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Comparison of the Diagnostic Performance of Urine and Serum Protein Biomarkers in Ovarian Cancer

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Abstract

This paper compares the diagnostic accuracy performance of combinations of biomarkers extracted from urine and serum samples for the early diagnosis of ovarian cancer. The sample population consisted of 23and 18 patients with benign disease and cancer, respectively. The concentrationsof 15 ovarian cancer-specific biomarkers were measuredbyantibody microbead-assisted multiplexed immunoassaytechnology. To determine the optimal combination todistinguish benign disease from cancer, the area under the receiver operating characteristic (ROC) curve (AUC) for various marker combinations, consisting of two biomarkers and three biomarkers,was estimated. The diagnosticperformance was evaluated using logistic regression. The biomarker combination with the highest AUC value comprised threebiomarkers with values of97.14% and 91.43%, for thebiomarkers extracted from urine and serum, respectively. This result demonstrates thatcombinations of biomarkers extracted from urine have higher diagnostic accuracy performance than those of biomarkers extracted from serum.

Keywords: *Biomarker, Tumor Markers, Multianalyte, Ovarian Cancer, IVDMIA, Logistic Regression*

1. Introduction

Thesymptoms of ovarian cancer in an early stage are not noticeable, and it is difficult to distinguish benign tumor from cancer by using nonradioactive diagnostic tests such as ultrasonography. Therefore, the diagnosis of ovarian cancer is generally established by expensive and unnecessary surgical diagnosis. Epithelial ovarian cancer, which accounts for 90% of ovarian cancer cases, is usually detected after the early stageand as a result, the survival rate at 5-years from diagnosis is less than 40%. Therefore, early detection of ovarian cancer is essential [1-2]. Cancer diagnosis with biomarkers is relatively simple since only urine or blood samplesneed to be used, and cancer detection can be achieved atan early stage without the use of expensive diagnostic methods [3].

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The U.S. Food and Drug Administration (FDA) approved protein biomarkers for diagnosing cancer and announced regulations and instructions in 2007 according to IVDMIA (In Vitro Diagnostic Multivariate Index Assay). IVDMIA by definition involves combining the values of multiple variables using an interpretation function to yield a single, patient-specific result such as "classification," "score," and "index," which is intended for use in the diagnosis of disease or other conditions, or in the cure, alleviate, treatment or prevention of disease [4]. In cancer diagnosis, IVDMIA is used to improve diagnostic accuracy by combining multiple biomarkers and statistically quantifying the analysis, since no single biomarker has a cancerspecificity close to 100% for a particular cancer.

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™ is the first IVDMIA of protein biomarkers cleared by FDA (2009) developed by Vermillion that uses five serum proteins to diagnose ovarian cancer. They tested patients with pelvic tumors who needed surgery and diagnosed the presence of benign or malignant disease on a scale of 0–10 [5].

Although serum proteinsare used in biomarker research for ovarian cancer diagnosis, urine biomarkers have more advantagesasthey are easier to handle clinically and they offer a noninvasive method for cancer diagnosis that enables the detection of patients with ovarian cancer among those with benign tumors [5-8, 10-12]. This paper compares the early diagnostic accuracy performance of biomarker combinations extracted from urine and serum.

2. Data Collection

The sample population consisted of 23 patients with benign disease and 18 with cancer, and 15 biomarkers from urine and serum were analyzed for each patient. The 41 samples were collected from Korean women and were also provided by the Seoul ASAN Medical Center, Korean Gynecologic Oncology Group (KGOG).Clinical characteristics of the patients included in the study**,** including median age and International Federation of Gynecology and Obstetrics (FIGO) stage,areshown in Table 1.

