# Trends in Medical Research

ISSN 1819-3587

Academic Journals Inc.

www.academicjournals.com

Trends in Medical Research 2 (3): 128-134, 2007
ISSN 1819-3587


# Specificity of Serum Tumor Markers (CA125, CEA, AFP, Beta HCG) in Ovarian Malignancies

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**Abstract:** The aim of present study was to investigate the role of serum CA125 in patients with ovarian cancer. Ovarian cancers comprise of heterogeneous group of malignancies arising from epithelial cells/mesencymal cells or germ cells. Present study included 100 patients who were diagnosed as epithelial ovarian carcinomas (75 cases), mucinous adeno carcinoma of ovary (20 cases) and germ cell ovarian carcinomas (05 cases). Upon presentation, all these cases were generally diagnosed using CA125; however other serum tumor markers were also used in this study to determine the specificity of the disease. Ninty percent of cases showed elevated levels of CA125, about 10-15% of cases showed specificity towards other serum markers like CEA, AFP and beta HCG. The survival period as monitored by these tumor markers ranged from 5-60 months being inversely proportional to the levels of CA125. Diagnosis with various tumor markers was useful in determining various sub types of ovarian cancers. It is concluded that the specificity of each type of tumor marker is typical for each subtype; however it is important to use more than one tumor marker to determine accurately the diagnostic and prognostic status of the cases.

**Key words:** Ovarian carcinoma, tumor markers, CA 125, CEA, AFP, Beta HCG, ovarian germ cell carcinoma

## INTRODUCTION

Ovarian cancer is the fourth leading cause of death from cancers in women and accounts for second highest mortality rate of all the gynecological cancers (Brewer *et al*., 2003). Ovarian cancer accounts 14,300 deaths in US each year, more than all other gynecological malignancies combined (Jemal *et al*., 2003). Despite aggressive treatment via radical surgery, radiotherapy or chemotherapy, the mortality rate remains >50% for diagnosed patients. Ovarian cancer has an overall cure rate of approximately 45%, a relatively discouraging prognosis (Gaspar *et al*., 2003). Tumor markers are important indicators of the clinical progress of women with ovarian cancer. Three quarters of patients present with FIGO stage 3-4, disease chiefly because there is no reliable screening technique for early detection (Benedet *et al*., 2000). Epithelial carcinoma of ovary which constitutes over 90% of ovarian malignancies arises from coelomic epithelium and proves fatal to the majority of patients. Most of the remaining types are either germ cell or sex cord stromal cancer. Epithelial cancer can be further classified based on cell type into serous, mucinous, endometroid, clear cell, mixed epithelial and undifferentiated squamous cell carcinoma. Of these histological subtypes, serous cell is the most common type (Meyer and Rustin, 2000).

CA125 is currently the only tumor marker to have a well-defined and validated role in the monitoring of ovarian carcinoma (Meyer and Rustin, 2000). Decreased levels are associated with response to therapy and increasing levels with tumor progression (Baron and Maihle, 2005). The CA125 antigen is a high molecular weight glyco protein, which is expressed by a large proportion of epithelial ovarian cancers. It is detected by OC monoclonal antibody, which was first described by

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Bast *et al*. (1981). Since its discovery, CA125 has become well established as a tumor marker for Non-mucinous epithelial ovarian carcinoma and has an important role in diagnosis, with it's incorporation into the risk of malignancy index. It is raised in approximately 50% of stage -1 epithelial ovarian cancers and 75-90% of patients with advanced disease. False positive results have been noted in some medical disorders, both malignant and benign (Buamah, 2000) though these false positive results decrease the specificity and sensitivity of CA125 to some extent, the requests for CA125 testing are increasing day-by-day (Moss, 2005).

The aim of present investigation has been to determine the role of serum CA125 in patients with ovarian cancer. Though it has some false positive prediction, its sensitivity and specificity makes it a useful biomarker. While CA125 is the marker of choice for epithelial ovarian cancer, other markers may complement its use. The complementary markers include CEA, AFP and Beta HCG etc (in ovarian germ cell tumors) (Bonfrer *et al*., 1999).

## MATERIALS AND METHODS

### Study Group

The study group was of Indian origin, belonging to local region of Andhra Pradesh. The procedure to enroll in this study was in accordance with ethical standards of responsible committees of the institutes/hospitals. The present study comprised 127 patients, the diagnosis included 75 ovarian carcinoma patients, 20 benign ovarian disease patients and the remaining were suffering from other malignancies. The 127 diagnosed patients were selected from those enrolled between August 2004-2006 at Indo-American Cancer Institute and Research Centre (IACI and RC).

