CHROMOGRANIN A SERUM LEVELS IN ELDERLY PATIENTS WITH OVARIAN CANCER

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ABSTRACT

Background and aims*: The observation of neuroendocrine activity during clinical course of ovarian cancer, suggested the use of neuroendocrine serum markers to detect this tumor. We analyze the incidence of pre-operative circulating Chromogranin A (CgA) in a population of ovarian cancer patients.*

Materials and methods*: We investigated the role of serum concentrations of CgA and CA125, in 134 women with ovarian cancer, enrolled between 2000-2008. We also examined the values of CgA and CA125 at different clinical stages of ovarian cancer.*

Results*: We observed significant increase in CgA and CA125 serum levels when comparing patients with ovarian cancer in stage I vs stage II ($p<0.001$), stage I vs stage III ($p<0.001$), stage I vs stage IV ($p<0.001$), stage II vs stage III ($p<0.001$), stage II vs stage IV ($p<0.001$). In patients with ovarian carcinoma in stage IV a correlation between CgA and CA125 with a difference of 0.718 was observed ($p<0.001$).*

Conclusions*: A high level of CgA signalizes extensive disease and larger tumor burden. CCgA may be associated rather with proliferation than with tumor secretion activity.*

Key words*: ovarian cancer, chromogranin A, tumor marker, CA125.*

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Introduction

Ovarian and breast cancers have become the most prevalent malignancy in women, with the majority of patients presenting with advanced-stage disease[1,2].

The ovarian cancer is the 5th most common cancer among women and the leading cause of death from gynecological cancer. Progress in understanding the biology of ovarian tumors is complicated by the fact that their tissue of origin is still unclear. The histotypic variability among ovarian cancers and their differentiation which mimics the Müllerian duct-derived oviductal has been ascribed to the capacity of ovarian cancer to differentiate along the developmental pathways of these epithelia. The Müllerian ducts forms as invaginations of the coelomic epithelium in regions adjacent to the future ovarian epithelium. The coelomic hypothesis implies that ovarian carcinomas are better differentiated than the cells from which they originate.

Management of these lesions comes from an understanding of certain basic aspects of the disease process. A number of tumor associated antigens detectable in the serum of patients with ovarian cancer have been described, the most useful one is CA125. CA125, a mucin-like glycoprotein, increases in response to changes of the celomic epithelium. This marker is most closely associated with ovarian carcinoma[3]. Because of the difficulty in accurately assessing tumor response in a neoplasm often confined to the peritoneal cavity, interest has developed in defining ways to use CA125 levels to determine response[4, 5]. CA125 also raises in presence of a few benign pathologies (endometriosis) or other peritoneal irritation conditions. Chromogranin A (CgA)

is a hydrophilic acidic one-chain peptide containing 439 aminoacids. The chromogranin family comprises acidic glycoproteins stored in the matrix of large dense secretion granules, containing protein hormones in a wide variety of endocrine and neuroendocrine cells[6-10]. CgA modulates processing of proteolytic hormones during their transport in neuroendocrine vesicles and is involved in the process of storage and release of hormone peptides[11]. It is also present in the widespread neuroendocrine system of the bronchial and gastrointestinal tracts and of the skin (Merkel cells)[12]. High-serum levels of CgA have also been demonstrated in patients with various malignancies such as pancreas[13], prostate[14], lung colon[15], and liver[16, 17]. The aim of our study was to analyze the incidence of pre-operative circulating CgA in a population of ovarian cancer patients.

Methods and methods

134 females with ovarian cancer were enrolled between 2000-2008 (Table 1). Inclusion criteria considered were: 1) no previous hormonal or radiation therapy; 2) no previous surgery on the ovaries; 3) histological proven ovarian carcinoma. None of these patients had previous or concomitant history of other malignant disease, adrenal incidentaloma, and/or uncontrolled blood hypertension. Patients with heart failure, kidney failure, type A chronic atrophic gastritis, autoimmune diseases and concomitant use of proton pump inhibitors or nitrates were not included since these conditions are associated with increased levels of CgA.

Total number	134
Age (years)	65-75
Smokers (yes/no)	61/73
BMI (kg/m2)	28±2.8
Heart rate (beats/min)	68±11
SBP (mmHg)	160±10.2
DBP (mmHg)	84±12
Stage I	(n=24 18%)
Stage II	(n=30 23%)
Stage III	(n=54 40%)
Stage IV	(n=26 19%)

Table 1: Demographic characteristics and clinical stages of ovarian cancer according to the F.I.G.O. classification of the subjects included in the study.

