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Comparison of the Diagnostic Performances of CA125 and Multiple Marker Combinations for Early Diagnosis of Ovarian Cancer

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Abstract

Studies have proven the diagnostic efficiency of multiple markers for ovarian cancer diagnosis with in vitro diagnostic multivariate index assays. We compared the diagnostic performance of multiple marker combinations and carbohydrate antigen (CA) 125 alone, a widely utilized tumor marker, for early ovarian cancer detection. Overall, 257 serum samples from Korean women, including 204 patients with benign tumors and 53 patients with ovarian cancer, were analyzed. We used 22 serum markers showing specific reaction to ovarian cancer was the concentration value obtained from xMAP™ bead-based technology (Luminex Corp.). To identify markers that combined well with CA125, a multivariate model that comprised combinations of 2–3 marker, including CA125, was created, and the best marker combinations were selected by assessing area under the receiver-operating curves (AUCs). The AUC values of CA125 alone, CA125+M2, and CA125+M2+M4 were 69.96%, 76.63%, and 81.28%, respectively. It was confirmed that multiple marker combinations including CA125 showed better diagnostic performance than CA125 alone for early-stage ovarian cancer diagnosis.

Keywords: *Biomarker, Multianalyte, Ovarian Cancer, Diagnostic, Logistic Regression*

1. Introduction

Early-stage ovarian cancer is largely asymptomatic. However, epithelial ovarian cancers, which account for approximately 90% of ovarian cancer, are mostly detected in stage III or beyond, and the 5-year survival rate after diagnosis is less than 40% [1]. Diagnosis also involves expensive unnecessary surgical diagnosis, since it is difficult to differentiate benign and negative tumors with ultrasonography. Therefore, it is very important to identify low-cost diagnostic methods that can detect early-stage ovarian cancer [2].

Diagnosis of cancer with biomarkers is relatively simple using urine or blood samples, and can detect the cancer in an early stage with expense compared to the other diagnosis methods [3].

The United States Food and Drug Administration (FDA) approved the use of cancer-

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associated protein biomarkers for cancer diagnosis and established policies and guidelines for in vitro diagnostic multivariate index assays (IVDMIA) in accordance with the "In Vitro Diagnostic Multivariate Index Assay" document (IVDMIA, 2007). According to the FDA, IVDMIA is defined as a combined device used to calculate a tumor "classification," "score," and "index" from a patient-specific single analytic function by combining multiple biomarker values in order to diagnosis, cure, alleviate, treat, and prevent diseases [4]. Since there are no such biomarkers with near-100% specificity for a certain cancer, IVDMIA is particularly meaningful in cancer diagnosis because it enables increased diagnosis accuracy by combining multiple biomarkers and quantifying the analyses with statistical techniques. The advantages of IVDMIA in comparison with a single biomarker assay are based on the premise that the single-valued index, with its aggregated information from complementary biomarkers, will outperform each of its component biomarkers used individually [5]. OVA1 [5] is the first in IVDMIA of protein biomarkers for ovarian cancer cleared by FDA(2009) developed by Vermillion. It uses five of serum proteins to diagnose the ovarian cancer (CA-125, transferrin, transthyretin (prealbumin), apolipoprotein AI, and beta-2-microglobulin). In particular, it distinguishes between cancerous and non-cancerous pelvic masses in patients who need surgery on a scale that ranges from 0 to 10. HealthLinx Ltd. developed OvPlex [6] as an ovarian cancer diagnosis method; this is a multivariate classification model that incorporates 5 serum proteins, CA-125, C-reactive protein, serum amyloid A, interleukin 6, and interleukin 8. Fujirebio Inc. developed ROMA (Risk of Ovarian Malignancy Algorithm) [7] by combining CA125 with HE4 results better, whose diagnostic performance was superior to that of CA125 only.

CA125 is most widely used serum biomarker for diagnose for ovarian cancer[8]. CA125 was elevated in only 50% of stage I ovarian cancer cases and in 69% of ovarian tumours while being far more prominently expressed in patients with late stage serous tumours [9]. Thus, CA125 has low sensitivity in early-stage disease and additional ultrasonography or combinations of other markers are required [9-11]. In recent years, a number of studies have confirmed that combinations of CA125 and other biomarkers provided improved diagnostic performance for early-stage ovarian cancer [5-7, 9-11].

Most of the women with clinical features that correspond to ovarian cancer have benign conditions, and thus it is important to detect early ovarian cancer in women with benign conditions [11].