### Demographic Recording

Clinical and bio-chemical characteristics recorded in the demographic proforma were age, sex, history of previous malignancies, history of patient families, cytology (FNAC, Pap smear reports) and histopathology (biopsy) results were obtained from the pathology departments for the clinical evaluation of the diagnosis. These data were systematically recorded. We used univariant analysis for determining the association between these factors.

### Estimation of Serum Tumor Markers (CA125, CEA, AFP, Beta HCG) Levels

Whole blood was collected from the diagnosed patients as above and centrifuged for 10-15 min at 2000-2500 rpm. The supernatant serum was used for the measurement of tumor markers (CA125, CEA, AFP, beta HCG etc.). Tumor markers levels were measured with a solid phase sandwich ELFA method, using commercially available kits (Biomerieux, S.A).

### Procedure of Tumor Markers (CA125, CEA, AFP, Beta HCG) Measurement

The serum samples (200 μL for each marker) were automatically analyzed by the automatic hormone analyzer, mini VIDAS of Biomerieux company, S.A.

### Clinical Data Collection

Clinical data were recorded, including patient age, race and using the classification of Federation of Gynecology and Obstetrics (FIGO) stage, type of adeno carcinoma, histological grade, primary therapy, surgical cyto-reduction, number of chemo cycles, CA125 and other tumor marker levels at each visit and response to therapy and clinical outcome. Complete response was defined by a normal physical examinations, normal CT scan of abdomen, pelvis and normal serum tumor markers level. The applied cut off value for CA125 was 35 IU $mL^{-1}$. A partial response was defined by a decrease of at least 50% in the sum of the largest dimensions of tumors as measured by CT scan. A smaller decrease

or any increase in tumor size during primary chemotherapy was defined as a non-responder. The duration of Overall Survival (OS) was the interval between initiation of treatment (surgery or chemotherapy) and death.

**Statistical Analysis**

Comparison of serum tumor markers (CA125, CEA, AFP, beta HCG etc.) activity against ovarian cancer was done according to age; type and stage of the tumor (and response to tumor). These parameters were calculated on percentage basis and shown in graphical representations. Based on the false positive and false negative productivity, the sensitivity and specificity of CA125 were also measured to make the statistics more effective. Sensitivity was defined as the proportion of patients with ovarian carcinoma correctly identified by CA125 and specificity as the proportion of patients without ovarian carcinoma correctly identified by CA125.

Serum tumor markers thus estimated in ovarian carcinoma and in benign tumors were statistically analyzed by student t-test of equal variance. The statistical analysis was done by the software, Systat 11.

The calculation of sensitivity and specificity measured were shown as follows:

- Sensitivity of CA125 for ovarian carcinoma cases = $\frac{\text{True positive}}{(\text{True positive} + \text{False negative})} \times 100$

- Specificity of CA 125 for ovarian carcinoma cases = $\frac{\text{True positive}}{(\text{True negative} + \text{False positive})} \times 100$

## RESULTS

The study comprised 127 female patients, who were suspected with ovarian cancers. The diagnosis included 75 serous epithelial ovarian cancer patients, 20 mucinous adeno carcinoma of ovary cases, 05 germ cell ovarian cancer patients, 20 benign ovarian disease patients and remaining 07 were suffering from other malignancies like breast metastases, carcinoma of endometrium etc. Out of 100 ovarian cancer patients 70% of patients were categorized under FIGO stage 3-4 and the remaining 30% were diagnosed with stage 1-2.

**Significance of Serum CA125 in Ovarian Cancer**

The best available marker for epithelial ovarian cancers is the mucin CA125.The reference interval is 0-35 IU $L^{-1}$. Among the 75 ovarian carcinoma cases 38(50.66%) patients were categorized under the age group of above 50 years; 23 (30.66%) patients were under the age group of 41-50 years and rest of the 12 (16.0%) women were below the age of 40 years. Only 2 ovarian carcinoma cases were found below 30 years of age. Hence it is clear that the older women have higher incidence of ovarian carcinoma. The average age of diagnosis was 50 years (based on Indian population also confirmed by western standards). The diagnosis of these patients was confirmed by the results of transvaginal ultrasound, CT scan of abdomen and pelvis, followed by FNAC or percutaneous biopsy. These reports are available at IACI and RC medical record files. When CA125 levels were correlated with the fiual diagnosis in patients for suspicion of ovarian cancer, 68 (90.66%) of patients had shown abnormal CA125 levels. The clinical characteristics of these 102 patients are presented in Table 1. Among 75 epithelial ovarian carcinoma cases, 24 (32%) patients with FIGO stage 1-2 showed increased serum CA125 levels and remaining cases were diagnosed with FIGO stage 3-4. Hence the possibility (32%) of early diagnosis (FIGO Stage1-2) of may also be possible with

the measurement of serum CA125 levels. The sensitivity of CA125 for ovarian cancer in this study group (Indian population) was 90.66% and specificity determined as 81.48%.