Table 1. Clinical characteristics of the 41 study patients

Characteristics	No. of patients	Median age (range)
No. of patients	41	42(20-66)
Benign tumor	23	39(20-58)
Ovarian Cancer	18	47(30-66)
FIGO stage		
I	7	41(20-52)
II	0	0
III	6	51(42-63)
IV	5	52(41-66)

Protein biomarker concentrations were measured by antibody microbead-assisted multiplexed immunoassays (xMAP™) with Luminex technology. The samples were incubated with Luminex-beads that were bound to 15 biomarkers, 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) [2].

3. Methods

To identify the optimum biomarker combination foraccurate early diagnosis of ovarian cancer, the performance ofdifferent biomarker combinations comprising two and three biomarkersextracted from urine and serum were compared. It is expected that, for an appropriate model, multiple biomarkers will be needed for detecting subclinical cancer with adequate sensitivity and specificity. Evaluation of the area under the curve (AUC) of a ROC curve is a common method for evaluating both sensitivity and specificity, and thus the classification performance [9].

In this study, the score calculated by logistic regression wasused to evaluate the ROC AUC and to select the biomarker combination with the highest AUC.

A 5-fold cross validation was repeated 1000 times for both urine and serum biomarker combinations, and the combinations of biomarkers from urine and serumwith the top five average values of AUC were selected.

4. Results

The performance was measured usinglogistic regression, and the AUC, 95% confidence interval (CI), sensitivity, specificity, classification accuracy, positive predictive value (PPV), and negative predictive value (NPV) were evaluated.

Table 2shows the top five individual biomarkers from urine in terms of diagnostic accuracy performance, and Table 3 shows those from serum.

Table 2. Top five biomarkers extracted from urine in terms of diagnostic accuracy performance (%)

Markers	M15	M4	M5	M3	M6
AUC	90.48	70.71	68.57	67.38	65.24
95% CI	54.00~100	46.46~87.58	44.08~88.39	39.05~83.84	40.93~85.12
Sensitivity	50.00	50.00	30.00	50.00	20.00
Specificity	100	90.48	90.48	71.43	57.14
Accuracy	83.87	77.42	70.97	64.52	45.16
PPV	100.00	71.43	60.00	45.45	18.18
NPV	80.77	79.17	73.08	75.00	60.00

Table 3. Top five biomarkers extracted from serum in terms of diagnostic accuracy performance (%)

Markers	M6	M7	M15	M4	M13
AUC	83.81	83.81	72.14	69.05	69.05
95% CI	59.67~95.38	59.83~96.25	40.83~94.04	40.76~87.27	44.69~87.93
Sensitivity	80.00	50.00	40.00	80.00	30.00
Specificity	76.19	85.71	100	52.38	100
Accuracy	77.42	74.19	80.65	61.29	77.42
PPV	61.54	62.50	100	44.44	100
NPV	88.89	78.26	77.78	84.62	75.00

The M15 biomarker showed a higher value than otherurine biomarkers. The AUC and accuracy of the M15 urine biomarker was 90.48% and 83.87%, respectively.The AUC and accuracy were 83.81% and77.42% for the serum protein biomarker M6 and 83.81%and 74.19%for the serum protein biomarker M7, respectively.The M15 urine biomarker (AUC, 90.48%)outperformed the M6 and M7 serum biomarkers (AUC,83.81%for both).Figure 1 shows the ROC curves of the top two individual biomarkers from urine and serum.

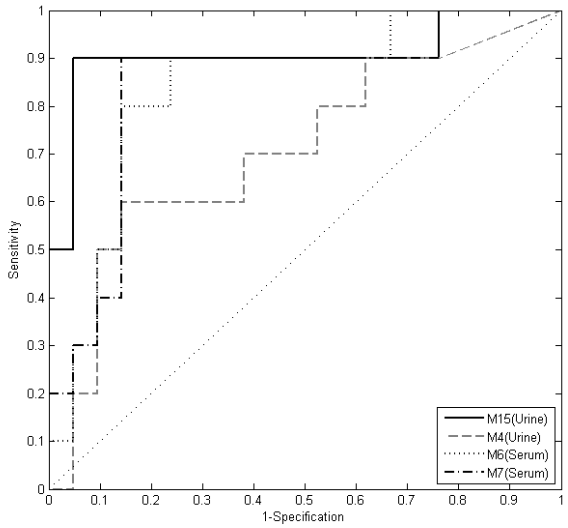

Figure 1. Receiver operating characteristics curves of the top two-biomarker combinations for urine (M1, M4) and serum(M6, M7)

Table 4 shows the diagnostic performance of the top five two-biomarker combinations from urine, and Table 5 shows those from serum.