Therefore, this study aims to evaluate the performance of a multivariate model, in which CA125 is combined with other biomarkers, for the diagnosis of benign and malignant ovarian tumors. The multivariate marker combination model was assessed with logistic regression, and the diagnostic performance was evaluated on the basis of the area under the receiver operating characteristic curve (ROC-AUC)[12].

2. Sample Collection

This study was performed with serum samples from 254 Korean women, including 204 patients with ovarian cysts and 53 ovarian cancer patients. Of the latter group, 21 patients had early-stage disease. The samples were provided by the Seoul ASAN Medical Center and the Hallym University Medical Center.

Table 1 lists the details of the clinical samples analyzed in the study. A total of 22 different ovarian cancer-specific protein biomarkers were used in the study [2].

Table 1. Details of the Analyzed Serum Samples

Characteristics	No. of patients
No. of patients studied	257
Ovarian Cyst	204
Ovarian Cancer	53
Age (Mean ± S.D.)	44.5 ± 13.42
(Range)	21-80
FIGO stage	
I	20
II	1
III	24
IV	8

The concentration of the biomarkers in serum was calculated by microbead-based antibody multiplexed immunoassay using Luminex technology. The serum samples were incubated with Luminex-beads that were bound to 22 biomarkers, including CA125, and the fluorescence from each antibody on the beads was measured with Luminex. The measured fluorescence intensities were converted to concentrations according to the standard curves generated by Bio-Rad (5-parameter curve fitting) [11].

Table 2 describes the median concentrations and concentration ranges of the individual biomarkers (M1-M4) that combined well with CA125, classified into benign, early-stage malignant, and late-stage malignant ovarian tumors.

Owing to the costs of the diagnostic analyses, the number of markers per combination was limited to less than 3. The marker names are not listed in order to avoid patent infringement.

Each of biomarkers displayed significantly elevated serum concentrations in ovarian cancer patient compared with either benign [9].

Table 2. Median Concentrations (ranges) of Serum CA125 and the Individual Markers (M1-M4) to be Combined with CA125

	Benign	Early-stage Malignant (stage I, II)	Late-stage Malignant (stage III, IV)
	(n = 204)	(n = 21)	(n = 32)
CA125	18.21 (0.3–2001.30)	43.73 (1.0–970.04)	207.06 (5.48–6136.49)
M1	775.58 (69.11–4330.48)	651.91 (261.96–3281.68)	486.67 (204.65–2223.01)
M2	34.17 (1.87–209.07)	56.14 (21.01–824.79)	53.82 (6.01–734.34)
M3	25.67 (0.04–2600.0)	14.61 (4.88–2600.0)	8.33 (0.04–2600.0)
M4	6726.55 (1008.3–33629.4)	3406.99 (1029.35–15619.82)	1909.70 (404.88–21772.11)

3. Methods

To identify CA125-inclusive marker combinations with superior performances, a multivariate model that comprised combinations of 2-3 marker, including CA125, was

created, and the multiple marker combinations with excellent performances were selected by assessing the ROC AUC [14].

To determine the combination of markers that can best distinguish benign tumors from cancer, each subset of the markers need to be evaluated, and narrow the subset down to the optimum subset [15].

In order to reduce the time needed for marker selection, 5-fold cross validation was repeated 100 times, and the top 20 combinations were initially selected. These 20 combinations then underwent 1000 rounds of 5-fold cross validation for a second screening of the top combinations according to the average AUC. The cross validation was repeated 1000 times to minimize deviations between the samples and the population.

All combinations of CA125 with 2-3 biomarkers from the 21 individual markers were included in the selection. Logistic regression was used to evaluate the multivariate marker combination model, and the diagnostic performance was investigated by ROC AUC analysis.

Figure 1 illustrates the multivariate modeling procedure used to identify the single best marker combination.

For the combinations of 2 and 3 markers, including CA125, diagnostic performances were compared between the all-stage ovarian cancer group, including both the early and late stages, and the benign group, as well as between the early-stage (stage I/II) ovarian cancer group and the benign group.

