CHAPTER - 5
In the previous Chapters, we have discussed the role of steroid receptors, Epidermal growth factor receptors (EGFRs) and steroid and peptide hormones in epithelial ovarian cancer patients. It was found that at least one hormone was abnormal which might have resulted into hormonal imbalance. Thus, we think that hormonal abnormalities may precede and presumably favour the onset of epithelial ovarian cancer or they may be related with the evolution of the disease. This study would be rather incomplete without the addition of tumor markers which are essential for monitoring patients as well as for management of this neoplasm.

Ovarian carcinoma cells exhibit distinct cell surface antigens that distinguish them from cells of normal adult tissues (Gall et al, 1973; Knauf and Urbach, 1977 and Imamura et al, 1978). Therefore, there is a great potential for the use of cell surface antigens in this disease that could accurately monitor the disease course and aid in patient's management.

The most promising marker in epithelial ovarian cancer is CA 125. It is an antigenic determinant defined by the murine
monoclonal IgG1 OC125 developed by somatic hybridization of spleen cells from mice immunized with an epithelial ovarian carcinoma cell line (OVCA 433; Bast et al, 1981). It is associated with a high molecular weight glycoprotein that is expressed in coelomic epithelium during embryonic development (Kabawat et al, 1983a). However, there are limitations of CA 125 with regard to preoperative discrimination of benign versus malignant tumors and sensitivity to detect mucinous adenocarcinomas (Bast et al, 1983; Cruickshank et al, 1987; Schilthuis et al, 1987 and Vasilev et al, 1988). Furthermore, CA 125 lacks sensitivity in detecting residual disease < 1 cm at the time of second look laparotomy (Berek et al, 1986; Brioschi et al, 1987 and Rubin et al, 1989).

In overcoming these difficulties, the combination of CA 125 with another cell surface antigen lipid-bound sialic acid (LSA) has been studied. Serum LSA, is a nonspecific sialoglycoprotein which probably reflects release of cancer cell membrane glycolipids (Katopodis et al, 1982 and Schwartz et al, 1987). Both CA 125 and LSA were reported to correlate well with the disease course (Patsner et al, 1988).

In the present study, we have evaluated the individual as
well as combined usefulness of CA 125 and LSA in epithelial ovarian cancer patients, considering stage, histologic type and grade of the tumor, presence/absence of residual disease, disease progression/remission as well as survival. Also the effect of chemotherapy on the marker was studied.

Furthermore, much of the work on CA 125 has been predominantly concentrated on serum measurements. To define its usefulness as a prognosticator, the marker has also been studied in cystic/ascitic fluids, and cytosols of primary epithelial ovarian tumors.

STUDY DESIGN:

CA 125 and lipid-bound sialic acid (LSA) were estimated in 72 histologically confirmed epithelial ovarian cancer patients and 20 controls. The patients were followed for a minimum period of 18 months. The clinical data, stage, histologic typing and grading, residual disease and treatment schedule were described in Chapter I. Surgery was followed by chemotherapy and the preoperative levels of these markers were compared to: (i) those during chemotherapy (i.e. after 2 cycles of chemotherapy) and (ii) those after completion of chemotherapy.

Blood samples for CA 125 and LSA were collected.
preoperatively to obtain baseline level of individual patients, postoperatively (10-14 days after operation) and monthly/bimonthly on each occasion. Serum was separated within 2 hours, aliquoted and preserved at -70°C till analysis.

CA 125 was estimated in cystic fluids ($N = 11$), ascitic fluids ($N = 13$) and cytosols ($N = 46$). The ascitic fluid was collected usually 1-2 days before operation, while cystic fluid was collected from the tumor specimens. Cystic/ascitic fluids were centrifuged and preserved at -70°C till analysis. Studies were performed retrospectively.

CA 125:

CA 125 was assayed using IRMA kits, supplied by Cis, France, using manufacturer’s instructions. The reference samples of the kit were considered for internal quality control purpose and an intraassay and an interassay coefficient of variation (CV) was 3-5% and 5-8% respectively. The sensitivity of the assay was 2.2 U/ml. The cut off value for CA 125 was 35 U/ml. CA 125 values in cytosols (prepared for receptor studies) were expressed as U/mg cytosol protein. Protein concentration in the cytosol was determined by the method of Lowry et al. (1951).
LIPID - BOUND SIALIC ACID (LSA):

LSA was assayed by the method of Katopodis et al. (1982). 50 ul of the serum was added in screw capped glass tubes with 150 ul glass distilled water. The tubes were vortexed for 5 seconds and placed on ice. 3 ml of cold (4°C) chloroform : methanol (2:1, v/v) was added to each tube, the tubes capped, and each tube was vortexed for 30 seconds. 0.5 ml of cold glass distilled water was added to each tube, the tubes recapped, and all the tubes were mixed for 30 seconds by repeated inversion. All the tubes were centrifuged for 5 minutes at 1200 x g, at room temperature. 1 ml of the resulting upper layer was transferred to another screw capped glass tube. 50 ul of phosphotungstic acid (1 gm/ml distilled water) was added to each tube and all the tubes were vortexed and kept at room temperature for 5 minutes. The tubes were centrifuged at room temperature for 5 minutes at 1200 x g. The supernatant was removed and the remaining pellets were redissolved in 1.0 ml of distilled water (kept at 37°C) by vortexing until no gross particles were seen (about 1 minute of vortexing per tube). To each tube was added 1 ml of freshly prepared resorcinol reagent: 10 ml of 2% (w/v) stock resorcinol in water, 9.75 ml of water, 0.25 ml of 0.1M CuSO₄, made to 100 ml with
concentrated HCl. Each tube was capped, vortexed and placed in a boiling waterbath for 15 minutes followed by 10 minutes in an ice bath. 2 ml of butylacetate/ n-butanol (85:15 v/v) was added to each tube and the tubes were vortexed and centrifuged at room temperature for 10 minutes at 1200 X g. The extracted chromophore was read on a Beckman DU 8B spectrophotometer at 580 nm. The concentration of LSA was calculated by the use of a standard curve of N- acetyl-neuraminic acid ( Sigma Chemical Co) prepared by treating the standard tubes with resorcinol reagent. The rest of the procedure was similar to that of LSA. A regression curve was calibrated. The intraassay and interassay coefficient of variation (CV) was 4-6% and 6-8% respectively. A level of 20 mg/dl or above was defined as abnormal ( Katopodis et al, 1982 ).

TUMOR MARKERS IN EPITHELIAL OVARIAN CARCINOMA MONITORING :

Depending on disease course, the patients were grouped into two : (i) patients with progressive disease and (ii) who responded to treatment at the end of 18 months. The details of it were described in Chapter II.

The sensitivity, specificity and predictive values of the markers in follow-up patients were calculated according to
Tondini et al. (1988) and Caponigro et al. (1990) with the following definitions.

True +ve: Patients in whom marker increased with progression.

False -ve: Patients in whom marker decreased with progression.

True -ve: Patients in whom marker decreased with response.

