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A Comparison of Optimal Biomarker Combinations for Benign-Cancer and Normal-Cancer Distinguishment in Ovarian Cancer Screening

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Abstract

This paper compares the performance of the combination of biomarkers to distinguish benign tumor from cancer and normal from cancer, from 21 urine biomarkers. Samples consist of 79 healthy women, 119 patients with benign tumor, and 137 patients with ovarian cancer. The concentrations of the 21 biomarkers were extracted using Luminex-PRA. The area under the curve (AUC) of ROC was evaluated to determine the optimum marker combination showing the best performance. The performance of the selected combination was confirmed with logistic regression. The highest AUC value of distinguishing benign tumor from cancer with a combination of two biomarkers and three biomarkers are 87.97% and 91.38%, respectively. And the highest AUC value of distinguishing normal from cancer with a combination of two biomarkers and three biomarkers are 87.39% and 90.68%, respectively. Interestingly, the benign-cancer classification shows a little higher performance than the normal-cancer classification.

Keywords: *Ovarian Cancer, Benign-Cancer, Normal-Cancer, Biomarker Combination*

1. Introduction

Ovarian cancer is the fourth leading cause of cancer deaths among women in the United States [1]. Early stage ovarian cancer has an excellent prognosis if treated but ovarian cancer typically causes very few specific symptoms in an early stage and over 60% of patients who are diagnosed with ovarian cancer ultimately die from the disease.

In addition, seventy percent of patients are diagnosed with advanced stages, where 5-year survival rates are less than 30%. Consequently, Given the limitations of treatment for advanced ovarian cancer and the success of treatment for early stage disease, the great way of improving clinical outcome to detect at an early stage should be required [2, 3].

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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 [4].

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 [5-7].

Of the various types of samples, diagnostic techniques using urine are advantageous in terms of clinical application to real patients because these techniques are non-invasive, inexpensive, and easy to perform, likely leading to earlier detection for malignancies

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%) [6].

Many researches for early diagnosis have been developed for distinguishing between benign tumor and cancer [8, 9]. At present, no screening techniques are recommended for early detection of ovarian cancer in the general population. However, in public medical examination, many of patients want to know whether they have some problem or not.

Because their anatomical location is deep down the pelvis, tumor-related abnormal functioning of the ovaries is asymptomatic until the tumor becomes enlarged or disseminates. In postmenopausal women, the problem is exacerbated because ovaries become dysfunctional after menopause.

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 [10].

Therefore, ovarian cancer is more likely to be detected in an advanced rather than an early stage [11].

The ability to accurately detect early stage disease would potentially improve ovarian cancer survival dramatically and apply normal-abnormal classification.

Unlike many related researched above, this research is for the patients who do not have any symptoms in their ovary.

In this paper, we try to compare the performance of classification between benign tumor and cancer and between normal and cancer, and to check that normal-cancer classification is also stable like benign-cancer classification

2. Data Collection

Samples consist of 79 healthy women, 119 patients with benign tumor, and 137 patients with ovarian cancer. The total 335 urine samples of Korean women were provided from ASAN Medical Center. The concentrations of urine protein biomarkers were measured using the multiplex immunoassay method with Luminex antibody microbeads: thereby, we used a multiplexed immunoassay kit consisting of cancer biomarkers specific to ovarian cancer [12]. Analyses were performed following the protocol of the manufacturer provided by Luminex Corp., and the samples were analyzed using the Bio-Plex Suspension Array System [13]. The biomarker expression levels are shown in terms of median fluorescent intensities generated from analyzing microbeads in quantities of 50-100 for an analyte of each sample. Analyte concentrations were quantified on the basis of the median fluorescent intensity using the standard curves generated by Bio-Rad (5-parameter curve fitting) [14].

3. Methods

To use urine samples in a clinical study, various variables should be calibrated to correct the collection time differences. The relatively stable creatinine protein level was used as a reference for calibration to correct the concentration differences in the total protein level in the urine samples [15].

A cancer diagnostic model with high specificity and sensitivity should be selected to classify different types of cancer. The conventional method of measuring the specificity and sensitivity is to determine an area under the receiver operating characteristic curve (AUC), which is used to evaluate the efficacy of cancer classification [16].

This paper uses Logistic regression to evaluate ROC AUC values and selects the marker combination that has the highest value. To minimize the biomarker selection time, the primary top 20 biomarker combinations were determined after performing evaluation 100 times with 5-fold cross validation. The 5-fold cross validation was re-performed 1000 times for the top 20 biomarker combinations to rank them based on AUC values. This cross validation was repeated for reducing the deviation between the population and sample. The repetition of 1,000 times could reduce the deviation of sampling processes.

