

## Abstract

**Purpose:** We have identified, through mRNA expression profiling, three novel serum cancer biomarkers, DD-C101, DD-P108, and DD-O110. We measured the levels of these three biomarkers in over 1200 serum samples in three independent studies, to evaluate their sensitivity and specificity for ovarian cancer detection and their efficacy in disease prognosis.

**Methods:** Dual monoclonal antibody sandwich ELISA's for DD-C101, DD-P108, and DD-O110 have been developed. The assays were used to test serum samples from age-matched healthy women and from patients with benign gynecological diseases or ovarian cancer in two independent studies. The sensitivity and specificity of each of the novel biomarkers and of CA125, and combinations of these biomarkers, were analyzed by Receiver Operating Characteristic (ROC) curves in univariate and multivariate models. In the third study, 78 ovarian cancer patients were also analyzed for the three biomarkers, and their levels were compared against CA125 values and the outcome of disease.

**Results:** Levels of DD-C101, DD-P108, and DD-O110 were elevated in ovarian cancer patients, when compared to healthy controls or women with benign gynecological diseases. In the first study, DD-C101, DD-P108, and DD-O110 had Areas Under the Curve (AUC's) of 0.90, 0.72 and 0.76, respectively, in the ROC analysis. DD-C101, which had the highest AUC, showed a sensitivity of 44%, when holding specificity at 98%. In the second study, DD-C101, DD-P108, and DD-O110 yielded comparable AUC's to the first study. In stage I and II ovarian cancer (28 patients), DD-C101, DD-P108, and DD-O110 showed AUC's of 0.76, 0.66 and 0.83, respectively, indicating that the biomarkers may have utility for early detection of ovarian cancer. The multivariate analysis of our biomarkers and CA125 in all stages, and in stage I + II patients, resulted in AUC's which were significantly higher than the AUC for each biomarker alone. In the third study, correlation of the marker levels with 2-yr survival data shows that the elevation of DD-P108 and DD-O110 levels correlate with patient survival, which can be useful in prognosis and in selecting appropriate therapy for the patients.

**Conclusions:** These findings suggest that DD-C101, DD-P108, and DD-O110 are promising serum biomarkers for ovarian cancer that could improve the sensitivity of traditional cancer markers and may be applicable for prognosis of ovarian cancer. Additional studies are needed to confirm and expand the current findings.

## Methods & Materials

**ELISA**  
**DD-C101** - recombinant protein was used to develop mouse monoclonal antibodies (mAbs). A sequential sandwich ELISA was developed using two DD-C101-specific mAbs. High binding polystyrene plates (Corning Life Sciences) were coated with the capture mAb. Twenty  $\mu$ L of serum samples were used in the assay. Calibration was accomplished by using recombinant DD-C101 standards at concentrations of 10, 5, 1, 0.5, 0.05, and 0 ng/mL. Antigen was detected by a biotinylated detection mAb, followed by streptavidin-alkaline phosphatase, and pNPP substrate.

**DD-P108** - recombinant protein was used to develop mouse monoclonal antibodies (mAbs). A sequential sandwich ELISA was developed using two DD-P108-specific mAbs. High binding polystyrene plates (Corning Life Sciences) were coated with the capture mAb. Twenty  $\mu$ L of serum samples were used in the assay. Calibration was accomplished by using recombinant DD-P108 standards at concentrations of 200, 100, 50, 25, 10 and 0 ng/mL. Antigen was detected by a biotinylated detection mAb, followed by streptavidin-alkaline phosphatase, and pNPP substrate.

**DD-O110** - recombinant protein was used to develop mouse monoclonal antibodies (mAbs). A sequential sandwich ELISA was developed using two DD-O110-specific mAbs. High binding polystyrene plates (Corning Life Sciences) were coated with the capture mAb. Fifty  $\mu$ L of serum samples were used in the assay. Calibration was accomplished by using recombinant DD-O110 standards at concentrations of 25, 10, 2.5, 0.5, 0.2, and 0 ng/mL. Antigen was detected by a biotinylated detection mAb, followed by streptavidin-HRP, and DAKO TMB Plus substrate (DAKO, Carpinteria, CA).

