

Development of Diagnosis Model using Urine Biomarkers for Early Ovarian Cancer

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Abstract

This paper develops a new diagnosis model using urine biomarker proteins for early ovarian cancer diagnosis. The optimum marker combination that best classifies the benign tumor and cancer was determined from 15 biomarkers and the performance was evaluated. Samples consist of 121 patients with benign tumor, and 55 patients with ovarian cancer. 15 urine biomarkers showing specific reaction to ovarian cancer was the concentration value obtained from xMAP™ bead-based technology (Luminex Corp.). The area under the curve (AUC) of ROC was evaluated to determine the optimum marker combination showing the best performance in difference between patients diagnosed with ovarian cancer and benign. The performance of the selected combination was confirmed with logistic regression. For each combination of two, three, and four biomarkers showed the highest AUC of 85.71%, 86.38%, and 86.7%, respectively. The highest accuracy of distinguishing benign to cancer was 82.58% for a single biomarker, and 84.27%, 84.83%, and 85.29% for each combination of two, three, and four biomarkers, respectively. It was confirmed that the ovarian cancer diagnosis model utilizing the optimum biomarker combination determined in this research showed better performance and higher accuracy than using a single biomarker.

Keywords: biomarker, Ovarian Cancer, IVDMIA, Logistic Regression

1. Introduction

The symptoms of ovarian cancer in an early stage are not noticeable, and it is hard to distinguish the benign tumor from cancer using nonradioactive diagnosis such as ultrasonography. Therefore the diagnosis of ovarian cancer is generally accompanied by expensive, unnecessary surgical diagnosis. Epithelial ovarian cancer, which takes up 90% of ovarian cancer, is usually detected after stage III, and as a result, the survival rate after 5 years from diagnosis is less than 40%. Therefore early detection of ovarian cancer is becoming paramount [1, 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 U.S. Food and Drug Administration (FDA) approved protein biomarkers as a way of diagnosing cancer, and announced the regulations and instructions in 2007 according to 'IVDMIA: In Vitro Diagnostic Multivariate Index Assay'. IVDMIA by definition is combining the values of multiple variables using an interpretation function to yield a single,

patient-specific result such as “classification,” “score,” “index,” that is intended for use in the diagnosis of disease or other condition, or in the cure, migration, treatment or prevention of disease [4]. In cancer diagnosis, IVDMIA is used to improve the accuracy of the diagnosis by combining multiple biomarkers and quantifying the analysis by statistic means, since there is no single biomarker that has a cancer-specificity close to 100% for a specific 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 in IVDMIA of protein biomarkers cleared by FDA (2009) developed by Vermillion that uses five of serum proteins to diagnose the ovarian cancer. They tested pelvic tumor patients who needed surgery and diagnosed whether the tumor was benign or malignant on a scale of 0-10 [5].

Correlogic Systems, Inc. eliminated the limitation of the conventional single biomarker diagnosis using an antibody diagnosis based on microbeads, and integrated it into the biomarker diagnosis, inventing the multivariate ovarian cancer diagnosis [6].

To classify ovarian cancer and pelvic tumor, Amonkar used the Random Forest algorithm and found 11 optimum biomarker combinations of 85.7% sensitivity from 204 biomarkers [7]. Nolen determined the optimum subset from 55 biomarkers using Brand and Bound algorithm, and constructed a diagnosis model using with the CART classification tree, and obtained 95% sensitivity for breast cancer classification [8].

Yurkovetsky proposed a classification model that can determine the four optimum biomarker combinations from 96 biomarkers using the Metropolis algorithm with Monte Carlo simulation [6].

Borgia achieved 88% sensitivity through CART classification model, using the six optimum marker combinations that were selected from 15 biomarkers by Random Forest. The 15 biomarkers were chosen through evaluating the AUC of ROC for 47 markers [9].

In general, serum is used in biomarker researches concerning ovarian cancer diagnosis. However, urine biomarkers have higher advantage in that it is clinically easier to handle and is a perfect non-surgical cancer diagnosis method that enables the detection of ovarian cancer patients among the benign tumor patients [10, 11, 12].

Petri compared the ROC AUC of the serum and urine biomarkers from the same sample pool, and demonstrated that there is no significant accuracy difference between serum biomarkers (83%) and urine biomarkers (84%) [11].

Most women with a clinical presentation consistent with ovarian cancer have benign conditions. Therefore methods to distinguish women with ovarian cancer from those with benign conditions would be beneficial [7].

This paper aims to develop an accurate and reliable classification model by determining the marker combination that best distinguishes benign tumor from cancer, from 15 urine biomarkers that specifically reacts to ovarian cancer [9]. The ROC AUC of every combination of 2~4 markers possible was evaluated, and the diagnosis performance of the optimum marker combination was confirmed with Logistic Regression.