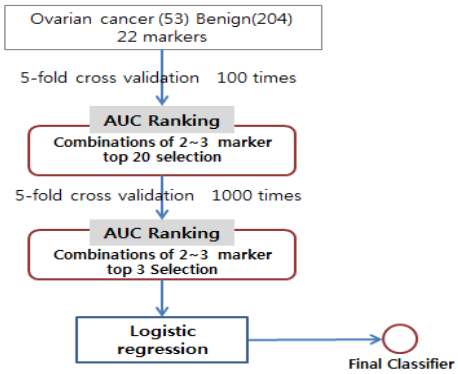


Figure 1. Multivariate Classification Modeling Procedure

4. Results

Table 3 and Figure 2 represent the AUC and ROC curve, respectively, of the marker combinations in the all-stage ovarian cancer group and the benign group.

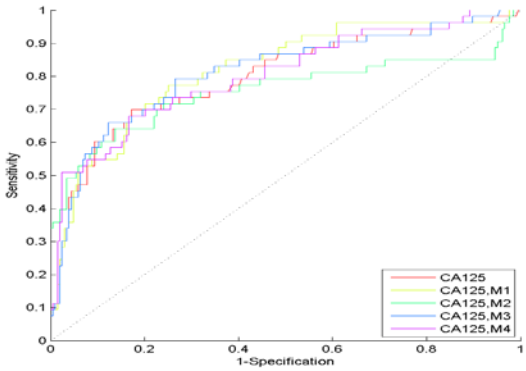
CA125 alone, CA125+M1, and CA125+M3 gave AUC values of 79.72%, 81.83%, and 81.25%, respectively. It was observed that the sensitivities, on the basis of 75% specificity, were 71.69% for CA125 alone, 75.47% for CA125+M1, and 73.58% for CA125+M3, indicating that the combinations performed better than CA125 alone. CA125+M2 and CA125+M4, on the other hand, performed similarly to CA125 alone.

Of the 3-marker combinations, ROC-AUC values of 82.16%, 85.52%, and 83.33% were obtained for CA125+M1+M2, CA125+M1+M4, and CA125+M2+M4, respectively, which indicated better performances than those of CA125 alone. Of the 2-marker combinations, CA125+M1 was identified as the best marker combination for differentiating the all-stage ovarian cancer group from the benign group, with an AUC of 81.83% and a sensitivity of 75.69%.

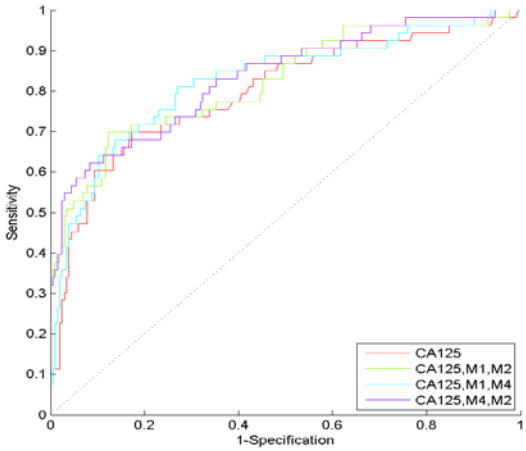
Among the 3-marker combinations, CA125+M1+M4 had a lower AUC (82.52%) than CA125+M2+M4 but had the best sensitivity (75.47%) of all the marker combinations.

Table 3. Performances in the All-stage Ovarian Cancer and Benign Groups

Markers	ROC-AUC	95%CI	SN at 75% SP
CA125	79.72	71.69-87.07	71.69
CA125, M1	81.83	73.49-88.14	75.47
CA125, M2	75.52	64.76-84.62	71.7
CA125, M3	81.25	73.53-87.70	73.58
CA125, M4	80.31	71.50-86.33	69.81
CA125, M1, M2	82.16	73.38-88.15	73.58
CA125, M1, M4	82.52	73.62-88.47	75.47
CA125, M2, M4	83.33	75.02-89.05	69.81



(a) 2-marker ROC Curves



(b) 3-marker ROC Curves

Figure 2. ROC Curves for the All-stage Ovarian Cancer and Benign Groups

Table 4 and Figure 3 describe the AUC and ROC curves, respectively, of the marker combinations in the early stage (stage I/II) ovarian cancer and benign groups. CA125 alone, CA125+M1, CA125+M2, CA125+M3, and CA125+M4 had AUC values of 69.96%, 71.27%, 76.63%, 70.79%, and 73.81%, respectively, indicating that all marker combinations performed better than CA125 alone.