False +ve: Patients in whom marker increased with response.

\[
\text{Sensitivity} = \frac{\text{True +ve (# of patients)}}{\text{True +ve + False -ve (# of patients)}}
\]

\[
\text{Specificity} = \frac{\text{True -ve (# of patients)}}{\text{True -ve + False +ve (# of patients)}}
\]

\[
\text{Predictive value (positive test)} = \frac{\text{True +ve (# of patients)}}{\text{True +ve + False +ve (# of patients)}}
\]

\[
\text{Predictive value (negative test)} = \frac{\text{True -ve (# of patients)}}{\text{True -ve + False -ve (# of patients)}}
\]
STATISTICAL ANALYSIS:

The difference in markers were analysed to obtain statistical significance, using an exact contingency table test for ordered data and Fisher's two-sided exact test (Mehta and Patel, 1983). P values less than 0.05 were considered to be significant. Both, progression-free and actual survival were calculated according to life table analysis (Kaplan and Meier, 1958).

Spearman's rank correlation coefficient (r) was used to calculate correlation between:

(i) Preoperative levels of CA 125 and LSA and (ii) Cytosolic CA 125 and survival.

Karl Pearson correlation coefficient (r) was used to calculate correlation between:

(i) Serum CA 125 and cystic/ascitic fluid and (ii) Serum and cytosolic CA 125.

RESULTS:

PART A:

INCIDENCE OF MARKERS IN EPITHELIAL OVARIAN CANCER PATIENTS:

CA 125 and LSA were significantly elevated in epithelial
ovarian cancer patients compared to controls (Table 1). In 68/72 (94.4%) and 25/72 (34.7%) patients, CA 125 and LSA were above upper normal limit respectively (Fig.1). However, no direct or inverse correlation was observed between these two markers (r = + 0.014).

RELATION OF MARKERS TO DISEASE STAGE:

CA 125 was high in stage II patients when compared to controls but the difference was statistically nonsignificant. However, when stage II patients were compared with stage III and IV, the marker was significantly low in stage II patients (P < 0.001; Table 2a). High levels were also observed in patients with recurrent disease, but the difference was statistically nonsignificant in comparison to stage II patients.

LSA showed an increasing trend as stage advanced. Statistical significance was observed between patients with stage II and stage IV and recurrent disease only (Table 2a).

Patients with serous tumors, when grouped according to disease stage, showed an increasing trend of both the markers as stage advanced. The differences were, however statistically significant only for CA 125 (P < 0.0001; Table 2b).
MARKERS AND HISTOLOGIC TYPES:

CA 125 concentrations were significantly high in serous tumors when compared to mucinous tumors (P < 0.001; Table 3). However, 4/5 (80%) patients with mucinous tumors had CA 125 levels above the upper normal limit. Out of these 4 patients, one had stage II, one had stage III and two had stage IV disease. When LSA was correlated with the histologic types, no such correlation was noted.

MARKERS ACCORDING TO HISTOLOGIC GRADE OF THE TUMOR:

Intergroup variation was not observed in CA 125 and LSA levels (Table 4a). Serous tumors when subgrouped according to the degree of differentiation, also showed no intergroup variations (Table 4b).

COMPARISON OF PRE- AND POST- OPERATIVE MARKER LEVELS:

The postoperative marker levels were divided into 3 groups according to the presence/absence of residual disease: (i) no residual disease (ii) < 2 cm residual disease and (iii) > 2 cm residual disease. The postoperative CA 125 level was significantly low in patients without residual disease and those with < 2 cm residual disease when compared to those with > 2 cm residual disease. Similarly, these levels were
significantly low when compared to preoperative levels, except >2 cm residual disease (Table 5). On the other hand, no such relationships were observed with LSA.

**MARKER LEVELS IN RELATION TO DISEASE STATUS:**

(i) Patients with progressive disease (N=12):

In 12/12 (100%) patients, CA 125 showed excellent correlation with disease progression which might be due to the fact that 57/72 (79%) of our patients had advanced disease. A rising CA 125 titre could accurately predict disease progression with a lead time of 3-5 months (Figs. 2-6). In case of LSA, the rise in titre was not many folds as observed for CA 125 (Figs. 2, 3, 4 and 6).

(ii) In responders (N=11):

In 11/11 (100%) patients, response to treatment was evident by a decrease in CA 125 levels. In case of LSA, response to treatment was not predicted as accurately as CA 125 in all patients (Figs. 7-11).

**SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUE OF MARKERS:**

The sensitivity, specificity and predictive value of a positive test and a negative test, and diagnostic efficiency
of CA 125 in follow-up patients were 100% while for LSA, they were 66.6%, 27.2%, 50%, 42.8% and 47.8% respectively (Table 6).

EFFECT OF CHEMOTHERAPY ON MARKER LEVELS:

(I) In patients with progressive disease:

In patients with progressive disease, 2 cycles of chemotherapy following surgery, resulted into a significant decrease of preoperative CA 125 level ($P < 0.02$). Completion of chemotherapy courses resulted in a nonsignificant elevation of CA 125 when compared to the levels after 2 cycles of chemotherapy (Table 7). No such relationship was seen with LSA.

(II) In responders:

In responders, CA 125 decreased after 2 cycles of chemotherapy, followed by a further decrease after completion of chemotherapy and the differences were statistically significant when compared to preoperative values ($P < 0.001$; Table 7). LSA did not show such a trend.

MARKER LEVELS IN PATIENTS ALIVE AND THOSE WHO DIED:

Preoperative CA 125 and LSA levels were higher in patients
who died as compared to those alive at the end of 18 months. The difference was statistically significant only for CA 125 ($P < 0.02$; Table 8).

**MARKER LEVELS ACCORDING TO DISEASE STAGE IN PATIENTS ALIVE AND THOSE WHO DIED:**

In the group of patients alive at the end of 18 months, CA 125 and LSA were low in stage II patients when compared to stage III+IV disease. The difference was statistically significant only for CA 125 ($P < 0.01$; Table 9).

Amongst the patients who died, significant differences were not observed in CA 125 and LSA with stage of the disease. However, patients who died had high CA 125 values in all stages as compared to patients alive at the end of 18 months. The difference was statistically significant only in patients with recurrent disease ($P < 0.05$; Table 9). In the group of patients who died, stage IV patients had significantly high levels of LSA as compared to stage IV patients alive at the end of 18 months ($P < 0.02$).

**SURVIVAL BASED ON PREOPERATIVE MARKER LEVELS:**

Patients with preoperative CA 125 and LSA levels within the normal range, had better progression-free and actual
survival in comparison to patients with elevated levels of CA 125 and LSA. These differences were statistically nonsignificant (Table 10; Figs. 12-15).

EFFECT OF 2 CHEMOTHERAPY CYCLES ON MARKERS VERSUS SURVIVAL:

Patients with CA 125 levels within the normal range ( < 35 U/ml) after 2 chemotherapy cycles had better progression-free and actual survival compared to those with elevated levels after 2 cycles of chemotherapy (Fig. 16 and 17). The difference was statistically significant only with progression-free survival (P < 0.05; Table 11). Significant difference in progression-free and actual survival was not observed in patients with LSA <20 mg/dl after 2 chemotherapy cycles when compared to those with LSA > 20 mg/dl after 2 cycles of chemotherapy (Fig. 16 and 17).

When both the markers were combined, it was observed that both progression-free and actual survival were significantly better in patients with CA 125 and LSA within the normal range compared to those with elevated marker levels (Table 11; Figs.18 and 19).