Among 75 epithelial ovarian carcinoma cases there were 2 cases with CA125 concentration above 500 IU $L^{-1}$ and these were not diagnosed as ovarian cancers, 2 cases were of metastatic disease from breast of unknown primary tumor and carcinoma of cervix. Also 3 cases out of 20 benign ovarian tumors, showed abnormal levels of serum CA125, which shows a decrease in sensitivity of CA125 for ovarian cancer. The CA125 levels in ovarian cancer ranged from 09-13,952 IU $mL^{-1}$ (represented in Table 1 and Fig. 1). These results confirm the high rates of sensitivity and specificity associated with CA125 in the diagnosis of ovarian cancer, though it has some false positive predicivity.

**Role of CA125 levels in prognosis:**

The mean age of the patient was 50 years. The median CA125 measurement was 600 IU $L^{-1}$ (ranges from 9-13952). Among the patients who underwent surgery, CA125 levels decreased postoperatively. Overall, 29 (of 30) patients had a postoperative decrease in CA125 after tumor debulking. To evaluate the prognostic role of CA125 in ovarian carcinoma, clinical characteristics were compared with CA125 levels for their association with survival.

The biochemical progression was defined as the occurrence of a value greater than 30 U $mL^{-1}$. The CA125 in those patients with complete clinical response stayed maximum until 61 months (Maximum survival period), where as those of non-responder rose back again within 3 months and the partial responders within 2 years. The trend of patients with complete clinical response differed significantly from that of partial/non responders for both the differences in coefficients. Partial/non responders had shorter survival and follow up, when compared to complete clinical responders. Details are presented in Fig. 2. Overall increase or decrease of CA125 levels correlated with progression/regression of disease in 29 of 30 patients.

**Significance of Serum CEA in mucinous adenocarcinoma of ovary**

The Carcio embryogenic Antigen (CEA) generally increases in patients with mucinous adeno carcinoma, endometroid carcinoma, benign tumors of epithelial origin and benign skin disorders. Most epithelial neoplasms of ovary also express CEA. The cut off value for CEA is 5 ng $mL^{-1}$.

Table 1: Clinical characteristics of the study group and CA125 activity against ovarian cancer

| Type of tumor | Total cases | CA125 elevated cases | Percentage of CA125 elevated cases | CA125 range (IU $mL^{-1}$) | Mean±SE (IU $L^{-1}$) | CV |
|---|---|---|---|---|---|---|
| Ovarian carcinoma | 75 | 68 | 90.66 | 09-13952 | 1077.31±199.04 | 1.733 |
| Benign tumors | 20 | 03 | 15.00 | 04-72 | 20.43±7.75 | 1.200 |

(p-value <0.001; Student t-value > $t_{0.05}$, Total number of cases studied: 102, Reference value of serum CA125 levels in normal cases: <35 IU $mL^{-1}$

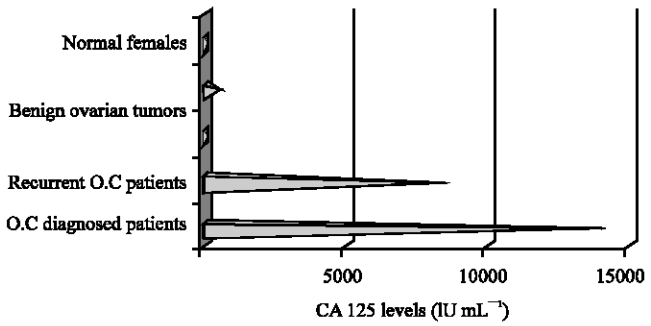


Fig. 1: CA125 ranges in disease status

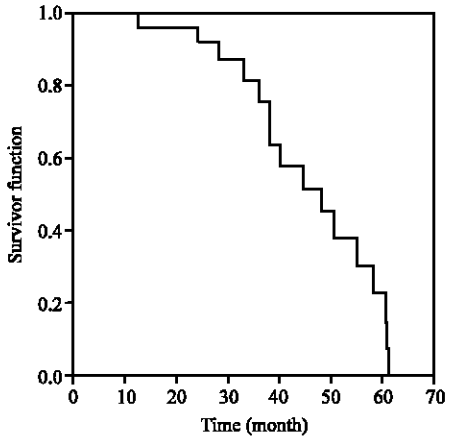


Fig. 2: Kaplan-Meier survival plot of ovarian carcinoma patients

Table 2: Clinical characteristics of the study group and CEA, AFP, Beta HCG activities against ovarian carcinomas

| Type of tumor marker | Total No. of cases | No. of patients with increased tumor marker levels | Percentage of cases with increased tumor marker levels | Ranges (ng $mL^{-1}$) |
|---|---|---|---|---|
| Serum CEA | 20 | 80 | 40 | 0.5-119 |
| Serum beta HCG | 05 | 04 | 80.0 | 151-76500 |
| Serum AFP | 04 | 02 | 50.0 | 10.50-700 |

In this investigation out of 127, only 20 ovarian adeno carcinoma patients were referred for both CEA and CA125 investigations. The mean age of the patients was 50 years. Among the 20 ovarian adeno carcinoma patients 08 (40%) cases had shown elevated CEA levels along with CA125 and the remaining 12 (60%) cases had normal CEA levels, though they were suffering from adeno carcinoma of ovary. The CEA levels in ovarian adeno carcinoma cases range from 0.5-119 ng $mL^{-1}$ and the mean value was 16.53 (Table 2).