2. Data Collection

Sample pool consists of 121 patients with benign tumor and 55 patients with ovarian cancer, and 15 urine biomarkers were tested. The 176 urine samples of Korean women were provided from ASAN Medical Center. Table1 shows the information of the clinical samples.

Table 1. Data of the clinical samples

Characteristics	No. of patients
No. of patients studied	176
Ovarian Cyst	121
Ovarian Cancer	55
Age (Mean \pm S.D.)	44.5 \pm 13.42
(Range)	21-80
FIGO stage	
I	24 (43.6%)
II	1 (1.8%)
III	21 (38.1%)
IV	9 (16.36%)
I	24 (43.6%)

The concentration of the biomarkers in urine was calculated by microbead-based antibody multiplexed immunoassay using Luminex, and the multiplex immunoassay kit including the cancer biomarkers specifically reacting to ovarian cancer was used [9].

Multiplex analysis were performed according to the manufacturers' protocols. Luminex Core Facility assays were performed as described previously [16]. Samples were analyzed using the Bio-Plex suspension array system (Bio-Rad Laboratories). Biomarker expression levels were expressed as median fluorescent intensities generated by analyzing 50 to 100 microbeads for each analyte in each sample. The concentrations of analytes were quantitated from median fluorescence intensities using standard curves generated by Bio-Rad five-parameter curve fitting) to the series of known concentrations for each analyte [8].

3. Methods

In order to clinically use the urine samples, the variables that might rise according to the time of samples collection have to be calibrated. To resolve the concentration difference of each biomarker proteins, calibration is done having the relatively stable creatinine as the standard [13].

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 [14].

In cancer classification, both sensitivity and specificity has to be evaluated when selecting the most appropriate classification model. Determining the classification performance of cancer considering both high specificity and sensitivity is normally done by measuring the AUC of ROC [15].

This paper uses Logistic regression to evaluate ROC AUC values and selects the marker combination that has the highest value.

To decrease the time consumed in marker selection, 5-fold cross validation was repeated for 100 times and the first top 20 was selected. To determine the top marker combination from the selected 20, 5-fold cross validation was repeated 1000 times and the top marker combinations were selected using the average AUC.

By repeating the cross validation 1000 times, the deviation between the total set of combinations and the subset chosen can be decreased. As shown in Figure 1, the AUC graph converges when repeated 1000 times.

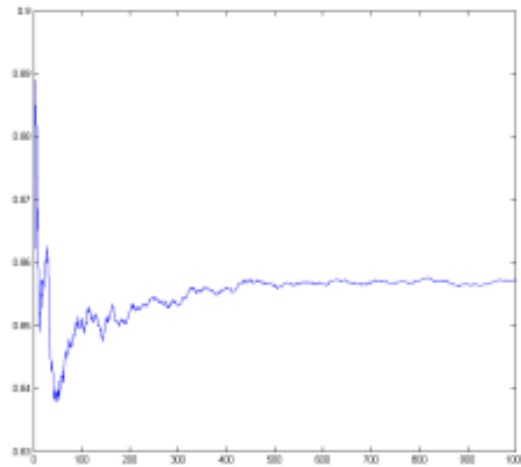


Figure. 1. AUC convergence graph

The combinations selected consist of 2~4 biomarkers out of 15, and the score threshold for Logistic Regression was set to be 0.5 to evaluate the diagnosis performance of the selected combinations.

To minimize sample set bias and to aid in the assessment of intermediate models, we employed ‘out-of-bag’(OOB) error estimation and an external 5-fold bootstrap validation with 10% holdout bootstraps. These bootstrap estimates allowed us to assess the potential value of many models using only the training data. In this way we were able to maintain the independence of the hold-out testing set of samples [7].

Figure 2 shows the modeling process to find the optimum biomarkers and classify.

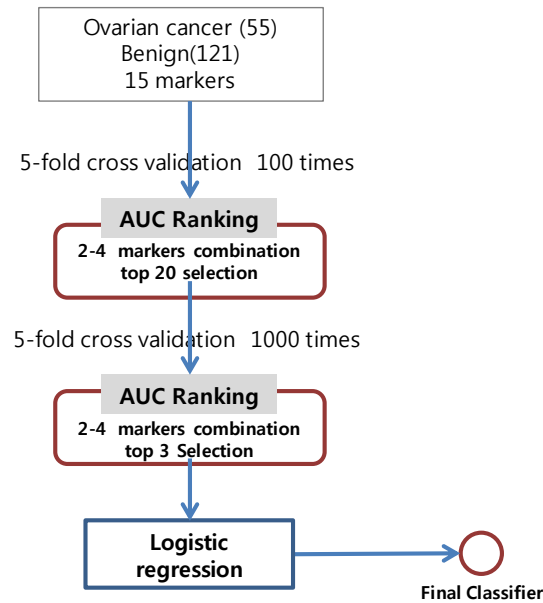


Figure 2. Process of Ovarian cancer Diagnosis modeling

4. Results

Table 2 shows the performance of the single biomarkers having the top three AUC values. Tables 3, 4, and 5 shows the performance of the combinations with the top three AUC values when two biomarkers, three biomarkers, and four biomarkers are combined, respectively.