SURVIVAL IN TOTAL PATIENTS AND PATIENTS WHO DIED, ACCORDING TO DISEASE STAGE:

Epithelial ovarian cancer patients showed a decreasing trend
of survival as stage advanced. Stage II patients had significantly better survival compared to patients with stage III+IV disease ( \( P < 0.001 \); Table 12). Amongst the patients who died, stage III patients had better survival than stage IV patients. However, the difference was statistically nonsignificant. When survival in total patients with stage III+IV disease was compared to survival in stage III+IV patients who died, it was observed that survival was better in total patients compared to those who died within 18 months ( \( P < 0.01 \) ).

PART B:

INCIDENCE OF CA 125 IN CYSTIC/ASCITIC FLUIDS:

The mean concentration of CA 125 in cystic/ascitic fluids was higher compared to serum level. However, the difference was statistically nonsignificant (Table 13).

In 12/24 (50%) patients, CA125 in cystic/ascitic fluids were elevated compared to their serum levels. In all 24 patients, CA 125 levels in cystic/ascitic fluids were above 35 U/ml, while values above 200 U/ml were found in 19/24 (79%) patients (Fig.20). Correlation coefficient (r) between cystic/ascitic fluid CA 125 and circulating level was + 0.123.
RELATION OF CA 125 IN CYSTIC/ASCITIC FLUIDS TO DISEASE STAGE:

There was no correlation between cystic/ascitic fluid CA 125 levels and stage of the disease (Table 14). However, in patients with recurrent disease, the levels were significantly elevated compared to the circulating CA 125 levels ($P < 0.05$).

CYSTIC/ASCITIC FLUID CA 125 AND HISTOLOGIC TYPES:

Significant differences were not seen in cystic/ascitic fluid CA 125 levels and the histologic types (Table 15). However, patients with serous, mucinous and mucin-secreting adenocarcinomas had higher mean values of cystic/ascitic fluid CA 125 than their circulating levels.

CYSTIC/ASCITIC FLUID CA 125 AND HISTOLOGIC GRADE OF THE TUMOR:

CA 125 concentrations in cystic/ascitic fluids were similar in the different histologic grades of the tumor (Table 16).

INCIDENCE OF CYTOSOLIC CA 125:

Cytosolic CA 125 was detectable in all 46 patients with epithelial ovarian carcinoma. The range of cytosolic CA 125
was from 3.29 - 1339.57 U/mg cytosol protein (Table 17; Fig. 21). The correlation coefficient (r) between cytosolic CA 125 and serum CA 125 was + 0.158.

CYTOSOLIC CA 125 ACCORDING TO DISEASE STAGE:

Cytosolic CA 125 showed an increasing trend as stage advanced. Elevated levels were also noted in patients with recurrent disease compared to stage II patients but the difference was statistically nonsignificant (Table 18).

CYTOSOLIC CA 125 AND HISTOLOGIC TYPES:

Cytosolic CA 125 in patients with mucinous tumors was significantly low compared to those with serous and endometrioid tumors (Table 19). Also patients with undifferentiated and mixed carcinomas had significantly low cytosolic CA 125 compared to those with serous tumors (P < 0.0001 and P < 0.01 respectively).

CYTOSOLIC CA 125 ACCORDING TO HISTOLOGIC GRADE OF THE TUMOR:

Patients with histologic grade I tumors had significantly low cytosolic CA 125 concentrations in comparison to patients with histologic grade II and III tumors (P < 0.01; Table 20).
Cytosolic CA 125 levels were low in ER/PR tumors than in ER/PR tumors but the difference was statistically significant only between PR and PR tumors (P < 0.05; Table 21).

CYTOSOLIC CA 125 VERSUS EGFR+ TUMORS:

No correlation was observed between cytosolic CA 125 level and EGFR positivity (Table 22).

SURVIVAL BASED ON CYTOSOLIC CA 125:

Significant difference in progression-free and actual survival was not found between patients with cytosolic CA 125 < 200 U/mg cytosol protein and those with cytosolic CA 125 > 200 U/mg cytosol protein (Table 23).

DISCUSSION:

The present study revealed elevated serum CA 125 levels in 68/72 (94.4%) patients with epithelial ovarian cancer as 57/72 (79%) had advanced disease and 55/72 (76.3%) had serous type histology. This was in accordance with the results of Bast et al. (1983 and 1984), Canney et al. (1984), Heinonen et al. (1985), Niloff et al. (1985) and Bhatavdekar et al. (1987).
A significant correlation with stage of the disease suggested that CA 125 reflected tumor burden. 34/35 (97.1%) and 22/22 (100%) patients with stage III and IV disease respectively had elevated CA 125 levels. The one stage III patient with CA 125 <35 U/ml had histology of mucinous cystadenocarcinoma.

With respect to histogenic origin of ovarian tumors, CA 125 was high in all histologic types including mucinous tumors which were originally reported as negative, both immunohistologically (Kabawat et al., 1983b) and serologically (Bast et al., 1983). Although, our patients with mucinous tumors had elevated CA 125 levels compared with controls, however, the antigen levels were significantly low than those found with serous tumors. Our results corroborate with those of Canney et al. (1984), Cruickshank et al. (1987) and Vergote et al. (1987).

Furthermore, CA 125 did not correlate with the degree of tumor differentiation. This lack of correlation between CA 125 and histologic grade agreed with the findings of Cruickshank et al. (1987). They suggested that CA 125 was not a tumor differentiation antigen. On the contrary, Vergote et al. (1987) observed an inverse relationship
between serum CA 125 levels and the degree of tumor differentiation.

A positive association was noted between postoperative CA 125 levels and absence/presence of residual disease. Postoperative CA 125 levels were significantly lower in patients without residual disease and those with residual disease < 2 cm compared to those with > 2 cm residual disease. Findings similar to ours were also reported by Vergote et al. (1987).

Changes in CA 125 levels correlated excellently with disease status. A rising CA 125 titre could accurately reflect disease progression in all 12/12 (100%) patients in the present study. Bast et al. (1983) observed 93% correlation between CA 125 and clinical course of the disease. It was observed that a rise in CA 125 preceded disease progression by approximately 3-5 months (Figs. 2-6).

In responders, CA 125 predicted successful response to treatment. In one patient (Fig. 10), a false +ve elevation of CA 125 was noted and the patient was free of disease. Similar observations were also noted by Bast et al. (1983). In their case, 2 patients had small bowel obstruction, 1 had episode of congestive heart failure and 1 patient's laparotomy was done.
Response to treatment is one of the factors which affects circulating marker levels. In the present study, CA 125 levels predicted response to chemotherapy in patients with progressive disease and in responders. Persistently elevated CA 125 levels in one stage III patient (Fig. 5) showed that the patient did not respond to chemotherapy and subsequently developed progressive disease.

We have also tried to assess the prognostic value of preoperative and postoperative (after 2 cycles of chemotherapy) CA 125 levels. The preoperative CA 125 level was significantly low in patients alive at the end of 18 months compared to those who died. Moreover, high preoperative levels were associated with shorter progression-free and actual survival. Kivinen et al. (1986) observed that high preoperative CA 125 values indicated a poor response to chemotherapy. It was also observed that if the postoperative (after 2 cycles of chemotherapy) CA 125 level was < 35 U/ml, then these patients had better progression-free and actual survival compared to those with CA 125 > 35 U/ml after 2 cycles of chemotherapy. Thus, the excellent sensitivity and specificity of CA 125 assay and excellent correlation with disease status suggested that CA 125 is a useful marker for monitoring disease and predicting survival in epithelial ovarian cancer patients.
LSA was elevated in 25/72 (34.7%) patients with epithelial ovarian cancer. Stratton et al. (1988) also found elevated LSA levels in only 32% of ovarian cancer patients, while 3 other studies have reported elevated LSA levels in more than 60% of ovarian cancer patients (Schwartz et al., 1987; Patsner et al., 1988 and Vardi et al., 1989). LSA was less specific compared to CA 125. However, LSA was elevated in one of the 4 patients with normal CA 125 levels. Thus, combined use of these markers helped to increase the diagnostic value in epithelial ovarian cancer patients.

