients with moderately differentiated ovarian cancer; and an average of 194.5 U/ml ($p = 0.001$), in patients with poorly differentiated ovarian cancer.

TABLE 2 – Association between serum CA 125 levels and histopathological findings

Variable	*n*-%	Serum levels of CA 125 (*p* value)
Benign neoplasms	46-77%	
Malignant neoplasms	14-23%	$p < 0.001$
Malignant neoplasms/degree of differentiation	14-100%	
Well differentiated	1-7.2%	
Moderately differentiated	5-35.7%	$p < 0.001$
Poorly differentiated	8-57.1%	
Invasion of the albuginea	14-100%	
Present	4-28.6%	$p < 0.005$
Absent	10-71.4%	
Invasion of pelvic structures or peritoneal implants	14-100%	
Present	2-14.3%	$p < 0.005$
Absent	12-85.7%	
Stage		
T1a	10-71.4%	
T1c	2-14.3%	$p = 0.1$
T2b	2-14.3%	

TABLE 3 – Determination of serum CA 125 levels associated with histological type in varied malignant neoplasms

Patient	Histological type	Serum CA 125 levels – U/ml
1	Serous adenocarcinoma	300
2	Mucinous adenocarcinoma	250
3	Mucinous adenocarcinoma	396
4	Mucinous adenocarcinoma	408
5	Mucinous adenocarcinoma	240
6	Serous adenocarcinoma	315
7	Mucinous adenocarcinoma	200
8	Serous adenocarcinoma	115
9	Mucinous adenocarcinoma	145
10	Serous adenocarcinoma	138
11	Serous adenocarcinoma	115
12	Mucinous adenocarcinoma	83
13	Mucinous adenocarcinoma	120
14	Mucinous adenocarcinoma	113

Cell differentiation degree presented a statistically significant relationship with the presence of necrosis ($p = 0.001$), extraovarian invasion ($p = 0.009$), and tumor size ($p = 0.008$). No significant relationship was observed between cell differentiation degree and pathological staging ($p = 0.6$).

DISCUSSION

Ovarian neoplasms encompass benign and malignant tumors, affecting mainly women of childbearing age. In general, malignant neoplasms correspond to tumors originated from the surface epithelium (coelomic), which, most of the cases, determine symptoms or signs in advanced stages of the disease. Ovarian carcinomas represent approximately 30% of malignant female genital tract tumors. About 70% of women diagnosed with ovarian carcinoma present tumor extension beyond the pelvis. The malignant ovarian tumors originated from the surface epithelium and/or stroma are graded as well differentiated (grade 1), moderately differentiated (grade 2) and poorly differentiated (grade 1); this classification is associated with prognostic factors and therapeutic modalities[8, 12, 20-24]. The tumor marker CA 125 is a glycoprotein synthesized by ovarian superficial cells; its serum measurement may be employed in the evaluation of disease progression or even in the early diagnosis of ovarian tumors. In general, serum CA 125 concentration is elevated in ovarian malignant tumors, principally in large lesions and/or advanced stages of the disease. The employment of the chemiluminescence method for the assessment of serum CA 125 levels presents a sensitivity of 27%, a specificity of 97%, intra- and inter-assay coefficients of variation of 10%, and a linearity of up to 600 U/ml[6, 9, 11, 14, 15, 18, 19, 25].

Benign tumors affect women between 20 and 45 years old, while malignant lesions predominate in patients older than 45 years[1, 3, 4]. In the present study, the mean age of patients was 50.24 ± 11.12 years, and the mean tumor size was 6,84 cm. Serum CA 125 levels ranged from 5 U/ml to 408 U/ml. Serum CA 125 level was associated with the biological behavior of the neoplasm (malignant or benign – $p = 0.002$), the degree of differentiation of malignant neoplasms ($p = 0.001$), age ($p = 0.009$), invasion of the albuginea ($p < 0.005$) and tumor extension to pelvic structures ($p = 0.005$). Relationship of serum CA 125 level was observed neither with tumor size ($p = 0.1006$), nor with pathological staging ($p = 0.1$). Duffy *et al*. describe that CA 125 measurement must be employed in postmenopausal patients, for its serum concentration is associated with the distinction between benign and malignant tumor processes, although it is not related to diseases in initial stage or restricted to the ovary[9]. Kolwijck *et al*. describe that the pre-operative serum CA 125 levels are significantly higher in advanced lesions and in serous tumors ($p < 0{,}001$)[15]. Rosai describes that the histologic grade and the disease stage are associated with serum CA 125 levels and the disease-free survival rate[21]. Reis *et al*. suggest that the routine transvaginal ultrasonography and the serial measurement of CA 125 with risk assessment for ovarian carcinoma is fundamental in the distinction between benign and malignant lesions, and in the detection of lesions in early stages[20].