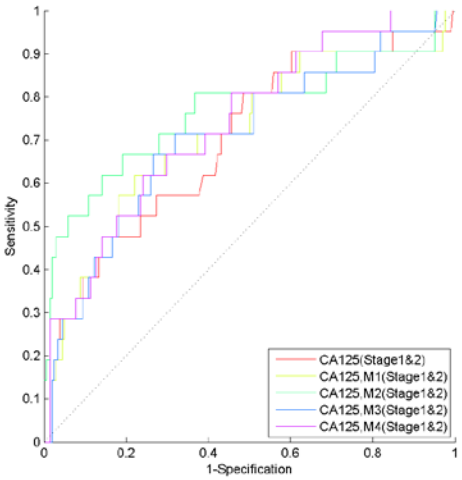
Of the 3-marker combinations, AUC values of 76.89%, 73.97%, and 81.28% were obtained with CA125+M1+M2, CA125+M1+M4, and CA125+M2+M4, respectively, which were better performances than that of CA125 alone.

With regard to sensitivity, on the basis of 75% specificity, generally good outcomes were observed, with sensitivity rates of 52.38% in CA125 alone, 66.67% in CA125+M2 and CA125+M1+M2, and 71.43% in CA125+M2+M4.

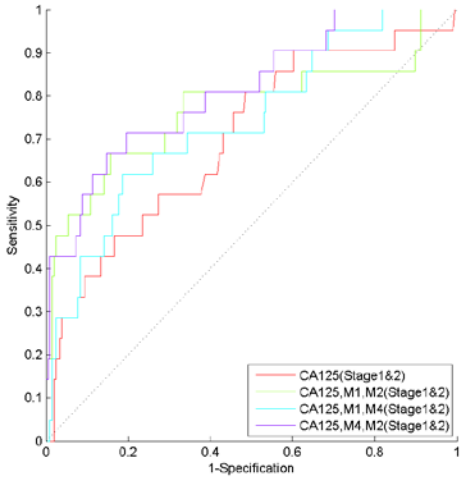
Of the 2-marker combinations, CA125+M2 demonstrated the best performance, with an AUC of 76.63% and a sensitivity of 66.69%, for differentiation between the early-stage ovarian cancer and benign groups. In the 3-marker combinations, CA125+M2+M4 demonstrated the best performance, with an AUC of 81.28% and a sensitivity of 71.43%.

Table 4. AUCs in the Early-stage Ovarian Cancer and Benign Groups

Markers	ROC-AUC	95% CI	SN at 75% SP
CA125	69.96	56.74-82.30	52.38
CA125, M1	71.27	55.88-82.48	61.9
CA125, M2	76.63	59.30-87.73	66.67
CA125, M3	70.79	53.90-81.79	57.14
CA125, M4	73.81	60.65-83.81	61.9
CA125, M1, M2	76.89	59.53-88.20	66.67
CA125, M1, M4	73.97	60.92-84.74	61.9
CA125, M2, M4	81.28	67.92-90.19	71.43



(a) 2-marker ROC Curves



(b) 3-marker ROC Curves

Figure 3. ROC Curves in the Early-stage Ovarian Cancer and Benign Groups

Given these results, most of the maker combinations indicated diagnostic performances superior to that of CA125 alone, but some marker combinations only performed similarly. When comparing the performances of the 2-marker and 3-marker combinations, the number of combined markers did not necessarily correlate with the performance level. This could suggest that combinations of complementary markers are essential to ensure more stable and sound performances.

In particular, in the early-stage ovarian cancer group, the sensitivity at 75% specificity was low in CA125 alone (52.38%), whereas an increased sensitivity (71.43%) was observed with CA125+M2+M4, a finding that is promising for early diagnosis.

The CA125+M1 combination gave an excellent performance in the comparison of the all-stage ovarian cancer and benign groups, as did the combination of CA125+M2+M4 in the comparison of the early-stage ovarian cancer and benign groups.

5. Conclusion

In the present study, we compared the performances of CA125, both alone and in combination with other markers, in order to assess its diagnostic efficiency in early-stage ovarian cancer.

The experimental results revealed that the marker combinations possessed higher AUC, and that the specificities and sensitivities of the combinations in early-stage ovarian cancer, according to the ROC curves, were comparable to those of CA125 alone. Such results indicate that the diagnostic efficiency could be improved by using the multivariate model with complementary multiple markers, rather than CA125 alone, especially for early-stage ovarian cancer.

Taken together, the experimental results of this study demonstrated that better diagnostic outcomes could be achieved by using CA125-containing marker combinations rather than CA125 alone, because the combinations of markers with CA125 support diagnosis by integrating information from the multiple markers.

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