LSA showed good correlation with disease stage but there was no correlation with histologic type or grade of the tumor. Although, LSA correlated with disease stage, it did not predict tumor burden as accurately as CA 125. LSA was elevated in 10/35 (28.5%) and 10/22 (45.4%) patients with stage III and IV disease respectively.

Postoperative LSA levels showed no correlation with the absence/presence of residual disease.

The sensitivity, specificity and predictive value of a positive test for LSA were 66.6%, 27.2% and 50% respectively. In case of LSA, the observation of each patient's individual marker level was the most important
criteria when monitoring the patients. The rise in LSA at progression was not many folds as observed for CA 125 and in 2 patients (Fig. 4), the levels were within normal limit throughout the disease course.

In responders, response to treatment was not predicted as accurately as CA 125. On the contrary, Patsner et al. (1988) observed that serum LSA levels correlated well with CA 125 levels both at progression and remission of the disease.

The prognostic value of LSA: (i) preoperatively and (ii) after 2 cycles of chemotherapy was also assessed. Preoperative LSA level was higher in patients who died compared to those alive at the end of 18 months but the difference was statistically nonsignificant. Moreover, patients with preoperative level more than 20 mg/dl had poor progression-free and actual survival compared to those with LSA <20 mg/dl.

The combination of CA 125+LSA post chemotherapeutic levels predicted both progression-free and actual survival more accurately in 18/18 (100%) patients. This may have an important role in patients' management. Low levels of CA 125+LSA (<35 &/or <20) after 2 cycles of chemotherapy will identify a subgroup of advanced ovarian cancer
patients, who will benefit from further chemotherapy. Conversely, patients with elevated CA125+LSA levels ( >35 &/or > 20) after 2 cycles of chemotherapy may be considered for palliative treatment. Thus, monitoring epithelial ovarian cancer patients with combination of these two markers will be of help in the surveillance and management of the patients.

We also have tried to correlate the cystic/ascitic fluid and cytosolic CA 125 levels with the patient's prognosis.

CA 125 level in cystic/ascitic fluids was higher than the circulating level. Our results corroborate with those of Hunter et al. (1990). In one patient who came with recurrent disease, we observed serum CA 125 < 35 U/ml and cytosolic CA 125 3.29 U/mg cytosol protein, while cystic fluid level was significantly high (2085.34 U/ml). The patient died within 16 months. Therefore, we think that cystic/ascitic fluid CA 125 may be used as a prognosticator. High concentrations of CA 125 in cystic/ascitic fluids compared to serum reflected accumulation of the antigen in the cystic/ascitic fluid and the presence of barriers between the antigen-producing neoplastic epithelial lining of primary ovarian tumors and the serum. Such barriers may play a role in the distribution
of tumor antigens over various body compartments. Also, it may be possible that CA 125 detected in the fluids would differ in molecular form from CA 125 present in the serum and that this may alter the antigenicity of CA 125 in each compartment (Fleuren et al, 1987). Moreover, the lack of correlation between fluid and serum CA 125 levels in the current study, may also relate to collection of cystic/ascitic fluid and serum at different points of time.

The prognostic value of CA 125 in the tissue (cytosolic) is not yet defined, although, it is present in the tumor tissue of over 80% of ovarian carcinomas studied immunohistochemically at first laparotomy (Kabawat et al, 1983b; Koelma et al, 1987 and Maughan et al, 1988). In the present study, measurable cytosolic CA 125 was present in all primary epithelial ovarian tumors including the 3/46 patients having normal serum CA 125 levels. Cytosolic CA 125 did not correlate significantly with serum CA 125 values. This probably suggests that CA 125, though present in the tumor was not always released in measurable amounts. Serum CA 125 may, thus, be dependent on tumor volume, rate of release into the blood and infiltration through basement membranes (Wahren et al, 1978).

Cytosolic CA 125 showed no correlation with stage of the
disease, although an increasing trend was observed as stage advanced. It was detected in all histologic types studied, including mucinous tumors. However, in mucinous tumors the levels were significantly low compared to serous and endometrioid tumors. Regarding tumor differentiation, cytosolic CA 125 showed good correlation with the histologic grade of the tumor. Maughan et al. (1988) using immunohistochemical method, detected CA 125 in all histologic types and poorly differentiated tumors.

Receptors transmit signals that can induce protein synthesis. In this regard, Veith et al. (1983) described the synthesis of a glycoprotein that was induced by estradiol in human mammary cell lines containing cER and cPR. We correlated cytosolic CA 125 with ER/PR content of the tumor and EGFRs. Cytosolic CA 125 showed high values in receptor positive than receptor negative patients and the difference was statistically significant for PR only. Similar relationship between receptor status and cytosolic content of CEA and TPA in breast cancer patients was noted by Gion et al. (1986). Cytosolic CA 125 values were similar in tumors having high ( > 100 fmol/mg protein) and low ( < 100 fmol/mg protein ) EGFRs.
Moreover, cytosolic CA 125 was unable to predict progression-free and actual survival in epithelial ovarian cancer patients.

From our present work, we think that hormonal imbalance played a vital role in the pathogenesis of epithelial ovarian cancer. Moreover, from our results we hypothesize that these hormonal abnormalities may be severe and/ or irreparable in advanced epithelial ovarian cancer than in early stages. Therefore, we would like to study, the hormone profile in the early stages of disease as well as in benign and borderline tumors and compare them with advanced cases.

CA 125 is an useful marker for monitoring epithelial ovarian cancer patients. We also have observed PRL to be an useful marker in the management of these patients. Thus, a combination of CA 125 + PRL could be used for monitoring these patients, so that we can treat them before relapse and finally the patients will have longer relapse free survival.

ABSTRACT

In ovarian cancers, the most promising tumor markers are (1) CA-125 (Bast et al, 1983), a high molecular weight cell
surface glycoprotein and (2) lipid-bound sialic acid (LSA), a nonspecific sialoglycoprotein. Part A evaluates the usefulness of CA-125 and LSA with clinically important prognosticators and in monitoring of the patients. In Part B, CA-125 in cystic/ascitic fluid and cytosolic CA-125 are correlated with their respective circulating levels and to see their usefulness as prognostic indicators.

Part A: The incidence of CA-125 and LSA was 94.4% and 34.4% respectively. Both the markers showed an increasing trend as stage advanced, while no such correlation was observed when compared with the various histologic types and grades of the tumor. However, mucinous tumors had low CA-125 levels compared to serous tumors. Postoperative CA-125 and LSA levels were correlated with the presence of residual disease. When the markers were correlated with disease outcome, it was observed that at recurrence, both of them were elevated compared to the preceding values. But in responders, CA-125 alone predicted a successful response to treatment. Also the effect of chemotherapy on the markers was observed. Post chemotherapeutic (after 2 cycles of chemotherapy) levels of CA-125 + LSA predicted both progression-free and actual survival in these patients.
Part B: CA-125 concentrations in cystic/ascitic fluid were higher than circulating levels, while cytosolic CA-125 levels were low compared to serum levels. An increased trend of cytosolic CA-125 was seen with stage and histologic grade of the tumor. Mucinous tumors had low levels when compared with serous and endometrioid tumors. Moreover, significantly high titres of cytosolic CA-125 were seen in PR tumors than PR tumors.
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Veith F, Zajdela A, Rochefort H.
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Evaluation of serum CA 125 levels in the monitoring of ovarian cancer.