The diagnosis of ovarian cancer was based on the clinical symptoms and serological features, on markedly elevated serum CA125 levels and typical findings on dynamic computed tomography. The diagnosis of ovarian cancer was based on histopathological findings. The International Federation of Gynecology and Obstetrics (FIGO) classification of ovarian cancer was used to classify the tumors[18]. An informed consent form was obtained from all patients for all the procedures carried out. The investigation was approved by the Local Ethics Committee and all the procedures followed were in accordance with the Helsinki Declaration of 1975.

Serum Collection and Storage

Blood samples were taken from the patients and sera were immediately frozen and stored at -20°C until analysis. A commercial solid-phase two-site immunoradiometric assay was used to detect serum CgA (CgA-RIA CT, CIS Biointernational ORIS Group, GIF-SUR-Yvette, France). Two monoclonal antibodies were prepared against sterically remote sites on the CgA molecule. The first was coated into the solid phase (coated tube), the second radiolabeled with iodine 125 and used as a tracer. CgA present in the standards or the samples to be tested are sandwiched between the two antibodies. Following the formation of the coated antibody, antigen-iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.

The radioactivity bound to the tube is proportional to the concentration of CgA in the sample. The normal range for serum levels of CgA in a control population is reported as 20-100 ng/ml. The coefficients of variation between and within assay were 6.4% and 4.1% respectively. CA125 was measured using an immunoradiometric analysis (IRMA) based on two monoclonal antibodies, one of which was labelled with I125, while the other was combed to magnetisable mono-dispersed polymer particles. Bound radioactivity was counted in Wallac Wizard 1470 Automatic Gamma Counter. The between and within assay coefficient of variation was 6.5% and 4.4% respectively. The sensitivity, specificity and accuracy of CgA and Ca125 assay were calculated as previously described,[19] using as cut-off the upper reference limits of our healthy subjects. For sensitivity and specificity 95% confidence intervals (C.I.) were also determined. Clinical chemistry tests were performed in the medical center laboratory using standard methods.

Fasting blood samples were taken at enrolment from the participants.

Statistical Analyses

All data are presented as mean±standard deviation. Discrete and continuous variables were compared using either Student's test or the Wilcoxon Mann-Whitney non-parametric test for unpaired data. Categorical variables were compared with either the χ square test or the Fisher exact test when requested. The Spearman's rank correlation coefficient test was used to test for univariate relationships between variables. The following tests at p value <0.05 level significance were used to evaluate the results and was considered statistically significant. Data were analyzed using the statistical package SPSS for Windows 7.5 (SPSS Inc. Chicago, Il, USA).

Results

The clinical and pathological characteristics of our study population are described in Table 2. The upper reference limits for CgA and CA125, defined as 2 SD above the mean levels of healthy subjects, were 35 ng/ml and 14 UI/L respectively. CgA values were above the upper reference limit in 74.2% of ovarian cancer patients. CA125 levels were significantly higher in ovarian cancer patients; CA125 values were above the upper reference limit in 78.2% of ovarian cancer patients.

Parameter	Mean value	Median	Range
Pre-operative serum CA125 (U/l)	165 ± 84	118	60-367
Pre-operative serum CgA (ng/ml)	110 ± 77.6	67	14-704

Table 2: Clinical and pathological characteristics of the patients included in the study.

Comparison between clinical stages of ovarian cancer

As concerns CgA levels significant differences were observed in the following comparisons: stage I vs stage II -54.6 ng/ml (p<0.001; C.I. -44.5 to -26.4); stage I vs stage III -118.02 ng/ml (p<0.001; C.I. -190.4 to -87.2); stage I vs stage IV -165 ng/ml (p<0.001; C.I. -267.6 to -104.71); stage II vs stage III -59.2 ng/ml (p<0.001; C.I. -84.6 to -32.2); stage II vs stage IV -87.4 ng/ml (p<0.001; C.I. -136.8 to -41.4). As concerns CA125 levels significant differences were observed in the following comparisons: stage I vs stage II -31.84 ng/ml (p<0.001; C.I. -44.56 to -23.48); stage I vs stage III -221.18 ng/ml (p<0.001; C.I. -241.44 to -177.08); stage I vs stage IV -231.24 ng/ml (p<0.001; C.I. -276.06 to -154.62); stage II vs Stage III -144.21 ng/ml (p<0.001; C.I. -187.36 to -71.84); stage II vs stage IV -136.10 ng/ml (p<0.001; C.I. -231.91 to -88.12). In the ovarian carcinoma CA125 and CgA were correlated in stage IV with a difference of 0.718 (p<0.001). Considering a cut-off of 35 ng/ml, the overall diagnostic accuracy for ovarian cancer of CgA was 73% with a sensitivity of 68% and a specificity of 64%. Considering a cut-off of 14 UI/L the diagnostic accuracy of CA125 was 91%, with a sensitivity of 78% and a specificity of 81%. The area under ROC (Receiver-operator characteristic) curve for CgA was 0.72 compared to 0.87 for CA125.