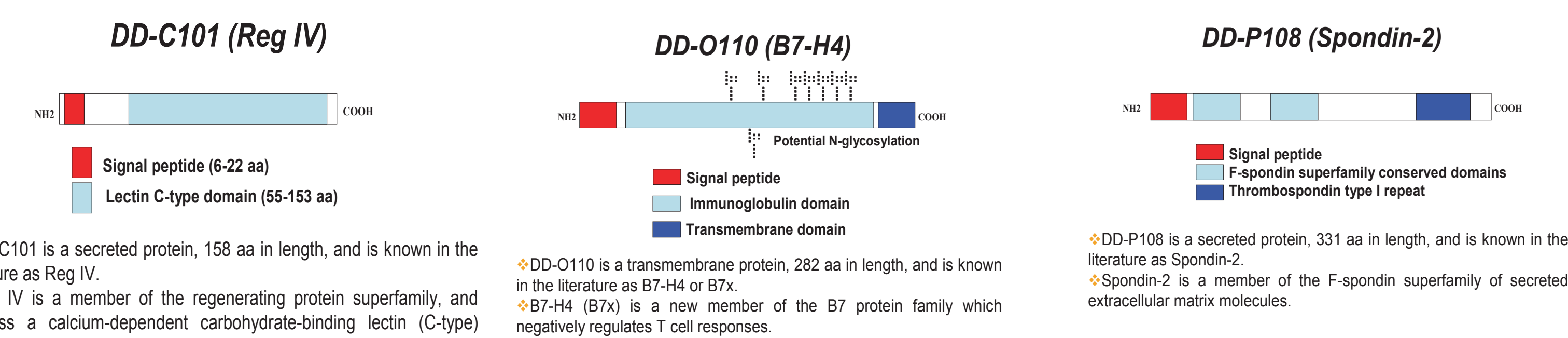
**Sample Selection for Clinical Studies**  
 Serum samples were obtained from commercial and academic sources. All samples were aliquoted upon arrival and stored at -80°C until use. In detection study 1, 236 samples were from patients with ovarian cancer with an average age of 59 years (15-85 years), 150 from patients with benign gynecological diseases (endometriosis, ovarian cysts, edema) with an average age of 32 years (20-52 years), and 260 from healthy women with an average age of 55 years (19-81 years). Serum samples in detection study 2 came from healthy women (average age 53 years), and women with enlarged ovaries (n=50), ovarian cysts (n=50) and endometriosis (n=100) with an average age of 35 years. The average age of women with ovarian cancer was 59 years. The stage distribution of the ovarian cancers was: stage I (n=13), stage II (n=15), stage III (n=26), stage IV (n=2), one cancer with unknown stage. Detection study 2 ovarian cancer samples were well-characterized, with more accurate clinical information. Thus, the stage correlation analysis was performed only with the detection study 2 samples. For the prognosis study, serum samples from 78 patients with ovarian carcinoma and 2-yr survival data were collected prior to each chemotherapy cycle, and were assessed for the levels of the tumor markers. The age median was 57 years (22-90 years). Thirty-eight patients died within 2 years of follow-up, while 40 patients survived.

**Statistical Analysis**  
 Statistical analyses were performed with JMP (SAS, Cary, NC) or MedCalc (MedCalc Software, Belgium). The scattergram distribution plots were generated with GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