Table 4. Top five combinations of two biomarkers extracted from urine in terms of diagnostic accuracy performance (%)

Markers	M3,M15	M14,M15	M7,M15	M10,M15	M1,M15
AUC	96.67	93.81	93.33	92.86	92.38
95% CI	78.18~100	70.65~100	69.21~100	68.59~100	71.43~100
Sensitivity	60.00	70.00	70.00	60.00	60.00
Specificity	100	100	100	95.24	95.24
Accuracy	87.10	90.32	90.32	83.87	83.87
PPV	100	100	100	85.71	85.71
NPV	84.00	87.50	87.50	83.33	83.33

Table 5. Top five combinations of two biomarkers extracted from serum in terms of diagnostic accuracy performance (%)

Markers	M6,M13	M6,M15	M6,M10	M6,M7	M4,M7
AUC	87.14	87.14	86.67	85.71	82.86
95% CI	61.56~97.62	61.85~96.43	67.54~95.45	61.70~96.97	47.53~96.25
Sensitivity	60.00	50.00	50.00	70.00	50.00
Specificity	85.71	90.48	90.48	90.48	85.71
Accuracy	77.42	77.42	77.42	83.87	74.19
PPV	66.67	71.43	71.43	77.78	62.50
NPV	81.82	79.17	79.17	86.36	78.26

For combinations of two biomarkers extracted from urine, (M3, M15) and (M6, M13),the AUCswere 96.67% and 93.81% and the accuracieswere 87.10% and 90.32%, respectively. For combinations of two biomarkers extracted from serum, (M6, M13) and (M6, M15), the AUCs were 87.14% and 87.14% and the accuracies were 77.42% and 77.42%, respectively.

These tables show that the urine marker combination(M3, M15) had better performance than the serum marker combination (M6, M13) .

Figure 2 shows the ROC curves of the different combinations consisting of two biomarkers from urine and serum.

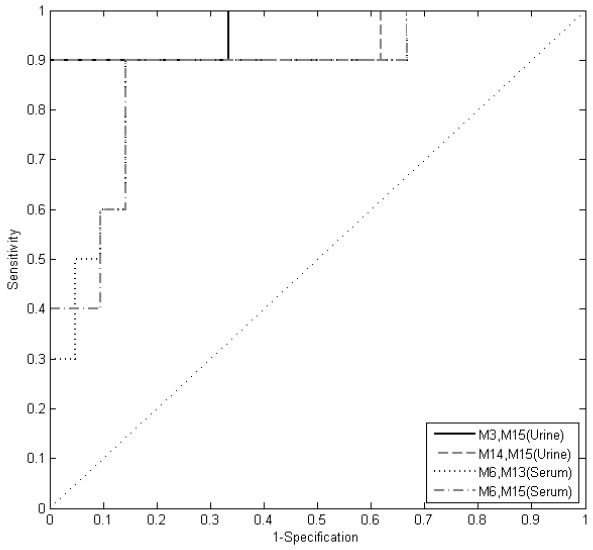

Figure 2. Receiver operating characteristic curves of the top two-biomarker combinations for urine (M3, M15 and M14, M15) and serum (M6, M13 and M6, M15)

Table 6 shows the performance of the top five combinations consisting of three urine biomarkers, and Table 7 shows those from serum.

