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Concentration Calibration of Urine Biomarkers for Diagnosis Improvement of Ovarian Cancer

Eun-Suk Yang, Yu-Seop Kim, Chan-Young Park, Hye-Jeong Song and Jong-Dae Kim*

Dept. of Ubiquitous Computing, Hallym University, 1 Hallymdaehak-gil, Chuncheon, Gangwon-do, 200-702 Korea
Bio-IT Research Center, Hallym University, 1 Hallymdaehak-gil, Chuncheon, Gangwon-do, 200-702 Korea

Abstract

This paper investigates the calibration effect of urine biomarkers by creatinine. The biomarkers were obtained from each urine sample of 256 patients (cancer: 137, benign: 119). The logistic regression of the combinations of 2 biomarkers calibrated by creatinine concentration was compared with that of the 3 biomarkers including creatinine. The average AUC over 1000 rounds of 5-fold cross validations was employed to evaluate the performance. The logistic regression of the calibrated biomarkers showed better performance than that without calibration for the top- ranked combinations.

Keywords: *Biomarker, ovarian cancer, calibration, logistic regression, exponential, urine, creatinine, AUC*

1. Introduction

The five-year survival rate of all types of ovarian cancer is 44%. In epithelial ovarian cancer, the five-year survival rate is 60–99% when diagnosed at early stages, but sharply declines to approximately 40% if diagnosed at stage III [1]. Because a single marker cannot provide sufficient sensitivity and specificity for diagnosis, there is an increasingly urgent need to find multiple biomarkers for the early diagnosis and treatment of ovarian cancer [2-3].

A biomarker refers to a marker that can clearly measure whether the organism is pathologically normal or abnormal, as well as the extent of response toward a certain drug. In particular, a biomarker can describe the pathological state of a disease, can measure the degree of the reaction of the organism when treated with a certain medication, and can predict the executable treatment for the disease. A desirable tumor marker would be a protein fragment that is detected from the urine or blood of a patient that would not be found in healthy people [4-6]. The University of Pittsburgh Cancer Institute recently reported the possibility of early diagnosis of ovarian cancer by using a biomarker found in urine [7].

In general, a urine sample must be used within 24 h of collection. However, among the various types of samples, a diagnostic technique using urine is favorable for patients because it is non-invasive, cost-effective, and relatively easy to diagnose [8].

The American Conference of Governmental Industrial Hygienists (ACGIH) have recently established a recommended list of Biological Exposure Indices for random urine collection. However, this method has a disadvantage related to variations of the excreted urine samples. Consequently, the values of the evaluated components of the urine will not be comparable due

* *Corresponding author: Jong-Dae Kim, Hallym University, Chunchon, Korea. Email: kimjd@hallym.ac.kr*

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to variations in the time when the sample was collected or hormone levels [8]. Because of this variability, the collected urine sample should be calibrated. In general, the sample is calibrated based on the value of creatinine measured in the urine [5, 7, 10]. Creatinine is a metabolite produced from the muscular tissue and is a core constituent of urine. According to the ACGIH, the total daily output of creatinine is approximately 1.2 g. Assuming that the average urine amount is 1.2 L (range: ~600–2500 mL), the average concentration of creatinine would be approximately 1 g/L. Based on this assumption, it is possible to calibrate the sample to a sample with an average creatinine concentration of 1 g/L. Therefore, although some urine samples might contain more or less than 1 g/L creatinine, calibration would enable effective comparisons among samples [9].

In previous studies [5, 7], the biomarkers were calibrated through dividing their concentrations by that of creatinine. In one study [7], the researchers tried to calibrate HE4 by the creatinine concentration, but they ultimately combined the log value of HE4 with that of the creatinine level ratio; these two values were combined through logistic regression. This was equivalent to the logistic regression of the log values of the HE4 and creatinine concentrations. Therefore, it is unclear whether using creatinine to calibrate other markers shows better performance than combining the creatinine equally with other markers through logistic regression. In our study, we compared the performance of the logistic regression of three markers, including creatinine, to that of two ratios obtaining by dividing the concentrations of the two markers by the creatinine concentration.

2. Materials and Methods

2.1 Data collection

Samples consist of 119 patients with benign tumor, and 137 patients with ovarian cancer. The total 256 urine samples of Korean women were provided from ASAN Medical Center. The concentrations of 20 urine protein biomarkers and creatinine 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 [11]. Analyses were performed following the protocol of the manufacturer provided by Luminex Corp., and the sample were analyzed using the Bio-Plex Suspension Array System [12]. 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) [13].