## Summary and Conclusions

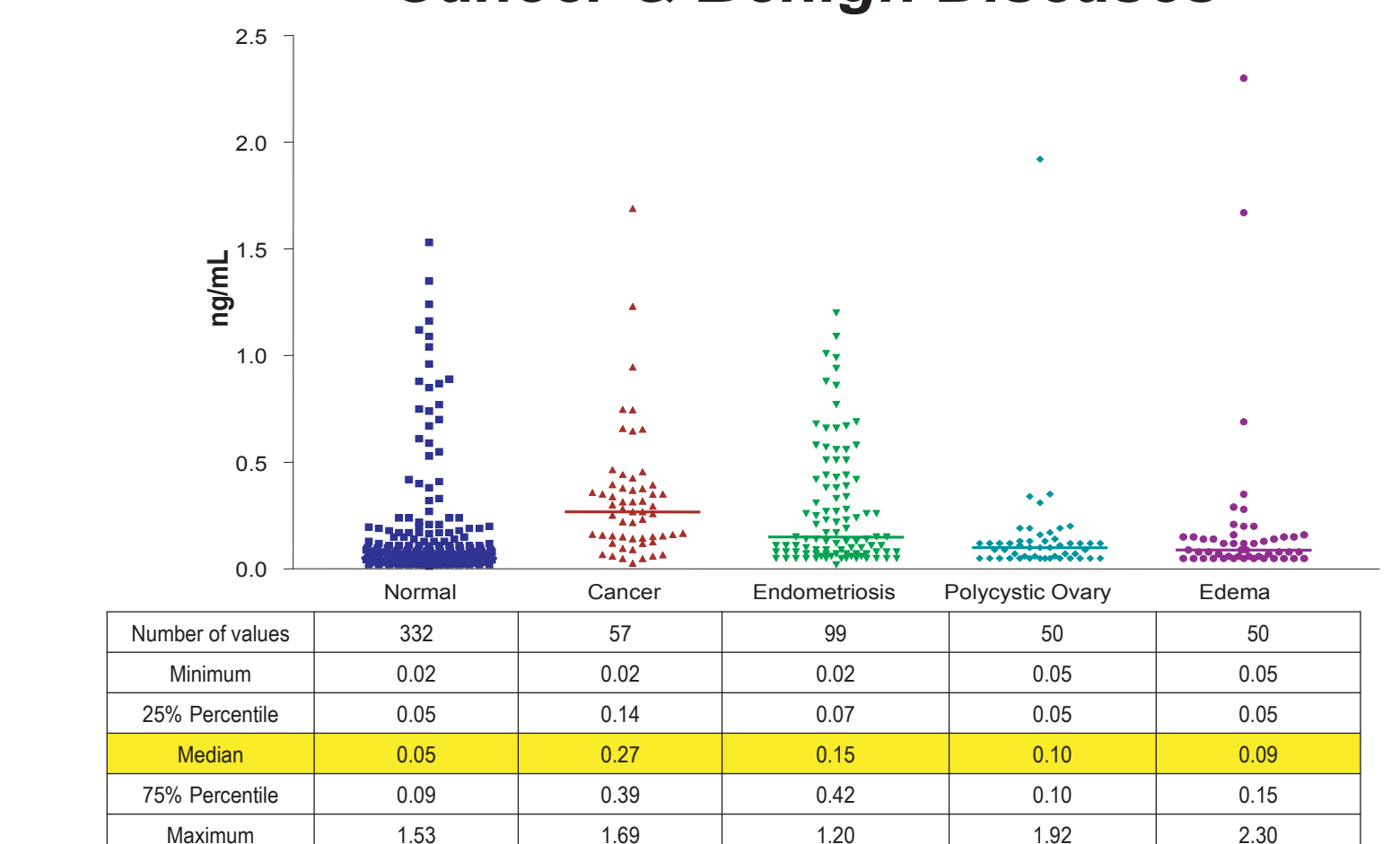
- Novel serum markers DD-C101, DD-O110, and DD-P108 showed elevation in ovarian cancer serum samples compared to normal controls and benign ovarian diseases.
- ROC analyses of the markers showed that DD-C101 and DD-O110 had sensitivity and specificity comparable to CA125, and may complement CA125 in early stage ovarian cancer detection.
- Correlation of the marker levels with 2-yr survival data shows that the elevation of DD-P108 and DD-O110 levels correlates with patient survival, which can be useful in prognosis and in selecting appropriate therapy for the patients.
- Additional studies are being planned to expand and confirm the current findings.