Carcinoembryonic antigen and other tumor markers in tissue and serum or plasma of patients with primary mammary carcinoma.
TABLES
Table 1: Incidence of serum CA 125 and LSA in epithelial ovarian cancer patients (M ± SE)

<table>
<thead>
<tr>
<th></th>
<th>CA 125</th>
<th>LSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 125 U/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSA mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>0.006.40 ± 0.00.91</td>
</tr>
<tr>
<td>Patients</td>
<td>72</td>
<td>752.75 ± 68.54</td>
</tr>
<tr>
<td>% elevation</td>
<td>094.4</td>
<td>34.7</td>
</tr>
</tbody>
</table>

* P < 0.0001
& P < 0.05
r = + 0.014

% elevation = Levels above upper normal limit.
Table 2a: Relation of markers to disease stage (M ± SE)

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>03</td>
<td>186.29 ± 119.45</td>
<td>11.26 ± 03.09</td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>788.99 ± 091.69</td>
<td>18.39 ± 02.09</td>
</tr>
<tr>
<td>IV</td>
<td>22</td>
<td>868.54 ± 128.75</td>
<td>21.57 ± 02.70</td>
</tr>
<tr>
<td>Rec.</td>
<td>12</td>
<td>576.41 ± 171.37</td>
<td>19.71 ± 02.54</td>
</tr>
<tr>
<td>III + IV</td>
<td>57</td>
<td>819.69 ± 75.27</td>
<td>19.62 ± 01.66</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>006.40 ± 00.91</td>
<td>15.78 ± 01.01</td>
</tr>
</tbody>
</table>

* P < 0.001
@ P < 0.02
$, P < 0.05
! P < 0.01
Table 2b: Relation of markers to disease stage in patients with serous tumors (M ± SE)

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>02</td>
<td>840.82 ± 618.96</td>
<td>13.26 ± 3.93</td>
</tr>
<tr>
<td>III</td>
<td>28</td>
<td>864.80 ± 996.69</td>
<td>18.30 ± 2.25</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td>894.43 ± 118.87</td>
<td>21.92 ± 3.41</td>
</tr>
<tr>
<td>Rec.</td>
<td>09</td>
<td>643.93 ± 213.74</td>
<td>18.68 ± 2.90</td>
</tr>
<tr>
<td>III + IV</td>
<td>44</td>
<td>875.57 ± 875.22</td>
<td>19.62 ± 1.91</td>
</tr>
</tbody>
</table>

*, &, # $ P < 0.0001

# P < 0.05
Table 3: Markers and histologic types (M ± SE)

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>N</th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>55</td>
<td>807.31 ± 73.35</td>
<td>19.23 ± 1.61</td>
</tr>
<tr>
<td>Mucinous</td>
<td>5</td>
<td>185.87 ± 75.76</td>
<td>21.81 ± 6.45</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>4</td>
<td>714.32 ± 323.02</td>
<td>15.93 ± 5.67</td>
</tr>
<tr>
<td>Undiff. Ca</td>
<td>2</td>
<td>504.00 ± 254.55</td>
<td>25.34 ± 7.13</td>
</tr>
<tr>
<td>Mixed</td>
<td>2</td>
<td>216.00 ± 24.04</td>
<td>22.71 ± 6.46</td>
</tr>
<tr>
<td>Mucin-secreting adenoCa.</td>
<td>4</td>
<td>1142.40 ± 427.24</td>
<td>15.46 ± 3.36</td>
</tr>
</tbody>
</table>

* P < 0.001
& P < 0.0001
Table 4a: Markers according to histologic grade (M ± SE)

<table>
<thead>
<tr>
<th>Histologic grade</th>
<th>N</th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>06</td>
<td>429.15 ± 200.21</td>
<td>23.27 ± 5.54</td>
</tr>
<tr>
<td>II</td>
<td>34</td>
<td>778.21 ± 102.27</td>
<td>18.80 ± 2.07</td>
</tr>
<tr>
<td>III</td>
<td>24</td>
<td>775.64 ± 110.83</td>
<td>19.01 ± 2.32</td>
</tr>
<tr>
<td>II + III</td>
<td>58</td>
<td>777.14 ± 075.48</td>
<td>18.88 ± 1.54</td>
</tr>
<tr>
<td>Unknown</td>
<td>08</td>
<td>818.63 ± 218.28</td>
<td>19.18 ± 3.91</td>
</tr>
</tbody>
</table>
Table 4b: Markers according to histologic grade in patients with serous tumors (M ± SE)

<table>
<thead>
<tr>
<th>Histologic grade</th>
<th>N</th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>01</td>
<td>1475.00</td>
<td>29.76</td>
</tr>
<tr>
<td>II</td>
<td>31</td>
<td>0711.79 ± 096.06</td>
<td>18.85 ± 2.26</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>0899.50 ± 125.37</td>
<td>20.07 ± 2.80</td>
</tr>
<tr>
<td>Unknown</td>
<td>07</td>
<td>0924.23 ± 222.46</td>
<td>17.49 ± 4.09</td>
</tr>
</tbody>
</table>
Table 5: Comparison of pre- and post-operative marker levels (Mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>CA 125</th>
<th>LSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>U/ml</td>
</tr>
<tr>
<td>Preoperative</td>
<td>28</td>
<td>704.46 ± 99.04</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual disease absent</td>
<td>02</td>
<td>198.23 ± 93.72</td>
</tr>
<tr>
<td>Residual disease &lt; 2 cm</td>
<td>04</td>
<td>151.34 ± 46.68</td>
</tr>
<tr>
<td>Residual disease &gt; 2 cm</td>
<td>22</td>
<td>632.23 ± 98.46</td>
</tr>
</tbody>
</table>

* P < 0.01

†, ‡ P < 0.001
Table 6: Sensitivity, specificity and predictive value of markers in follow-up patients

<table>
<thead>
<tr>
<th></th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100 %</td>
<td>66.6 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>100 %</td>
<td>27.7 %</td>
</tr>
<tr>
<td>Predictive value of positive test</td>
<td>100 %</td>
<td>50.0 %</td>
</tr>
<tr>
<td>Predictive value of negative test</td>
<td>100 %</td>
<td>42.8 %</td>
</tr>
<tr>
<td>Diagnostic efficiency</td>
<td>100 %</td>
<td>47.8 %</td>
</tr>
</tbody>
</table>
Table 7: Effect of chemotherapy on marker levels in patients with progressive disease and in responders (M ± SE)