Discussion

The present study showed the high incidence of elevated plasma levels of CgA at various stages of ovarian cancer. Although the biological functions of CgA are not well established, the growing body of evidence suggests that CgA may affect various components of the tumor stroma and contribute to regulate tumor growth[20]. Several stromal elements form the tumor microenvironment, that plays a relevant role in cancer cell initiation, proliferation, migration, invasion and dissemination to distant organ[20]. CgA as tumor marker is a diagnostic tool for basic diagnosis within other diagnostic procedures and/or for evaluation of the course of the disease and of the response to treatment[21]. Serum CgA levels have been also used as markers for other tumors, like neuroblastoma, phaeochromocytoma, small cell lung cancer and the carcinoids[22, 23]. Recently, the cellular replies with morphological and functional neuroendocrine features occurred in no endocrine tumors and the high serum levels CgA were described in patients with carcinoma of breast, liver and ovary[24].

The morphological classification of the ovarian tumors reflects the current knowledge on embryogenesis and histogenesis of this complex organ. It consists of four main types responsible of a neoplastic variety: surface epithelium or celomatic; germinal cells; sexual cords; specialized ovarian struma. The ovarian surface epithelium, when involved in metaplastic or neoplastic conditions,

meets a müllerian differentiation. Therefore the histopathological varieties of this group of neoplasia are: serous, mucinous, endometrioid, clear cells, transitional and squamous cells, sometimes they have mixed and hybrid tumors.

Other tumors are so scantily differentiated, thus they cannot be inserted in some of these categories and are defined undifferentiated. The epithelial tumors of ovary represent over the 90% of the malignant forms; the remaining share is formed by the germinal and struma neoplasia. The growth of tumor cell clones, expressing neuroendocrine markers during the process of de-differentiation is not only a common feature of colon, breast and prostate cancer but an important finding in ovarian cancer[25].

Bosman[26] noted that neuroendocrine differentiation could occur in carcinomas that lack neuroendocrine cells in their normal epithelial counter parts, such as mucinous cystadenocarcinoma of the ovary, ovarian teratoma and ovarian carcinoma. Neuroendocrine differentiation appeared to be associated with a poor prognosis and is common in the advanced stage of the disease. Newly researchers showed the presence of hormonal positive and amine peptides in cells of ovarian mucinous tumors as gastrin, calcitonin, serotonin (5-HT) and neurotensin. These substances are like those found in the fore-gut carcinoid[27, 28].

CgA had a low diagnostic accuracy in detecting ovarian cancer, being the overall accuracy inadequate to support this marker in a screening program. Circulating CgA levels in ovarian cancer patients could reflect CgA expression in ovarian tissues or could represent the expression of altered tumor microenvironment[29]. Elevated levels of plasma CgA may be expression of the neuroendocrine phenotype, as demonstration of somatostatin receptors on ovarian cell membrane. Human malignancies demonstrate a high degree of cellular heterogeneity and at different points in time, tumor progression. In spite of the biological interest of this phenomenon, its clinical significance remains an open question[30-32]. Several investigators found that tumors with neuroendocrine differentiation behave more aggressively than tumors without such differentiation[8]. Even if neuroendocrine tumor cells do not proliferate, they produce neuroendocrine growth factors with mitogenic activity that promote cell proliferation and induce anti-apoptotic features in non-neuroendocrine cells in close proximity to neuroendocrine cells through a paracrine mechanism[33-34].

The abnormal production of CgA suggest that this circulating glycoprotein can indeed affect several aspects of the complex interplay between tumor-associated vessels and neoplastic cells. According to previous studies, we found that CgA depicted a significant trend in association with high-grade disease.

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