## Background

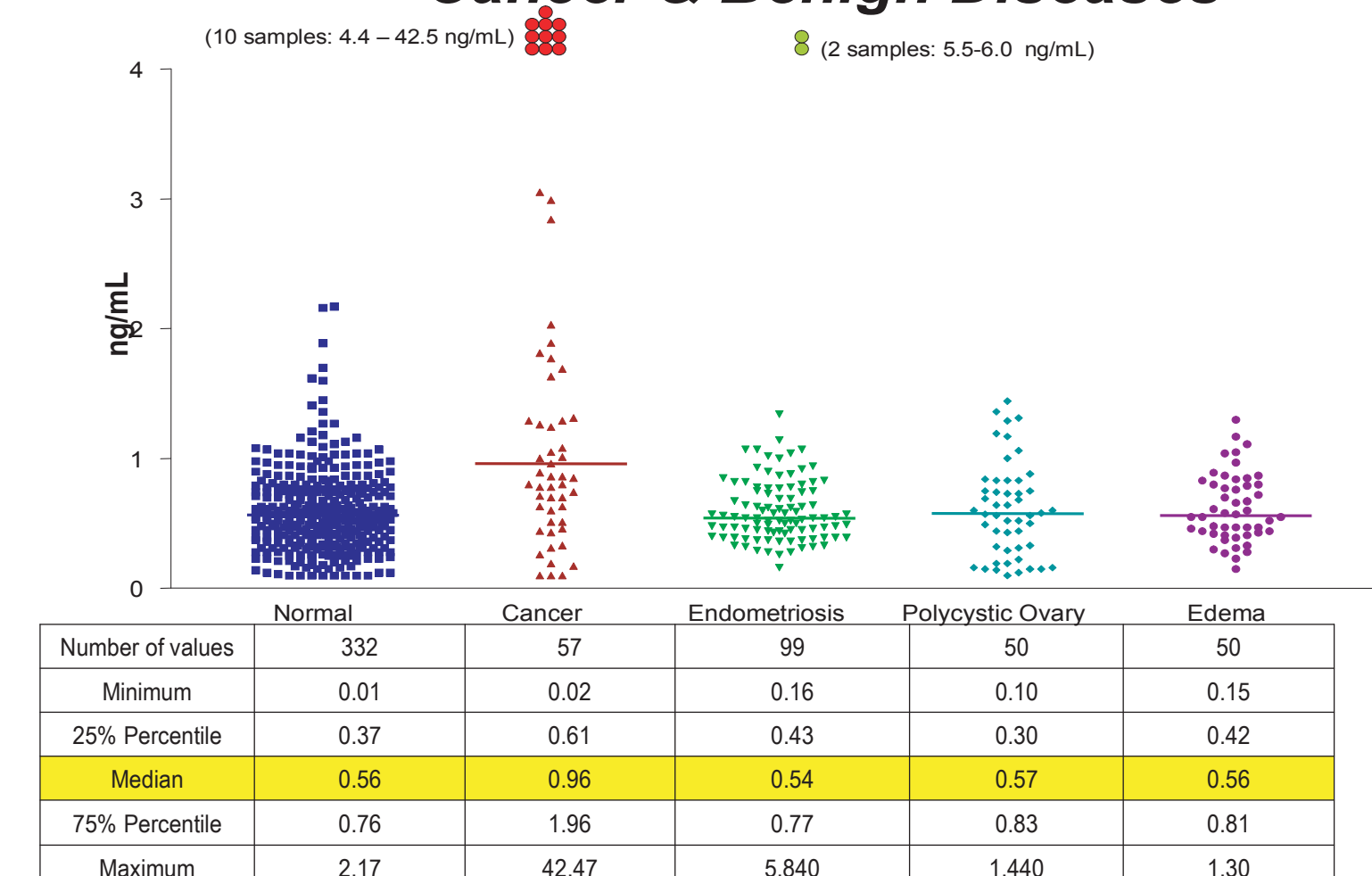


## Detection Study 2

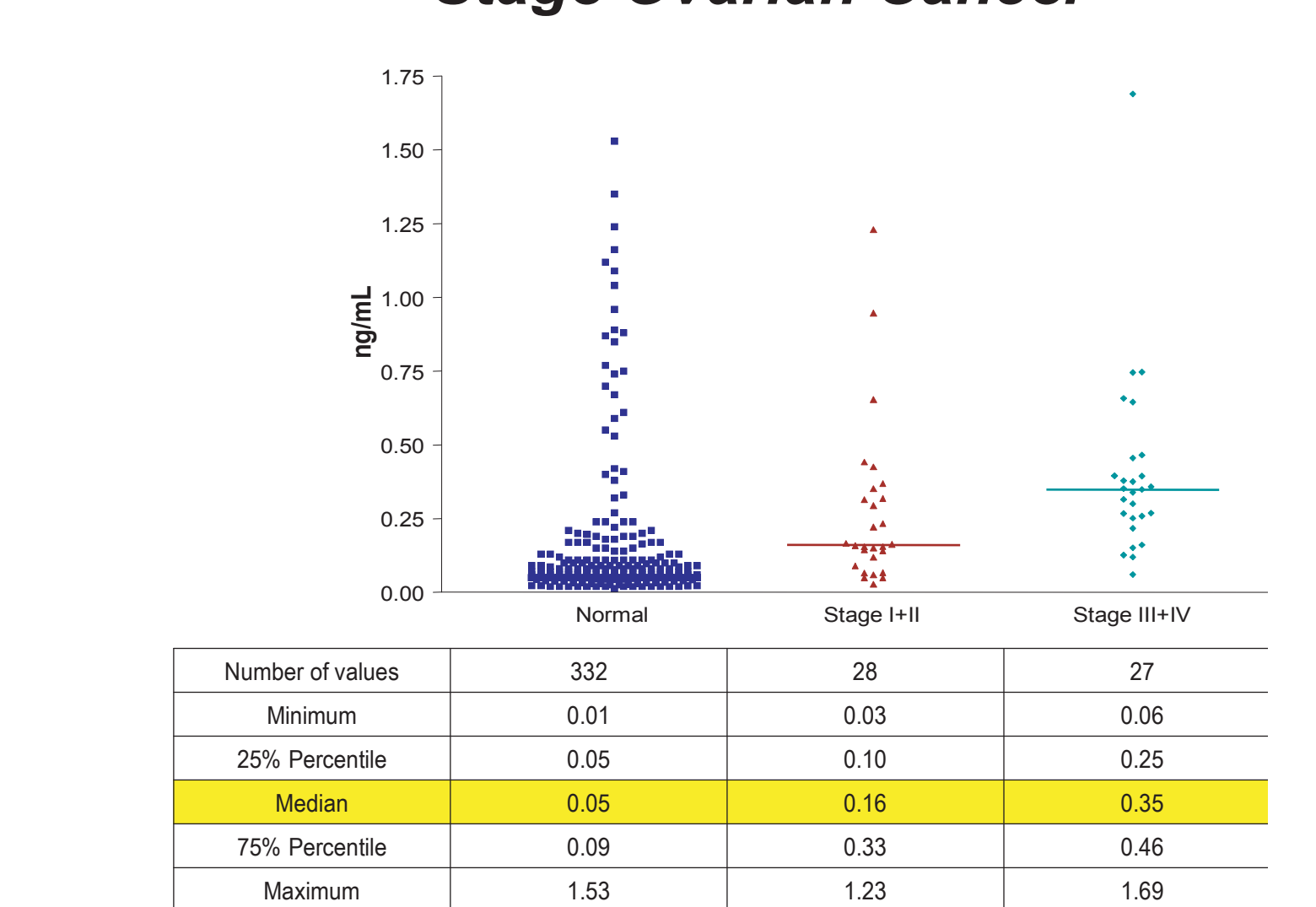
Serum Levels of DD-C101 in Ovarian Cancer & Benign Diseases



