Integration of Menopausal Information into the Multiple Biomarker Diagnosis for Early Diagnosis of Ovarian Cancer

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Abstract

In this study, a new diagnosis model based on multiple serum biomarkers and menopausal information was developed for the early detection of ovarian cancer. A total of 254 serum samples from 202 patients with benign tumors and 52 patients with ovarian cancer were used. The concentrations of 22 ovarian-cancer-specific serum biomarkers were determined using Luminex, and premenopausal and postmenopausal status was binary mapped as 0 and 1. To identify the best biomarker combinations effectively differentiating ovarian cancer from benign tumors, all combinations with 2 and 3 biomarkers were evaluated according to the area under the receiver operating characteristic curve (AUC). Menopausal information was included in the combinations of 2 and 3 biomarkers that showed the best classification performance, with AUC values of 84.53 and 87.01, respectively. The diagnosis model for ovarian cancer developed in this study based on the best biomarker combinations proved that combinations of biomarkers together with menopausal information show more accurate performance than mere combinations of biomarkers alone.

Keywords: *Biomarker, Marker, Menopause, Luminex, Ovarian Cancer, Logistic regression*

1. Introduction

Women aged 50 to 79 years are most susceptible to the development of ovarian cancer, a malignant tumor that affects the ovaries. Ovarian cancer is also the second most common gynecological cancer, after cervical cancer. Although the 5-year survival rate associated with ovarian cancer is 50–95% when diagnosed in the early stages, it is below 25% when diagnosed at later stages. Since most ovarian cancers are diagnosed at advanced stages, diagnostic methods that allow early detection are of great importance [1-2].

The United States Food and Drug Administration (FDA) approved the use of cancer-associated biomarkers for cancer diagnosis and established related regulations and guidelines, “In Vitro Diagnostic Multivariate Index Assay” (IVDMIA), in 2007. According to the FDA, IVDMIA is defined as a combination device with which to determine “classifications,” “scores,” and “indices” by using analytic functions based on specific patient

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outcomes and combining multiple biomarker values for the purpose of disease diagnosis, treatment, alleviation, cure, and prevention [3].

OVA-1, which was approved by the FDA in September 2009, is the first diagnostic algorithm to combine multiple biomarkers [4]. The single numerical score calculated by OVA-1 has been reported to be more reliable than any of the individual biomarkers. The score calculated by OVA-1 ranges from 1 to 10, and postmenopausal women with scores greater than 4.4 and premenopausal women with scores greater than 5.0 are considered to be at high risk of malignancy [5].

Currently, patient age and menopausal information are the most important factors that should considered when differentiating pelvic impairments associated with a risk of malignancy; this risk increases from 14% in premenopausal women to 45% in postmenopausal women [3].

In this study, the concentration of each biomarker was determined using Luminex [7]. Luminex uses a panel reactive antibody (PRA) assay, which is a solid phase-based assay developed by Luminex Corp. In the Luminex-PRA assay, human leukocyte antigen (HLA)-linked Luminex beads react with HLA antibodies in serum and the antibody fluorescence on each bead is measured using specialized equipment and software programs [8].

We identified the best combinations of 22 different ovarian cancer-specific serum biomarkers and compared the classification performance of the best biomarker combinations according to the presence or absence of menopausal information.

All combinations of 2 and 3 biomarkers were identified from the 22 biomarkers, and the classification performance was assessed according to the AUC (The area under the receiver operating characteristic curve) [9]. Logistic regression was performed to investigate the diagnostic performance of each biomarker combination [10].

Chapter 2 describes the data collection method used in the study, Chapter 3 explains the experimental methods, Chapter 4 illustrates the results regarding the classification performance of biomarker combinations, and Chapter 5 draws conclusions from the results of this study and discusses future studies.

2. Data Collection

This study was performed using serum samples from 254 Korean women (202 patients with benign tumors and 52 patients with ovarian cancer) that were provided by the ASAN Medical Center in Seoul. The serum samples were incubated with Luminex beads linked to 22 biomarkers, and the antibody concentration on each bead was determined using Luminex. Experimental data were prepared by normalizing the measured concentration values of each biomarker between 0 and 1 according to their minimum and maximum values in order to ensure a uniform range of the concentration values of each biomarker. Further, premenopausal and postmenopausal information, assigned values of 0 and 1, respectively, was integrated into the experimental data and was used to identify the optimal biomarker combinations.

3. Methods

To identify the best biomarker combinations with which to effectively differentiate ovarian cancer from benign tumors, all possible biomarker combinations were evaluated for their differentiating power [11]. When classifying cancers, it is critical to select models with optimum classification performance by evaluating both the sensitivity and specificity. The AUC, which can adequately assess both values, is a common method for verifying classification performance [12].