As shown in Figure 1, the AUC graph converges when repeated 1000 times.

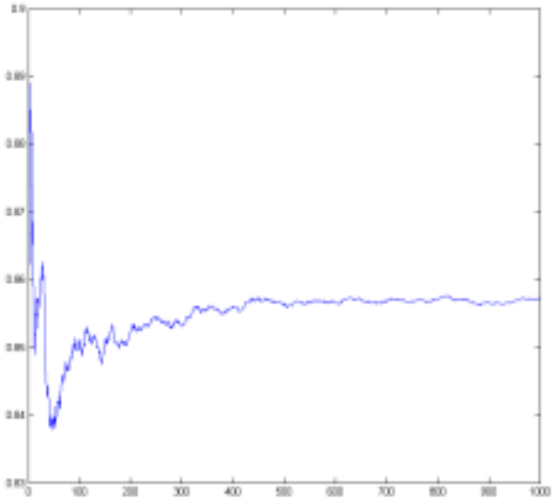

Figure. 1. AUC convergence graph

The combinations selected consist of 2 and 3 biomarkers out of 21 markers, 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 [10].

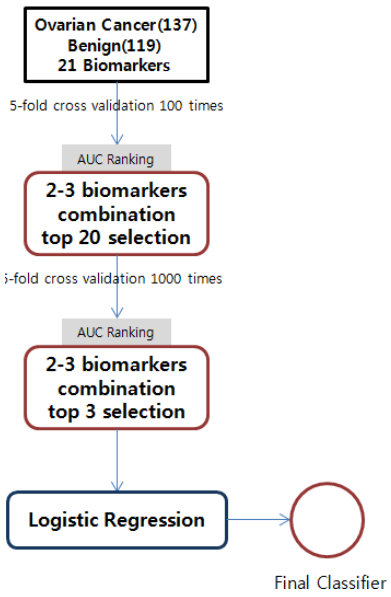

Figure 2. Process of ovarian cancer Diagnoisis modeling

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

4. Results

Table 1 shows the best biomarkers combination of two markers (M2,M5) to distinguish normal from cancer, the AUC was 87.97%, and accuracy was 78.7%, and, for the best biomarkers combination with three markers (M2,M5,M15), the AUC was 91.38% and accuracy was 83.8%. Table 2 shows the performances to distinguish benign tumor from cancer with the above combinations of two markers and three markers, are 82.71% of AUC and 74.22% of accuracy and 83.23% of AUC and 75.39% of accuracy, respectively.

Table 3 shows the best biomarkers combination of two markers (M5,M21) to distinguish benign tumor from cancer, the AUC was 87.39% , and accuracy was 81.64%, and for three markers (M5,M21,M19), the highest AUC was 90.68% and accuracy was 84.38%. Table 4 shows the performance to distinguish normal from cancer with the above combination of two markers is 81.83% of AUC and 74.54% of accuracy. For biomarkers combination of three markers has the AUC of 85.15%, and accuracy of 76.39%.

Table 1. Performance of the best biomarkers combination of two markers and three markers to distinguish normal from cancer having the top AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M2,M5	87.97	87.59	63.29	78.70	80.54	74.63
M2,M5,M15	91.38	89.05	74.68	83.80	85.92	79.73

Table 2. Performances to distinguish benign tumor from cancer with the above combinations of two markers and three markers having the top AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M2,M5	82.71	62.77	87.39	74.22	85.15	67.10
M2,M5,M15	83.23	62.77	89.92	75.39	87.76	67.72

Table 3. Performance of the best biomarkers combination of two markers and three markers to distinguish benign tumor from cancer having the top AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M5,M21	87.39	72.26	92.44	81.64	91.67	74.32
M5,M21,M19	90.68	74.45	95.80	84.38	95.33	76.51

Table 4. Performances to distinguish normal from cancer with the above combinations of two markers and three markers having the top AUC values (%)

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M5,M21	81.38	86.13	54.43	74.54	76.62	69.35
M5,M21,M19	85.15	86.86	58.23	76.39	78.29	71.88

Figure 3 shows the ROC curves of the combination of two markers and three markers to distinguish benign tumor-cancer and normal-cancer with best performance.