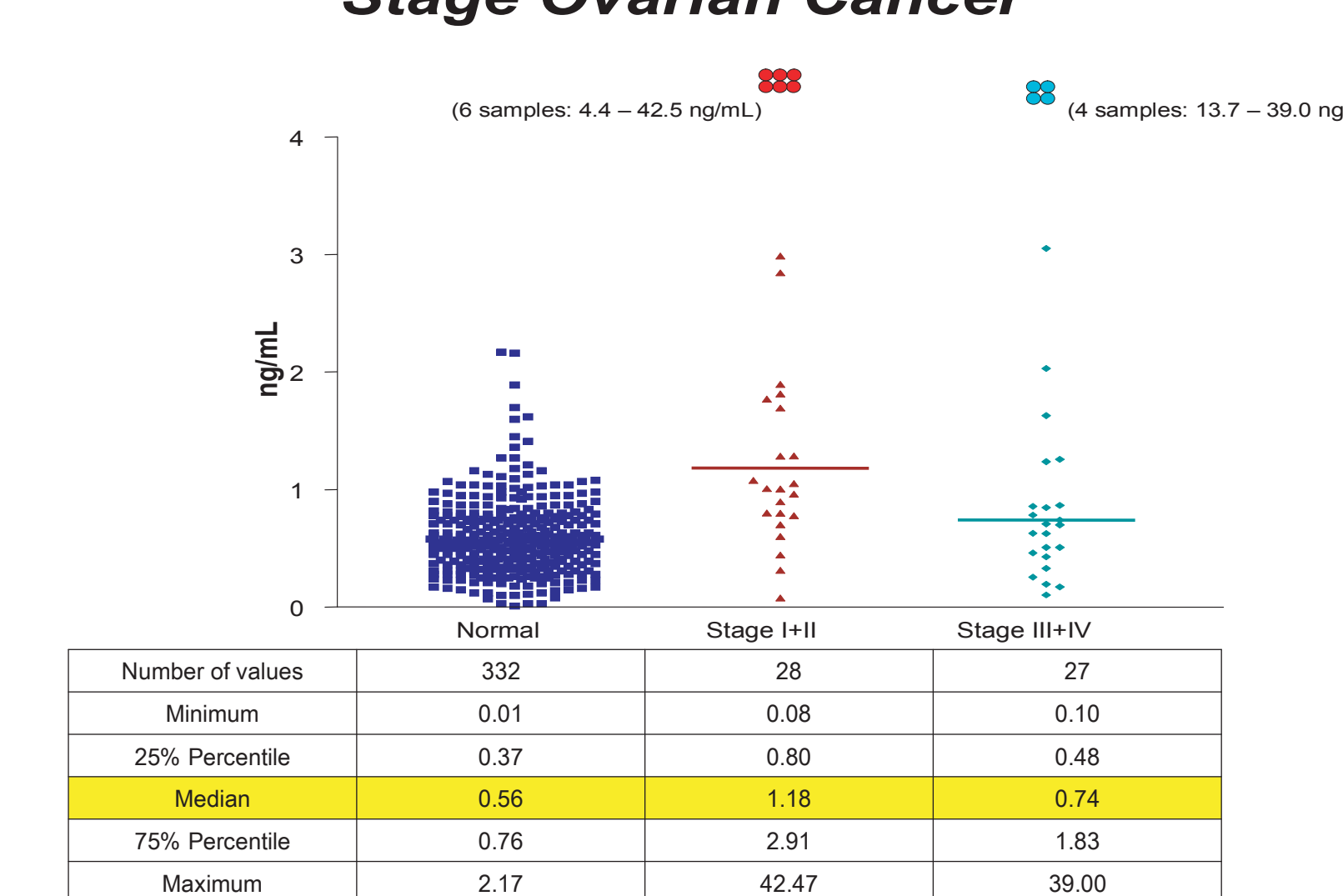
Serum Levels of DD-O110 in Ovarian Cancer & Benign Diseases