In this study, premenopausal and postmenopausal information was mapped as values of 0 and 1, respectively, to integrate this information into the biomarker combination. To select the best biomarker combinations, we selected all possible combinations of 2 and 3

biomarkers from among 22 biomarkers in 2 datasets: the biomarker only set and the biomarker plus menopausal information set. By using logistic regression, AUC values were determined for each combination and then rated. The score cut-point for logistic regression analysis was set at 0.5 to evaluate the diagnostic performance of the selected combinations.

To reduce the calculation time, first 5-fold cross validation was repeated 100 times, and the 20 best combinations according to the average AUC value were selected. The selected 20 combinations were subjected to 5-fold cross validation repeated 1000 times. Then the biomarker combinations were ranked according to the obtained average AUC values. In the second iteration 1000 times of the 5-fold cross validation was selected in order to decrease the deviation between the samples and the population. As described in Figure1, the AUC values were confirmed to converge when the cross validation was repeated 1000 times.

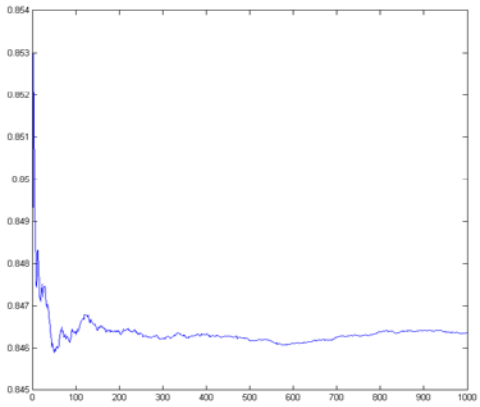

Figure 1. AUC Convergence Graph

Along with 'out-of-bag'' (OOB) error estimation and 10% holdout bootstraps, a 5-fold bootstrap validation was performed to investigate bias due to the small number of samples and to assess the intermediate model. Bootstrap estimation allows researchers to evaluate the potential value of a model using study data only and to ensure the independence of the holdout testing sets of samples [13].

Figure 2 represents the modeling process for the identification and classification of the best biomarker combinations.

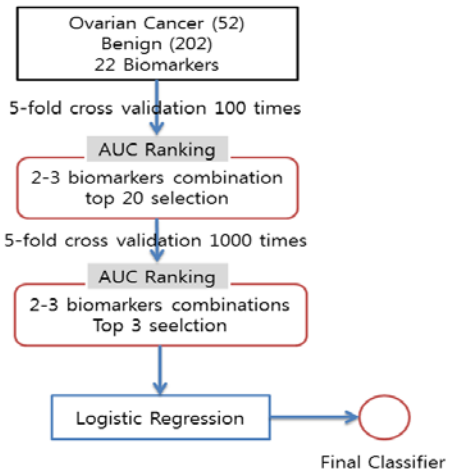

Figure 2. Process for Ovarian Cancer Diagnosis Modeling

4. Results

Table 1 indicates the diagnostic performances of the top 3 combinations with the highest AUC values among the combinations of 2 biomarkers without consideration of menopausal information. The combination of M1 and M6 showed the best performance with an AUC value of 82.25.

Table 1. Diagnostic Performances (%) of the Top 3 Combinations of 2 Biomarkers without Consideration of Menopausal Information

Marker	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M1,M6	82.25	21.15	98.02	82.28	73.33	82.85
M1,M14	80.20	19.23	98.51	82.28	76.92	82.57
M8,M14	79.62	36.54	94.55	82.68	63.33	85.27

Table 2 indicates the diagnostic performances of the top 3 combinations with the highest AUC values among the combinations of 2 biomarkers with consideration of menopausal information. The M1 combination together with menopausal information showed better performance, with an AUC value of 84.3, than did the combination without menopausal information. The M14 combination with menopausal information was also found to have the highest accuracy, with the value of 93.86, among the combinations of 2 and 3 biomarkers.

Table 2. Diagnostic Performances (%) of the Top 3 Combinations of 2 Biomarkers with Consideration of Menopausal Information

Markers	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M1, Menopausal	84.30	28.85	98.51	84.25	83.33	84.32
M14, Menopausal	83.58	44.23	94.06	93.86	65.71	86.76
M1,M6	82.25	21.15	98.02	82.28	73.33	82.85

Table 3 describes the diagnostic performances of the top 3 combinations with the highest AUC values among the combinations of 3 biomarkers without consideration of menopausal information. The M1, M9, and M14 combinations showed the best performance with an AUC value of 83.52.

Table 3. Diagnostic Performances (%) of the Top 3 Combinations of 3 Biomarkers without Consideration of Menopausal Information

Markers	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M1,M9,M14	83.52	28.85	97.03	83.07	71.43	84.12
M8,M14,M16	83.11	42.31	95.54	84.65	70.97	86.55
M1,M14,M22	82.84	28.85	97.52	83.46	75.00	84.19

Table 4 illustrates the diagnostic performances of the top 3 combinations with the highest AUC values among the combinations of 3 biomarkers with consideration of menopausal information. The M8 and M13 combinations with menopausal information showed the best performance, with an AUC value of 85.46, among the combinations of 2 and 3 biomarkers.