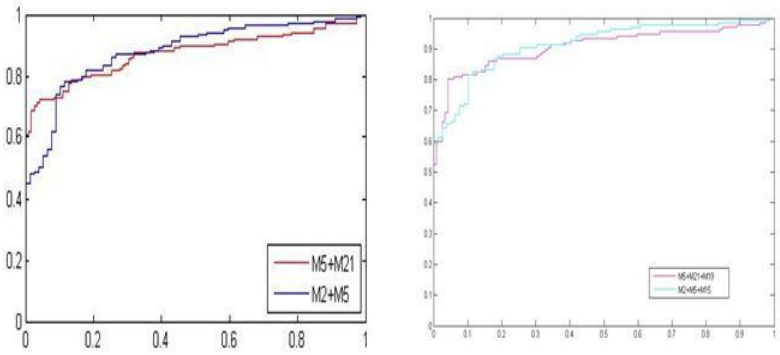

Figure 3. ROC curves of the multiple markers of the best combination.to distinguish benign tumor-cancer and normal-cancel

Figure 4 shows the ROC curves of the combination of two markers used benign tumor-cancel and normal-cancel data to distinguish normal-cancer, benign tumor-cancel with best performance.

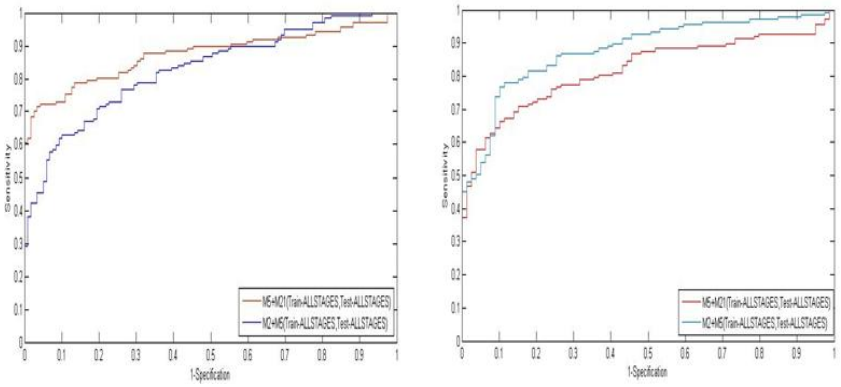

Figure 4. ROC curves of the combination of two markers used benign tumor-cancel and normal-cancel data of the best combination.

Figure 5 shows the ROC curves of the combination of three markers used benign tumor-cancel and normal-cancel data to distinguish normal-cancer, benign tumor-cancel with best performance.

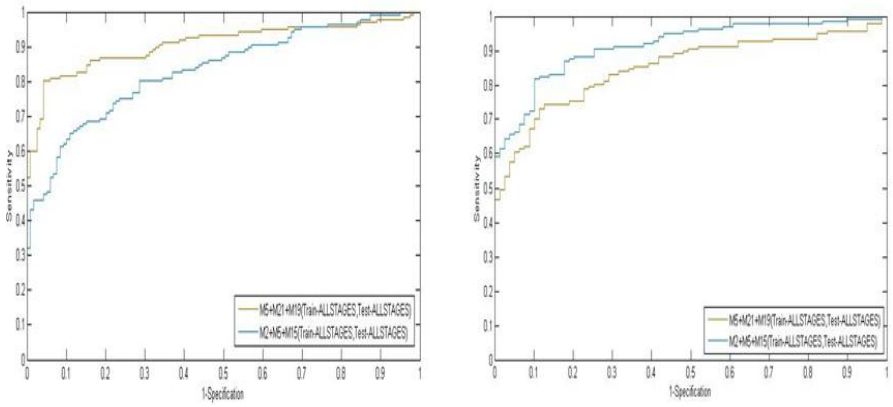

Figure 5. ROC curves of the combination of three markers used benign tumor-cancel and normal-cancel data of the best combination.

The normal-cancer classification and benign-cancer classification could not select the same marker combination. With this result, the normal-cancer classification and benign-cancer classification should be considered to be a totally different problem although they select a same marker, M5.

Generally, people could have intuition that the classification of normal and cancer would be easier than that of benign and cancer. However, the best biomarker combinations was failed to prove the intuition.

5. Conclusion

In this research, we tried to compare the biomarker combinations showing the highest performance to classify the normal patient and cancer patient and the highest performance of the benign patient and cancer patient.

The research shows that different biomarkers are selected, except M5, in case of normal-cancer and benign-cancer classification. It means that two classification problems should be treated as two different problems.

For the further study, we will develop another application to utilize the normal-abnormal classification as well as normal-cancer classification.

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