Ryu *et al*. describe that the stage, degree of differentiation, serum CA 125 level and the presence of residual tumor are relevant prognostic factors in cases of clear cell carcinoma of the ovary[22]. Osman *et al*. report that postoperative serum CA 125 levels are associated with stage, histologic grade and survival in cases of ovarian carcinoma[18]. Alonso *et al*. cite that serum CA 125 values in cases of serous ovarian cystadenoma may be so high as those found in malignant tumors of this anatomic site[1]. Montero *et al*. cite that serum CA 125 levels are similar among benign ovarian neoplasms such as mature cystic teratoma and cystadenoma[16].

In this study an average CA 125 value of 279.8 U/ml was found in the patients with moderately differentiated malignant neoplasms; and an average CA 125 value of 194.5 U/ml, in the patients with poorly differentiated malignant neoplasms. We also verified that the degree of differentiation was associated with the presence of necrosis areas ($p = 0.001$), extraovarian invasion ($p = 0.009$) and tumor size ($p = 0.008$). It seems that the smaller the neoplasm differentiation, what implies tumors with higher number of atypies, the faster rate of tumor growth and necrosis zones, the greater the capacity of these cells in the synthesis of glycoprotein CA 125, what suggests that during the process of carcinogenesis, cells of the ovarian epithelium acquire a functional capacity that is distinct, but relevant in the disease identification and progression.

In the present study, the authors describe a significant association between serum levels of tumor marker CA 125 and the degree of differentiation in malignant ovarian neoplasms with epithelial differentiation, suggesting that high levels of this glycoprotein are associated not only with malignant neoplasms, but also with lesions with more aggressive biological behavior.

RESUMO

Introdução: *As neoplasias primárias de ovário apresentam uma ampla variação dos aspectos histomorfológicos; sendo os tumores com diferenciação epitelial os mais frequentes. Entre os tumores malignos, o tipo histológico mais comum é o adenocarcinoma seroso, cujo diagnóstico é determinado em estágios avançados de doença em aproximadamente 75% das pacientes. O marcador tumoral CA 125 corresponde a uma glicoproteína sintetizada pelas células neoplásicas com diferenciação epitelial principalmente, e seu nível sérico parece estar associado ao potencial biológico dessas lesões.* ***Objetivo:*** *Estimar a associação entre o nível sérico de CA 125 e o grau de diferenciação em neoplasias ovarianas primárias.* ***Método:*** *Foram selecionados 60 casos distintos de tumores ovarianos primários, previamente analisados entre 2005 e 2010, de pacientes submetidas à dosagem sérica concomitante do marcador CA 125. Em cada caso foram determinados tamanho tumoral, tipo histológico, grau de diferenciação, presença de necrose tumoral, invasão neoplásica da albugínea ou tecidos extraovarianos, estadiamento patológico e nível sérico de CA 125.* ***Resultados:*** *Foi encontrada uma relação estatisticamente significativa entre nível de CA 125 e grau histológico (p = 0,001), idade (p = 0,009), comportamento biológico da neoplasia (maligno ou benigno – p = 0,002) e invasão extraovariana (p = 0,005). Não foi observada relação do nível de CA 125 com o tamanho tumoral (p = 0,1006) e o estadiamento patológico (p = 0,1). O grau histológico esteve associado à presença de necrose (p = 0,001), invasão extraovariana (p = 0,009) e ao tamanho tumoral (p = 0,008).* ***Conclusão:*** *Os níveis séricos de CA 125 estiveram associados ao grau histológico em neoplasias primárias ovarianas, principalmente nos tumores malignos de alto grau, sugerindo que os níveis elevados dessa glicoproteína estejam associados a lesões de comportamento biológico mais agressivo.*

Unitermos: *neoplasia ovariana; diferenciação tumoral; carcinoma; CA 125; método de quimioluminescência.*

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