<table>
<thead>
<tr>
<th></th>
<th>CA 125</th>
<th>LSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>U/ml</td>
</tr>
<tr>
<td>Patients with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>progressive disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>632.57 ± 145.04</td>
<td>16.91 ± 3.98</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>161.32 ± 88.66</td>
<td>21.23 ± 3.85</td>
</tr>
<tr>
<td>After chemotherapy</td>
<td>339.78 ± 119.63</td>
<td>19.94 ± 6.16</td>
</tr>
<tr>
<td>Responders</td>
<td>08</td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td>580.66 ± 105.36</td>
<td>16.64 ± 3.11</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>034.46 ± 12.15</td>
<td>17.51 ± 2.70</td>
</tr>
<tr>
<td>After chemotherapy</td>
<td>017.37 ± 001.69</td>
<td>17.22 ± 4.21</td>
</tr>
</tbody>
</table>

* P < 0.02
&, $ P < 0.001
Table 8: Marker levels in patients alive and those who died (M ± SE)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>CA 125 U/ml</th>
<th>LSA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients alive</td>
<td>22</td>
<td>535.06 ± 77.92</td>
<td>17.08 ± 2.19</td>
</tr>
<tr>
<td>Patients who died</td>
<td>50</td>
<td>848.54 ± 89.25</td>
<td>20.25 ± 1.76</td>
</tr>
</tbody>
</table>

* P < 0.02
Table 9: Marker levels according to disease stage in patients alive and those who died (N ± SE)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Patients alive</th>
<th>Patients who died</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA 125 (U/ml)</td>
<td>CA 125 (U/ml)</td>
</tr>
<tr>
<td></td>
<td>LSA (ng/dl)</td>
<td>LSA (ng/dl)</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>II</td>
<td>03</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>186.29 ± 119.44</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>11.26 ± 3.09</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>647.78 ± 67.03</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>17.76 ± 2.82</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>573.86 ± 260.11</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>13.6/ ± 1.26</td>
<td>-</td>
</tr>
<tr>
<td>Rec.</td>
<td>02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>174.01 ± 863.08</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>24.18 ± 7.35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>288.81 ± 137.49</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22.36 ± 2.90</td>
<td>-</td>
</tr>
<tr>
<td>III + IV</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>639.09 ± 892.07</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>17.28 ± 2.51</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>896.45 ± 998.04</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20.61 ± 2.10</td>
<td>-</td>
</tr>
</tbody>
</table>

* P < 0.01
* P < 0.05
* P < 0.02
<table>
<thead>
<tr>
<th>Marker levels</th>
<th>N</th>
<th>Progression - free survival</th>
<th>Actual survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CA 125 (U/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35</td>
<td>03</td>
<td>10.66 ± 3.53</td>
<td>13.33 ± 3.03</td>
</tr>
<tr>
<td>&gt; 35 and &lt; 100</td>
<td>07</td>
<td>09.42 ± 2.96</td>
<td>12.64 ± 2.14</td>
</tr>
<tr>
<td>&gt; 100 and &lt; 1000</td>
<td>37</td>
<td>07.74 ± 1.21</td>
<td>11.28 ± 1.01</td>
</tr>
<tr>
<td>&gt; 1000</td>
<td>20</td>
<td>05.08 ± 1.10</td>
<td>09.11 ± 1.24</td>
</tr>
<tr>
<td><strong>LSA (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>47</td>
<td>08.13 ± 1.06</td>
<td>11.64 ± 0.86</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>25</td>
<td>05.83 ± 1.22</td>
<td>09.39 ± 1.23</td>
</tr>
</tbody>
</table>

* Mucinous tumors not considered.
<table>
<thead>
<tr>
<th>Marker levels</th>
<th>N</th>
<th>Progression - free survival</th>
<th>Actual survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CA 125 (U/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35</td>
<td>10</td>
<td>14.05 ± 1.34</td>
<td>16.05 ± 1.05</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>08</td>
<td>07.93 ± 2.19</td>
<td>12.34 ± 1.62</td>
</tr>
<tr>
<td><strong>LSA (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>10</td>
<td>12.88 ± 1.78</td>
<td>14.77 ± 1.38</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>08</td>
<td>10.12 ± 2.32</td>
<td>13.53 ± 1.60</td>
</tr>
<tr>
<td><strong>CA 125 + LSA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 35 &amp; or &lt; 20</td>
<td>07</td>
<td>15.14 ± 1.26</td>
<td>16.64 ± 0.85</td>
</tr>
<tr>
<td>&gt; 35 &amp; or &gt; 20</td>
<td>11</td>
<td>08.90 ± 1.84</td>
<td>12.97 ± 1.42</td>
</tr>
</tbody>
</table>

*, # P < 0.05
& P < 0.02
Table 12: Survival (in months) in total patients and patients who died according to disease stage (N ± SE)

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Actual survival in total patients</th>
<th>N</th>
<th>Actual survival in patients who died</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>03</td>
<td>18.68 ± 0.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>35</td>
<td>12.74 ± 0.93</td>
<td>20</td>
<td>8.80 ± 0.93</td>
</tr>
<tr>
<td>IV</td>
<td>22</td>
<td>87.21 ± 1.26</td>
<td>20</td>
<td>6.13 ± 1.13</td>
</tr>
<tr>
<td>Rec.</td>
<td>12</td>
<td>18.77 ± 1.34</td>
<td>10</td>
<td>8.77 ± 1.06</td>
</tr>
<tr>
<td>III+IV</td>
<td>57</td>
<td>18.68 ± 0.93</td>
<td>40</td>
<td>7.46 ± 0.76</td>
</tr>
</tbody>
</table>

* P < 0.01

†, ‡, §, ¶ P < 0.001
Table 13: Incidence of CA 125 in cystic / ascitic fluids (M ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Cystic / ascitic fluid CA 125 (U/ml)</th>
<th>Serum CA 125 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>24</td>
<td>812.59 ± 147.13</td>
</tr>
</tbody>
</table>

% elevation

- > 35  | 100 % | 95.8 % |
- > 200 | 079.1 % | 66.6 % |

r = + 0.123

Table 14: Relation of CA 125 in cystic / ascitic fluids to disease stage (M ± SE)

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>CA 125 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>01</td>
<td>1465.17</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>0674.31 ± 154.88</td>
</tr>
<tr>
<td>IV</td>
<td>08</td>
<td>0672.76 ± 278.36</td>
</tr>
<tr>
<td>Rec.</td>
<td>03</td>
<td>1521.06 ± 439.57</td>
</tr>
<tr>
<td>III + IV</td>
<td>20</td>
<td>0673.69 ± 145.02</td>
</tr>
</tbody>
</table>
Table 15: Cystic/ascitic fluid CA 125 and histologic types (M ± SE)

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>N</th>
<th>CA 125 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>17</td>
<td>819.61 ± 191.29</td>
</tr>
<tr>
<td>Mucinous</td>
<td>02</td>
<td>781.99 ± 483.07</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>01</td>
<td>752.93</td>
</tr>
<tr>
<td>Undiff. Ca</td>
<td>01</td>
<td>374.82</td>
</tr>
<tr>
<td>Mucin-secreting adenoCa.</td>
<td>03</td>
<td>959.01 ± 278.49</td>
</tr>
</tbody>
</table>

Table 16: Cystic/ascitic fluid CA 125 and histologic grade (M ± SE)