**Significance of Serum AFP and Beta-HCG in Ovarian Germ Cell Tumors**

AFP and Beta HCG are the main tumor markers for ovarian germ cell tumors. Both AFP and HCG are necessary in the follow up of patients with ovarian germ cell cancers. Out of 100 cases, at the time of ovarian cancer diagnosis only five cases were diagnosed as mixed germ cell tumors of ovary. Among these 5, four cases were referred for both the markers AFP and Beta HCG and the other one was referred only for beta HCG.

Among the 5 ovarian germ cell cases, four cases had shown elevated beta HCG levels and two cases out of four had shown increased AFP levels (Table 2). As the number of cases for ovarian germ cell tumors were less, it is difficult to generalize the usage of these two biomarkers but with the above results it may be confirmed that AFP and Beta HCG may play a significant role in the early diagnosis and prognosis, even though the occurrence of these types tumors is very less in the general population.

## DISCUSSION

The detection of serum antigens (CA125) confirms not only neoplasms of ovary but also some cases of metastatic breast cancer and some benign cases. The signs and symptoms of ovarian cancer are known to be vague and nonspecific in the early stages of disease. The advance stage patients are most often associated with abdominal distension, ascites, pelvic mass or an ovarian cyst and those with more non-specific symptoms was to attempt to identify patients who were more likely to have

ovarian disease (Buamah, 2000). Contemporary research efforts are aimed at further evaluatory role of CA125 to detect early stage cancer, to differentiate benign from malignant tumors, to predict responsiveness to chemotherapy and survival and to monitor disease recurrence and clinical progression (Van der Burg, 1990).

It was apparent that CA125 was mainly being used to investigate a broad range of symptoms and was often not used in conjunction with ovarian imaging. A normal result seems to have been taken as an indication of the absence of ovarian disease in many cases because 27% of patients being investigated had a CA125 within normal limits and had no ovarian imaging (Moses, 2005). This may be because the suspicion of ovarian malignancy was low or because the clinician involved was able to suspect ovarian carcinoma through his routine examination and advised the test for serum CA125. For example, a complex pelvic mass or ascites of unknown origin would presumably increase both the sensitivity and specificity of CA125 in diagnosis.

Eagle and Ledermann (1997) reported that CA125 levels were raised above 35 U ml in 78% of women with ovarian malignant masses, but also in 22% of those with benign masses. The predictive value of CA125 measurement in post-menopausal women is little higher and using cutoff 65 U $mL^{-1}$ the false positive rate was reported as 8%.

We had shown that the increased serum CA125 levels have a good diagnostic significance, as well as prognostic significance, though it has some false predicivity. The survival periods were found to be directly associated with CA125 levels in ovarian cancer patients. According to Eagle and Ledermann (1997), AFP and beta HCG are probably the best known tumor markers in clinical practice for germ cell neoplasia. The number and combination of tumor markers depends upon the type of ovarian disease. In addition, combination of CEA, AFP and Beta HCG in ovarian adeno carcinomas and ovarian germ cell tumors may give more effective results than using a single biomarker. There is evidence that combination of serum tumor markers are superior to single marker (CA125) alone in diagnosis of ovarian carcinoma. The low use of AFP and Beta HCG suggests that there could be very few cases of ovarian germ cell tumors in the population, but these are more frequent in younger women.

## CONCLUSION

This study evaluated 127 cases of ovarian carcinomas of South Indian women, using various serum tumor markers. The results confirm the high rates of sensitivity and specificity of CA125 and its association in the diagnosis and prognosis of ovarian cancer. Also the Kaplan-Meier survival graph indicated a survival period ranging from 6-60 months. It is evident from present results that combination of serum tumor markers is superior to CA125 alone in the diagnosis of ovarian malignancies.

## ACKNOWLEDGMENTS

We thank to all the participants in this study. Without their cooperation, this study could not have been possible. We also wish to thank the Oncologists and management of this institute who encouraged us to take up this study. Our special thanks to Dr. Mohan Vamsy, Dr. Susheela and Dr. Sudha Murthy for their valuable guidance.

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