Table 6. Top five combinations of three biomarkers extracted from urine in terms of diagnostic accuracy performance (%)

Markers	M7,M8,M15	M6,M7,M15	M10,M14,M15	M1,M14,M15	M5,M14,M15
AUC	97.14	96.67	95.71	94.29	93.81
95% CI	83.37~100	80.29~100	76.34~100	66.61~100	62.15~100
Sensitivity	80.00	80.00	70.00	70.00	70.00
Specificity	100	100	100	100	100
Accuracy	93.55	93.55	90.32	90.32	90.32
PPV	100	100	100	100	100
NPV	91.30	91.30	87.50	87.50	87.50

Table 7. Top five combinations of three biomarkers extracted from serum in terms of diagnostic accuracy performance(%)

Markers	M2,M6,M13	M2,M6,M15	M6,M10,M13	M6,M10,M15	M6,M14,M15
AUC	91.43	90.95	90.48	89.52	88.57
95% CI	76.67~99.09	70.44~97.47	72.17~98.10	65.72~97.27	63.39~98.18
Sensitivity	60.00	60.00	70.00	60.00	70.00
Specificity	90.48	90.48	90.48	90.48	90.48
Accuracy	80.65	80.65	83.87	80.65	83.87
PPV	75.00	75.00	77.78	75.00	77.78
NPV	82.61	82.61	86.36	82.61	86.36

The AUCs of the marker combinations consisting of three urine biomarkers (M7, M8, M15) and (M2, M6, M13) were 97.14% and96.67%, and the accuracies were 93.55% and 93.55%, respectively. The AUCs of the combinations (M2, M6, M13) and (M2, M6, M15) from serum were 91.43% and 90.95% and the accuracies were 80.65% and 80.65%, respectively.

These tables show that the urine marker combination (M7, M8, M15) has better diagnostic performance than the serum marker combination (M2, M6, M13).

Figure 3 shows the ROC curves of the three-biomarker combinations from urine and serum.

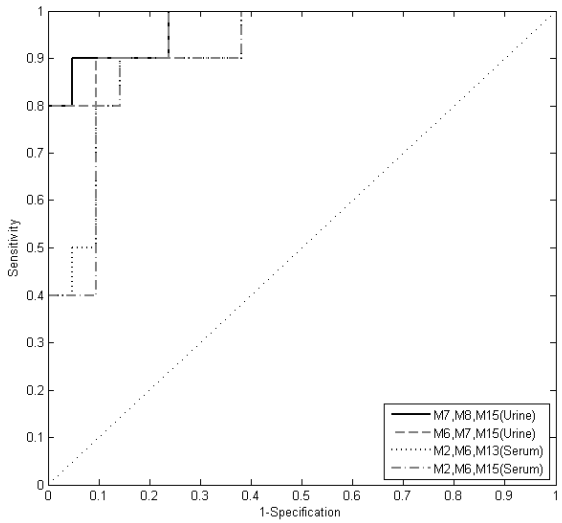

Figure 3. Receiver operating characteristic curves of the top three-biomarker combinations for urine (M7, M8, M15 and M6, M7, M15) and serum (M2, M6, M13 and M2, M6, M15)

5. Conclusion

The study compares the performance of two- and three-biomarker combinations for the early diagnosis of ovarian cancer extracted from urine and serum.Combinations from15 biomarkers specific to ovarian cancer wereevaluated, and the classification performance wascompared.

The optimal marker combination was determined by comparing the ROC AUC.

The highest AUC value for three-biomarker combinations was 97.14% and 91.43% for biomarkers extracted from urine and serum, respectively. This result demonstrates that the biomarker combinations extracted from the urine have higher performance than those from serum.

Multiple urine markers including M15 showed good diagnostic performance for the detection of ovarian cancer in patients with a pelvic mass. Multiple serum markers including M6 also showed good performance.

Acknowledgments

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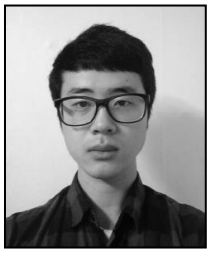

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