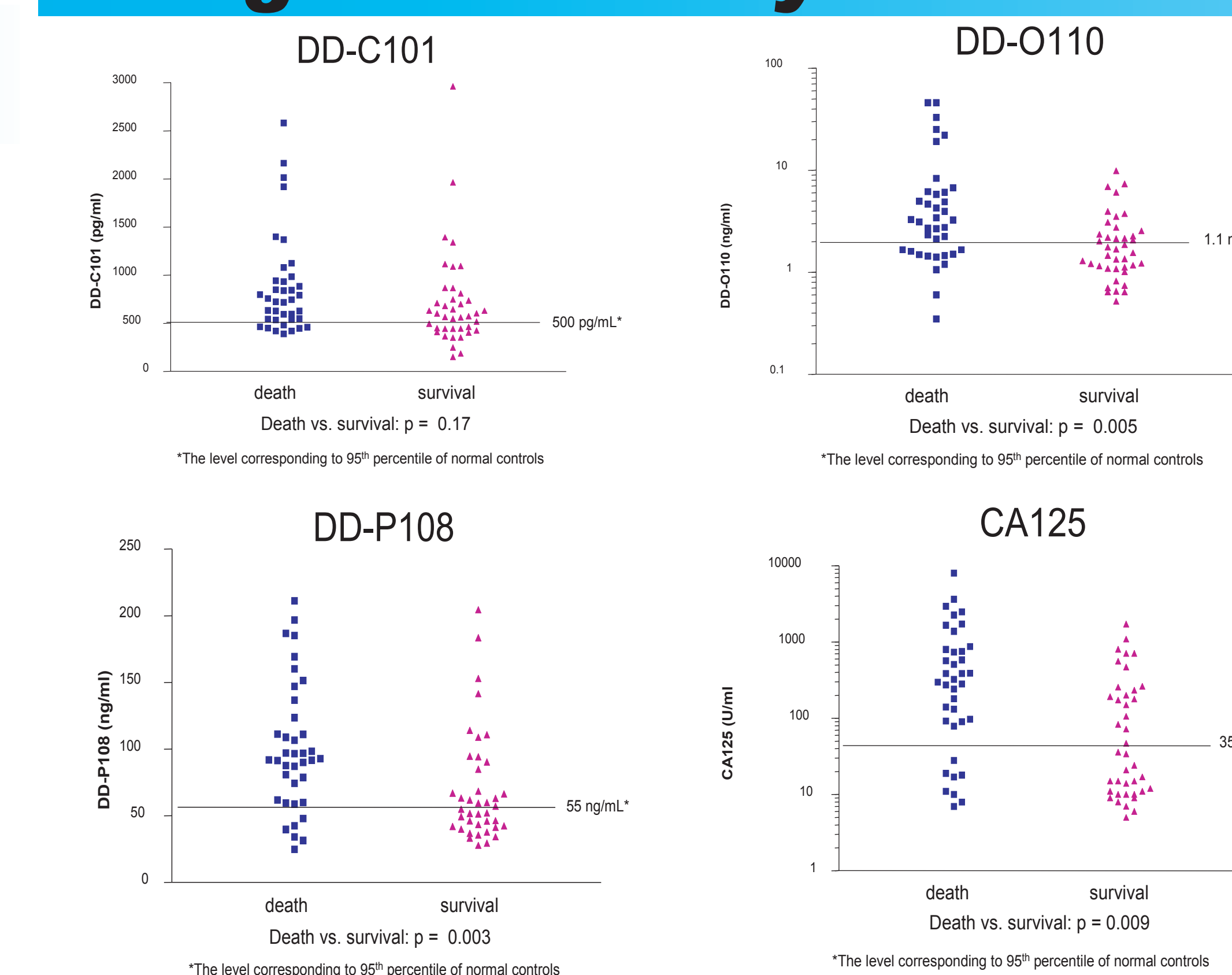
Serum Levels of DD-C101 in Early vs. Stage Ovarian Cancer