The performance was estimated using Logistic regression algorithm and measured the AUC, sensitivity, specificity, accuracy, PPV(positive predictive value), and NPV(negative predictive value) by Leave-one-out cross validation.

In this research, the marker combination was limited only up to four biomarkers considering the diagnosis cost, and did not reveal the marker name to avoid infringement of patent.

Table 2. Performance of the biomarkers having the top three AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M5	82.49	47.37	99.17	82.58	96.43	80
M15	76.95	38.6	88.43	72.47	61.11	75.35
M2	71.34	45.61	81.82	70.22	54.17	76.15

Table 3. Performance of the combination of two biomarkers having the top three AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M5,M15	85.98	54.39	96.69	83.15	88.57	81.82
M5,M3	83.65	52.63	99.17	84.27	96.77	81.63
M5,M12	84.99	52.63	98.35	83.71	93.75	81.51

Table 4. Performance of the combination of three biomarkers having the top three AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M3M5,M15	87.49	57.89	97.52	84.83	91.67	83.1
M3,M5,M12	86.68	54.39	99.17	84.83	96.88	82.19
M2,M3,M5	86.08	54.39	97.52	83.71	91.18	81.94

Table 5. Performance of the combination of four biomarkers having the top three AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M4,M5,M12, M15	88.5	57.89	98.35	85.39	94.29	83.22
M3,M5,M12, M15	88.4	57.89	98.35	85.39	94.29	83.22
M3,M5,M14, M15	87.43	57.89	97.52	84.83	91.67	83.1

The single marker M5 showed the best performance having the AUC of 82.49%, and accuracy of 82.58%. When combining two biomarkers, M5 and M15 combination showed high performance having the AUC of 85.98% and accuracy of 83.15%. For three biomarkers combined (M3,M5,M15), the highest AUC was 87.49%, and accuracy was 84.83%, and for four biomarkers combined (M4,M5,M12,M15) AUC was measured to be 88.5% and accuracy as 85.39%.

Figure 3 shows the ROC curves of the combination of biomarkers and single biomarker with high performance.

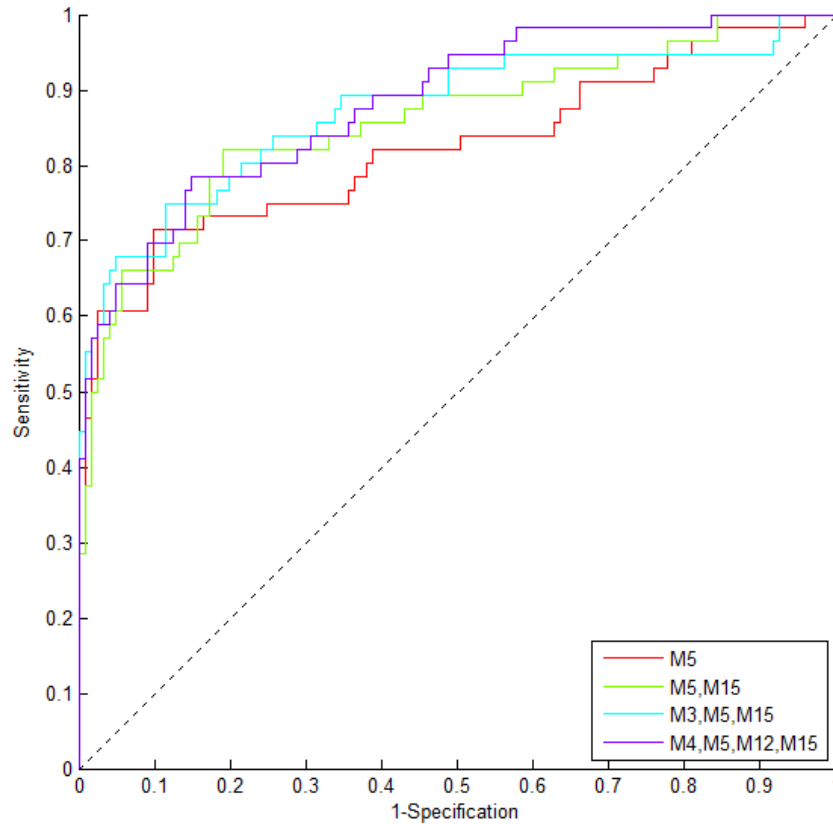


Figure 3. ROC curves of the single biomarker and the multiple markers of the optimum combination

Figure 4 shows the distribution of concentrations of individual biomarker showed the best performance in patient samples. Each biomarker also demonstrated a significant difference in concentrations between benign and malignant tumour groups. Concentrations of M5, M15 and M2 boimarkers were slightly elevated in patients with malignant disease compared with benign controls.