<table>
<thead>
<tr>
<th>Histologic grade</th>
<th>N</th>
<th>CA 125 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>02</td>
<td>781.99 ± 483.07</td>
</tr>
<tr>
<td>II</td>
<td>09</td>
<td>669.78 ± 220.61</td>
</tr>
<tr>
<td>III</td>
<td>11</td>
<td>751.17 ± 208.00</td>
</tr>
<tr>
<td>Unknown</td>
<td>02</td>
<td>1823.62 ± 148.12</td>
</tr>
</tbody>
</table>
Table 17: Incidence of cytosolic CA 125 (M ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Cytosolic CA 125</th>
<th>Serum CA 125</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (U/mg cytosol protein)</td>
<td>(U/ml)</td>
</tr>
<tr>
<td>Patients</td>
<td>46</td>
<td>299.62 ± 42.75</td>
</tr>
</tbody>
</table>

\[ r = + 0.158 \]

Table 18: Cytosolic CA 125 according to disease stage (M ± SE)

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Cytosolic CA 125 (U/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>02</td>
<td>168.24 ± 863.27</td>
</tr>
<tr>
<td>III</td>
<td>28</td>
<td>313.79 ± 858.16</td>
</tr>
<tr>
<td>IV</td>
<td>08</td>
<td>331.89 ± 883.02</td>
</tr>
<tr>
<td>Rec.</td>
<td>08</td>
<td>250.62 ± 803.87</td>
</tr>
<tr>
<td>III + IV</td>
<td>36</td>
<td>317.81 ± 848.87</td>
</tr>
</tbody>
</table>
Table 19: Cytosolic CA 125 and histologic types (M ± SE)

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>N</th>
<th>Cytosolic CA 125 (U/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>32</td>
<td>304.47 ± 041.06</td>
</tr>
<tr>
<td>Mucinous</td>
<td>04</td>
<td>071.98 ± 053.65</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>03</td>
<td>776.22 ± 266.81</td>
</tr>
<tr>
<td>Undiff. Ca.</td>
<td>02</td>
<td>043.11 ± 027.38</td>
</tr>
<tr>
<td>Mixed</td>
<td>02</td>
<td>144.46 ± 021.26</td>
</tr>
<tr>
<td>Mucin - secreting adenoCa</td>
<td>03</td>
<td>349.42 ± 203.52</td>
</tr>
</tbody>
</table>

*, $ P < 0.01
& P < 0.0001
# P < 0.05
Table 20: Cytosolic CA 125 according to histologic grade (M ± SE)

<table>
<thead>
<tr>
<th>Histologic grade</th>
<th>N</th>
<th>Cytosolic CA 125 (U/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>300.46 ± 43.58</td>
</tr>
<tr>
<td>II</td>
<td>21</td>
<td>293.63 ± 38.65</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>360.72 ± 84.34</td>
</tr>
<tr>
<td>II + III</td>
<td>41</td>
<td>326.36 ± 45.96</td>
</tr>
</tbody>
</table>

* , & , #

* , & P < 0.01

# P < 0.001
Table 21: Cytosolic CA 125 in ER / PR and ER / PR tumors (M + SE)

<table>
<thead>
<tr>
<th>Receptor status</th>
<th>N</th>
<th>Cytosolic CA 125 (U/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ ER</td>
<td>29</td>
<td>339.91 ± 54.57</td>
</tr>
<tr>
<td>- ER</td>
<td>17</td>
<td>230.90 ± 65.90</td>
</tr>
<tr>
<td>+ PR</td>
<td>33</td>
<td>341.17 ± 55.61</td>
</tr>
<tr>
<td>- PR</td>
<td>13</td>
<td>194.17 ± 42.00</td>
</tr>
</tbody>
</table>

* P < 0.05
Table 22: Cytosolic CA 125 versus EGFR+ tumors (M ± SE)

<table>
<thead>
<tr>
<th>EGFR+ (fmol/mg protein)</th>
<th>N</th>
<th>Cytosolic CA 125 (U/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 100</td>
<td>10</td>
<td>433.45 ± 121.71</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>8</td>
<td>415.86 ± 104.51</td>
</tr>
</tbody>
</table>

Table 23: Survival (in months) based on cytosolic CA 125 (M ± SE)

<table>
<thead>
<tr>
<th>Cytosolic CA 125 U/mg cytosol protein</th>
<th>N</th>
<th>Progression - free survival</th>
<th>Actual survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>21</td>
<td>8.73 ± 1.52</td>
<td>13.07 ± 1.00</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>25</td>
<td>8.54 ± 1.32</td>
<td>11.31 ± 1.32</td>
</tr>
</tbody>
</table>
LEGENDS TO FIGURES:
Fig. 1

Scatterogram showing preoperative values of serum CA 125 and LSA in epithelial ovarian cancer patients.
Stage III, grade III patient with papillary serous cystadenocarcinoma (PSCAC) was treated with surgery followed by chemotherapy (CT). She responded to chemotherapy but 8.5 months after completion of chemotherapy, she developed progressive disease.

Both serum CA 125 and LSA levels were high at the time of disease progression. Rising CA 125 titres at the end of 18 months correlated with disease progression.
Stage III
PSCAC
HG III
Res.dis.>2 cm

LSA

CA 125

MONTHS

CA 125 U/ml

Fig-2
Stage III, grade II patient was treated with surgery followed by chemotherapy. She responded to chemotherapy but after 7 courses developed progressive disease.

Serum CA 125 and LSA levels were high at the time of disease progression. LSA levels predicted disease progression with a lead time of 4-5 months.
Stage III
PSCAC
HG II
Res. dis. > 2 cm.

Fig. 3
Stage III, grade III patient with endometrioid tumor was treated with surgery followed by chemotherapy. One year later, she developed lung metastases with pleural effusion. Exploratory laparotomy was done and she had massive generalised carcinomatosis. She died within 15 days after operation.

Circulating CA 125 and LSA showed decreased levels postoperatively and after 2 courses of chemotherapy. Both the markers were high at the time of lung metastases. CA 125 levels predicted disease progression with a lead time of 8 months. LSA levels were within normal limit throughout the course of the disease.
LSA Stage III
Endometrioid
HG III
Res. dis. >2 cm.

Fig-4
Stage III, grade III patient was operated and further treated with chemotherapy. After 10 courses of chemotherapy, she complained of abdominal distension and finally died. After an initial postoperative decrease, serum CA 125 was persistently high throughout the course of the treatment. LSA was not useful as a marker in this patient.
Fig. 6

Stage III, grade III patient was treated with surgery followed by chemotherapy. After 10 months, she developed progressive disease and was operated again. She was further treated with radiotherapy (RT). She died one month after completion of RT.

The preoperative values of serum CA 125 and LSA were high. The levels decreased postoperatively and following 5 courses of chemotherapy. With disease progression, the levels of both the markers were high and predicted disease progression with a lead time of 4 months.
LSA stage III
CA 125
HG III
Res. dis. > 2 cm.

17 MONTHS
Stage III, grade II patient was treated with surgery followed by chemotherapy. She had < 2cm residual disease. She responded to chemotherapy and was free of recurrence till the end of 18 months.

The preoperative value of serum CA 125 was high. The level decreased postoperatively and following 12 courses of chemotherapy. At last follow-up the level was within normal limit. Serum LSA was not useful as a marker in this patient.
Fig. 8

Stage III, grade III patient was treated with surgery followed by chemotherapy. She responded to chemotherapy and was free of recurrence till the end of 18 months.