Serum Levels of DD-O110 in Early vs. Late Stage Ovarian Cancer



## Prognosis Study



### Quartile Distribution

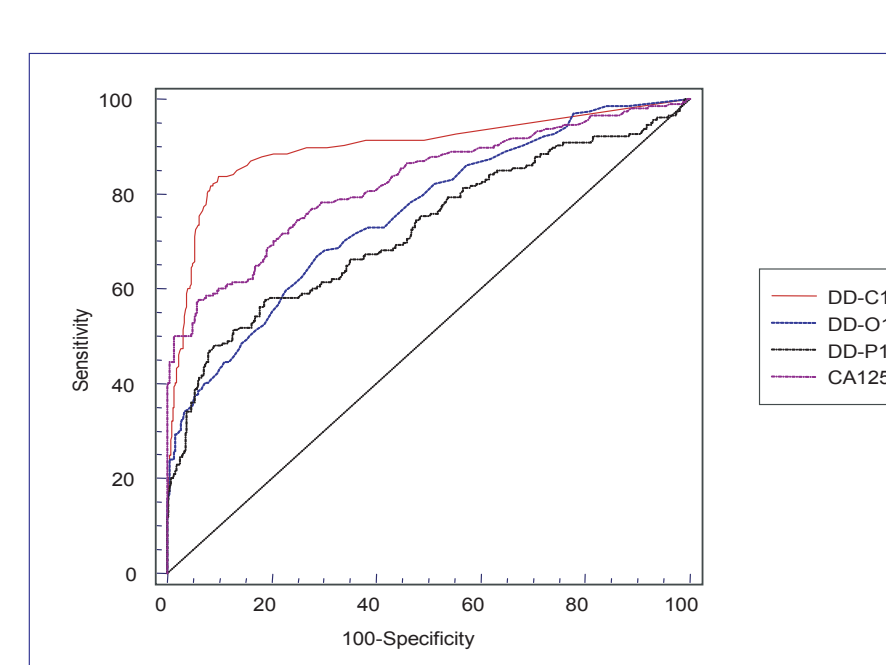
|         | DD-C101 (pg/mL) | DD-P108 (ng/mL) | DD-O110 (ng/mL) | CA125 (U/mL) |
|---------|-----------------|-----------------|-----------------|--------------|
| Maximum | 2960            | 211.4           | 46.1            | 8050.0       |
| 75.0%   | 875             | 108.8           | 4.1             | 563.8        |
| 50.0%   | 630             | 71.4            | 2.1             | 145.5        |
| 25.0%   | 460             | 46.3            | 1.3             | 15.0         |
| Minimum | 150             | 24.9            | 0.4             | 5.0          |

### Univariate Assessment of the Risk of Death by Quartile

|         | Wald p (Quartile 1 vs. 4) | Odds Ratio (Quartile 1 vs. 4) |
|---------|---------------------------|-------------------------------|
| DD-C101 | 0.028                     | 4.55                          |
| DD-O110 | 0.003                     | 11.25                         |
| DD-P108 | 0.010                     | 6.76                          |
| CA125   | 0.006                     | 6.93                          |

## Detection Study 1

ROC Analysis of Ovarian Cancer Markers



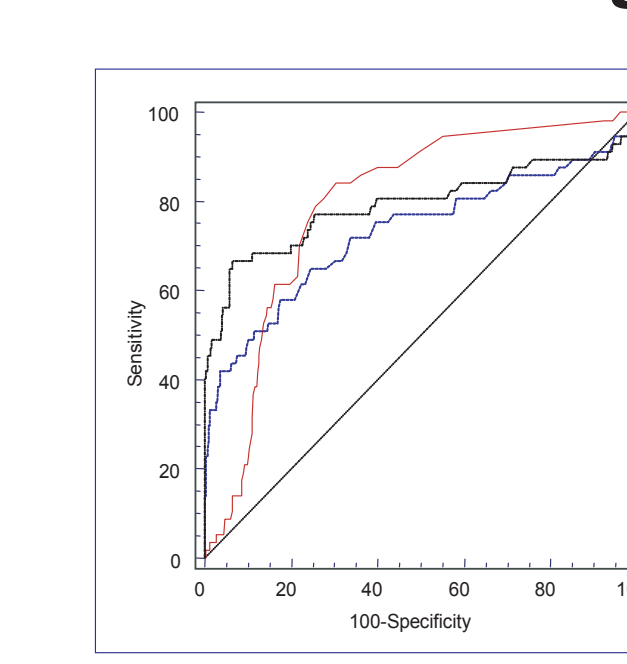
| Marker  | AUC  |
|---------|------|
| DD-C101 | 0.90 |
| DD-O110 | 0.76 |
| DD-P108 | 0.72 |
| CA125   | 0.82 |