Table 4. Diagnostic Performances (%) of the Top 3 Combinations of 3 Biomarkers with Consideration of Menopausal Information

Markers	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
M8,M14, Menopausal	85.46	51.92	96.53	87.40	79.41	88.64
M1,M14, Menopausal	85.40	46.15	96.04	85.83	75.00	87.39
M12,M14, Menopausal	85.21	51.92	96.53	87.40	79.41	88.64

Taken together, the above results confirmed that the combinations with menopausal information showed generally higher performance than those without menopausal information.

For performance assessment, the AUC, sensitivity, specificity, accuracy of classification, positive predictive value (PPV), and negative predictive value (NPV) were determined by leave-one-out cross validation using a logistic regression classification algorithm.

In this study, the number of biomarkers used for the combinations was limited to 2 and 3 because of the diagnostic cost. The biomarker names were not listed in order to avoid patent infringement with regard to specific biomarkers.

From Figure 3 to Figure 6 show representative ROC curves for the combinations with 2 or 3 biomarkers with and without consideration of menopausal information.

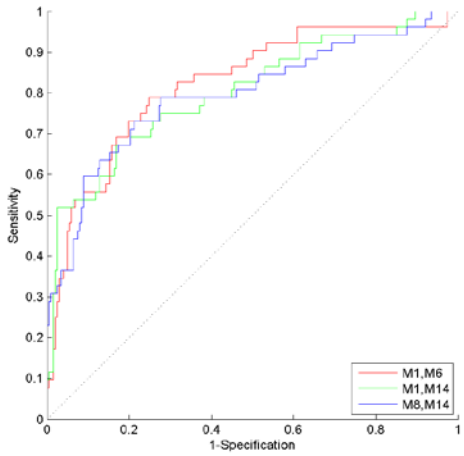

Figure 3. ROC Curves for the Combinations of 2 Biomarkers without Consideration of Menopausal Information

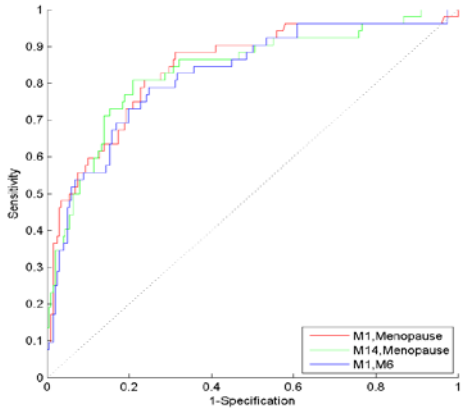

Figure 4. ROC Curves for the Combinations with 2 Biomarkers with Consideration of Menopausal Information

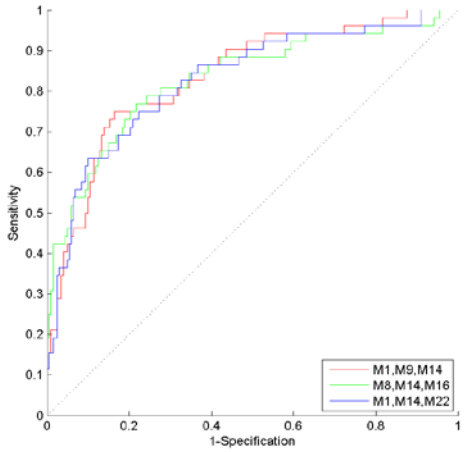

Figure 5. ROC Curves for the Combinations of 3 Biomarkers without Consideration of Menopausal Information

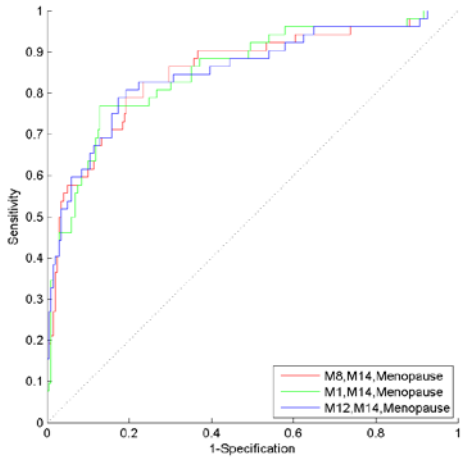

Figure 6. ROC Curves for the Combinations of 3 Biomarkers with Consideration of Menopausal Information

5. Conclusion

In this study, we developed a novel diagnosis model that used multiple biomarkers and menopausal information for the early detection of ovarian cancer. In particular, we established 2 sets of biomarker combinations that could differentiate cancer from benign tumors, using 22 ovarian cancer-specific serum biomarkers: the biomarker combination only set and the biomarker combination plus menopausal information set. The diagnostic performances of the identified combinations were then assessed and compared.

In conclusion, biomarker combinations with menopausal information showed superior diagnostic performance, with AUC values of 84.3 and 85.46 for the combinations of 2 and 3 biomarkers, respectively, than biomarker combinations without menopausal information.

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