The preoperative value of serum CA 125 was high. Following laparotomy and 2 courses of chemotherapy, the levels decreased to within normal limit. With further 11 courses of chemotherapy, the levels remained within normal limit and at last follow-up, the value was 16 U/ml. Serum CA 125 showed excellent correlation with the disease course. LSA was not useful as a marker in this patient.
The patient came with recurrent disease (Rec. dis.) and had grade III tumor. She was operated and had no residual disease. She was further treated with chemotherapy. She responded to chemotherapy and was well till the end of 18 months.

The preoperative value of serum CA 125 was high. There was a slight increase in the postoperative level. Following chemotherapy, the levels decreased and at last follow-up, the level was within normal limit. LSA was not useful as a marker in this patient.
Stage III, grade III patient was treated with surgery followed by chemotherapy. She responded to it and was free of recurrence till the end of 18 months.

The preoperative value of serum CA 125 was high. There was a further increase, postoperatively. Following 2 courses of chemotherapy, the level reached to within normal limit. Following further 8 courses of chemotherapy, the levels remained within normal limit. After the 9th course of chemotherapy, an increase in serum CA 125 was noted but she was free of recurrence. This false positive peak observed was due to viral infection. At last follow-up the level was slightly above the normal limit. LSA was not useful as a marker in this patient.
Stage III
PSCAC
HG III
Res. dis. > 2 cm
CA 125 U/ml

LSA mg/dl

Viral Injection

Lap
Stage III, grade III patient was treated with surgery followed by chemotherapy. She responded to it. After 16 months, second - look laparotomy was done and was given further chemotherapy. She was free of recurrence till the end of 18 months.

The preoperative level of serum CA 125 was high. Following laparotomy and 2 courses of chemotherapy, the levels decreased and reached to within normal limit. After further 8 courses, chemotherapy was stopped and the levels remained within normal limit. After 16 months, exploratory laparotomy was done and she was given further chemotherapy. At last follow-up, the level within normal limit. LSA was not useful as a marker in this patient.
Stage III
PSCAC
HG III
Res, dis. > 2 cm

MONTHS
Fig. 12

Preoperative CA 125 levels versus progression - free survival.
Preoperative CA 125 levels versus progression-free survival.

- **< 35 U/ml.**
- **> 35 < 100 U/ml.**
- **> 100 < 1000 U/ml.**
- **> 1000 U/ml.**

**Survival %**

**Months**

*Fig-12*
Fig. 13

Preoperative CA 125 levels versus actual survival.
Preoperative CA 125 levels versus actual survival.

Fig-13

18 Months

- < 35 U/ml.
- ≥ 35 < 100 U/ml.
- > 100 < 1000 U/ml.
- ≥ 1000 U/ml.
Fig. 14

Preoperative LSA levels versus progression - free survival.
Preoperative LSA levels versus progression-free survival.

<20 mg/dl.

>20 mg/dl.

Fig-14
Fig. 15

Preoperative LSA levels versus actual survival.
Preoperative LSA levels versus actual survival

- < 20 mg/dl.
- > 20 mg/dl.

Survival %

0  3  6  9  12  15  18 Months

Fig-15
Fig. 16

Effect of 2 chemotherapy cycles on marker levels versus progression-free survival.
Effect of 2 chemotherapy cycles on markers versus progression-free survival.

- **CA 125 > 35 U/ml.**
- **CA 125 < 35 U/ml.**
- **LSA > 20 mg/dl.**
- **LSA < 20 mg/dl.**

Survival %

0 3 6 9 12 15 18

Months

Fig-16
Fig. 17

Effect of 2 chemotherapy cycles on marker levels versus actual survival.
Effect of 2 chemotherapy cycles on markers versus actual survival.

- CA 125 > 35 U/ml.
- CA 125 < 35 U/ml.
- LSA > 20 mg/dl.
- LSA < 20 mg/dl.

Survival %

0 3 6 9 12 15 18 Months

Fig-17
Fig. 18

Effect of 2 chemotherapy cycles on CA 125 + LSA versus progression-free survival.
Effect of 2 chemotherapy cycles on CA 125 + LSA versus progression-free survival.

- < 35 & < 20
- > 35 & > 20

Survival %

0 3 6 9 12 15 18 Months

Fig-18
Fig. 19

Effect of 2 chemotherapy cycles on CA 125 + LSA versus actual survival.
Effect of 2 chemotherapy cycles on CA 125 + LSA versus actual survival.

- <35 & <20
- >35 & >20

Survival %

0  50  100

0  3  6  9  12  15  18  Months

Fig-19
Fig. 20

Scatterogram showing the distribution of serum and cystic/ascitic fluid CA 125
Cystic/ascitic fluid CA 125 U/ml

Serum CA 125 U/ml

Fig-20
Fig. 21

Scatterogram showing the distribution of serum and cytosolic CA 125.
Serum CA 125 U/ml

1700 *

1300

900

500

Cytosolic CA 125 U/mg protein

Fig-21
TUMOR MARKERS IN THE MANAGEMENT OF EPITHELIAL OVARIAN CARCINOMAS.

BY

NEELAM G. SHAH

Synopsis Submitted to THE GUJARAT UNIVERSITY for the degree Doctor of Philosophy in Life Sciences

ENDOCRINOLOGY DIVISION DEPARTMENT OF CANCER BIOLOGY THE GUJARAT CANCER AND RESEARCH INSTITUTE ASARWA. AHMEDABAD 380 016 INDIA
The incidence rate of ovarian cancer at The Gujarat Cancer & Research Institute, Ahmedabad, India in 1987 was 3.9% which was similar to those at Bangalore, Madras, Bhopal and Delhi. The age incidence of epithelial ovarian cancer rises from the second decade until the sixth or seventh decade, when it reaches plateau, indicating that although the ovary is too old to function, it never gets old to form a cancer. Ovarian cancer, which is increasing in incidence in highly industrialized countries, presents the most frustrating problem in gynecology. Ovarian tumors are difficult to diagnose and at initial diagnosis, about 60% - 70% have already reached stage III or IV. Now, much effort has been put into attempts to correlate differences in incidence rates with environmental, endocrinologic and genetic factors. Epidemiologic studies, also suggest ovarian cancer to be an endocrine-related tumor. Moreover, the part played by gonadotrophins and a third pituitary hormone - prolactin - in ovarian tumorigenesis is currently a matter of considerable interest, largely because of the possibility that hypergonadotrophism may be an etiological factor in ovarian neoplasia (Beamer, 1980).
Only a few studies of hormone concentrations and their receptors in epithelial ovarian cancer have been carried out and their results are often controversial. The hormonal abnormalities may precede and presumably favour the onset of epithelial ovarian cancer or they may be related with the evolution of the disease. An attempt has been made here to associate the important determinants in epithelial ovarian cancer biology, correlating steroid hormone- and growth factor- receptors, steroid and peptide hormones and markers in the epithelial ovarian cancer patients. This work is the first in Gujarat and also the first of its kind in India, where several important prognostic factors have been combined in an attempt to study the endocrine manoeuvres involved in epithelial ovarian cancer.

STUDY DESIGN:

A total of 72 patients with histologically confirmed epithelial ovarian cancer and 15 controls (frank menopausal) were enrolled from June 1984 to June 1989. The details of age, risk factors, FIGO (International Federation of Gynecology and Obstetrics) stage and appearance of clinical recurrence were noted from the disease progress charts of the patients maintained at The Gujarat Cancer & Research Institute, Ahmedabad, India.