## Multivariate ROC Analysis of Ovarian Cancer Markers

| Markers                       | DD-C101 | CA125 | DD-C101 +DD-O110 | DD-C101 +DD-P108 | DD-C101 +CA125 |
|-------------------------------|---------|-------|------------------|------------------|----------------|
| AUC                           | 0.90    | 0.82  | 0.92             | 0.92             | 0.94           |
| Sensitivity @ 98% Specificity | 44%     | 50%   | 53%              | 54%              | 65%            |

### All Stages

Univariate ROC Analysis - All Stages Ovarian Cancer



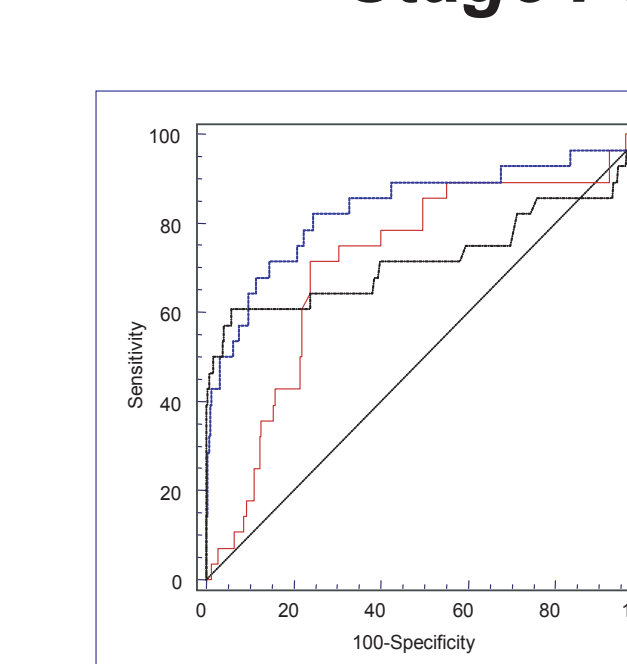
| Marker  | AUC  |
|---------|------|
| DD-C101 | 0.78 |
| DD-O110 | 0.73 |
| CA125   | 0.79 |

## Multivariate ROC Analysis - All Stages Ovarian Cancer

| Markers | CA125 | CA125 +DD-C101 | CA125 +DD-O110 | DD-C101 +DD-O110 |
|---------|-------|----------------|----------------|------------------|
| AUC     | 0.79  | 0.84           | 0.86           | 0.79             |

### Early Stages

Univariate ROC Analysis - Stage I & II Ovarian Cancer



| Marker  | AUC  |
|---------|------|
| DD-C101 | 0.73 |
| DD-O110 | 0.83 |
| CA125   | 0.72 |

## Multivariate ROC Analysis - Stage I & II Ovarian Cancer

| Markers | CA125 | CA125 +DD-C101 | CA125 +DD-O110 | DD-C101 +DD-O110 |
|---------|-------|----------------|----------------|------------------|
| AUC     | 0.73  | 0.79           | 0.85           | 0.83             |

### Case Distribution by Quartile

|         |           | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 |
|---------|-----------|------------|------------|------------|------------|
| DD-C101 | Total (n) | 20         | 20         | 19         | 19         |
|         | Death (n) | 6          | 10         | 10         | 12         |
| DD-P108 | Total (n) | 19         | 20         | 20         | 19         |
|         | Death (n) | 5          | 5          | 15         | 13         |
| DD-O110 | Total (n) | 19         | 20         | 20         | 19         |
|         | Death (n) | 4          | 9          | 10         | 15         |
| CA125   | Total (n) | 20         | 19         | 20         | 19         |
|         | Death (n) | 4          | 10         | 10         | 14         |

Total: 78 patients (38 deaths, 40 survival)

### Relative Risk in Quartiles (Death vs. 2 yr-Survival)

|         |               | Relative Risk | 95% CI       |
|---------|---------------|---------------|--------------|
| DD-C101 | Q1 vs. Q4     | 1.90          | 0.99 to 3.66 |
|         | Q1&2 vs. Q3&4 | 1.42          | 0.91 to 2.24 |
| DD-P108 | Q1 vs. Q4     | 2.33          | 1.14 to 4.77 |
|         | Q1&2 vs. Q3&4 | 2.64          | 1.55 to 4.49 |
| DD-O110 | Q1 vs. Q4     | 3.75          | 1.52 to 9.23 |
|         | Q1&2 vs. Q3&4 | 1.86          | 1.15 to 2.98 |
| CA125   | Q1 vs. Q4     | 3.04          | 1.39 to 6.66 |
|         | Q1&2 vs. Q3&4 | 1.67          | 1.05 to 2.64 |

|             | Disease | Survival | Total |
|-------------|---------|----------|-------|
| High Marker | a       | b        | a+b   |
| Low Marker  | c       | d        | c+d   |

$$RR = (a/[a+b]) / (c/[c+d])$$