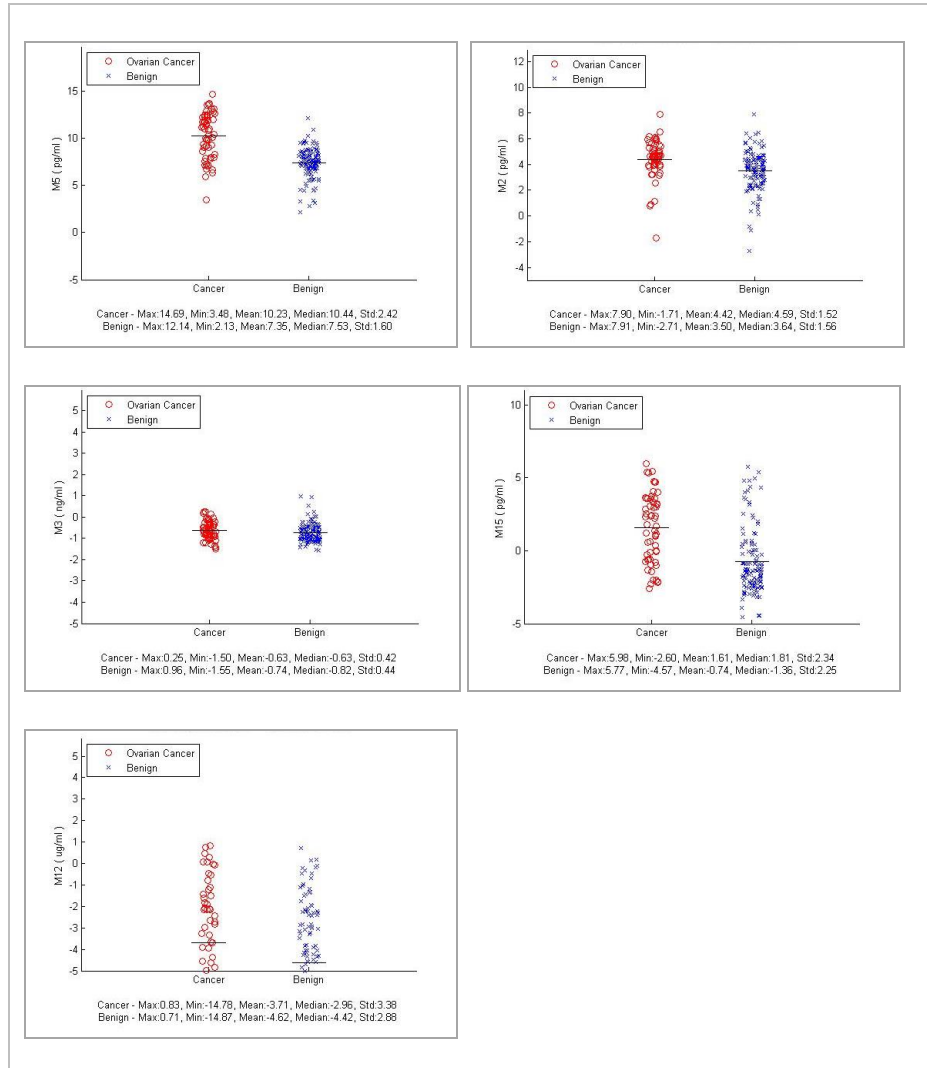


Figure 4. Distribution of serum levels of biomarkers presented as dot plots. Comparison of individual biomarker showed the best performance across all patient groups. dot indicate the individual values of each patient measurement shown on a log scale and horizontal lines show median concentration of each group

5. Conclusion

This paper develops a new diagnosis model using urine biomarker proteins for early ovarian cancer diagnosis. The optimum marker combination that best classifies the benign tumor and cancer was determined from 15 biomarkers and the performance was evaluated. The ROC AUC of each of the combinations of 2~4 biomarkers were evaluated to find the optimum combination, and it was demonstrated that the AUC when four biomarkers are combined was 88.5%, which is approximately 6% higher than that of the single biomarker(82.49%) ,indicating that the combination of biomarkers have improved performance.

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