2.2 Comparison method

In order to compare the combination group in which creatinine was combined equally with other markers to the combination group in which creatinine was used only for calibration, we found 20 combinations that showed good diagnostic performance for each combination group. In the group where creatinine was combined equivalently to the other markers, 2 of the 20 biomarkers were selected and combined with creatinine through logistic regression. In the group in which creatinine was used only for calibration, the concentrations of 2 of the 20 biomarkers were divided by the creatinine concentration and the ratios were combined through logistic regression. In order to compare the combinations of all groups, multiple rounds of an average of 5-fold cross validation were performed. For cross validation, the average of the area under the receiver operating characteristic (ROC) curve (AUC) of the test set was calculated using the logistic regression coefficient obtained from the training set. The

average AUC was again averaged over the rounds, and Figure 1 illustrates the convergence of the average AUC in accordance with the increase of the number of rounds. As the round average of the average AUC converged after several hundreds of the rounds, we used the average of 1000 rounds in order to ensure sufficient convergence.

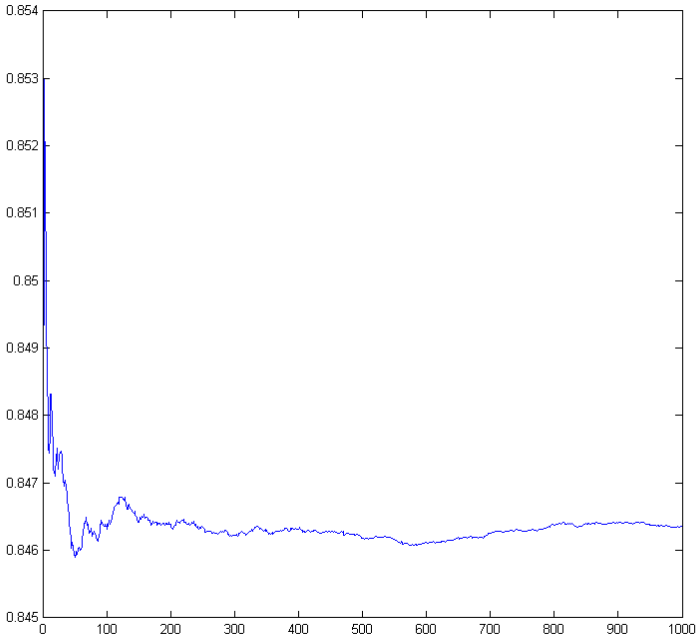


Figure 1. AUC convergence according to the number of rounds of the 5-fold cross validation

3. Results

Table 1 indicates the AUC values and 95% confidence intervals of individual markers that were calibrated by dividing the value by the creatinine level and those of the uncalibrated markers. In the table, the $AUC(\cdot)$ function refers to the AUC of the marker concentration or the score index obtained through the logistic regression, and $LR(\mathrm{m},C)$ refers to the score index obtained from the logistic regression of the concentrations of marker *m* and creatinine *C*. In general, the calibrated markers showed higher AUC values than the uncalibrated markers. There were 4 and 8 uncalibrated and calibrated markers, respectively, for which the AUC values were above 0.7, which indicates that the creatinine-calibrated markers showed improved performance. The performance of marker M13 showed the most improvement in performance, which improved by 0.1548 following calibration. Figure 2 shows the confidence intervals of the calibrated markers with AUC values above 0.7. The performance of 8 markers was substantially improved as a result of calibrating by the creatinine level, excluding marker M4, which showed similar performance using both methods.

Table 1. AUC value and 95% confidence interval of single marker

No	marker	$AUC(LR(\mathrm{m},C))$	95% CI	$AUC(\mathrm{m}/C)$	95% CI
1	M1	0.823	0.7637~0.8689	0.8929	0.8430~0.9318
2	M2	0.7931	0.7327~0.8434	0.8208	0.7620~0.8680
3	M3	0.7411	0.6789~0.7994	0.7751	0.7065~0.8261
4	M4	0.7669	0.6984~0.8193	0.7633	0.6990~0.8162
5	M5	0.6824	0.6179~0.7452	0.7383	0.6714~0.7968
6	M6	0.6887	0.6161~0.7508	0.7379	0.6777~0.7958
7	M7	0.6744	0.6101~0.7389	0.7331	0.6699~0.7944
8	M8	0.6325	0.5789~0.6847	0.7182	0.6530~0.7790
9	M9	0.6173	0.5476~0.6817	0.6839	0.6176~0.7455
10	M10	0.5714	0.4946~0.6331	0.6717	0.6040~0.7366
11	M11	0.5795	0.5069~0.6474	0.6588	0.5870~0.7235
12	M12	0.4828	0.5547~0.4059	0.6376	0.5746~0.7010
13	M13	0.6123	0.6767~0.5416	0.5889	0.6606~0.5113
14	M14	0.4528	0.3814~0.5192	0.5346	0.4575~0.6080
15	M15	0.4419	0.3731~0.5153	0.5121	0.4432~0.5830
16	M16	0.3766	0.3129~0.4463	0.4751	0.4065~0.5427
17	M17	0.5232	0.5928~0.4569	0.4286	0.4918~0.3577
18	M18	0.3939	0.3269~0.4630	0.4253	0.3542~0.4993
19	M19	0.5444	0.4740~0.6134	0.3778	0.4505~0.3075
20	M20	0.635	0.5677~0.7050	0.331	0.3998~0.2713

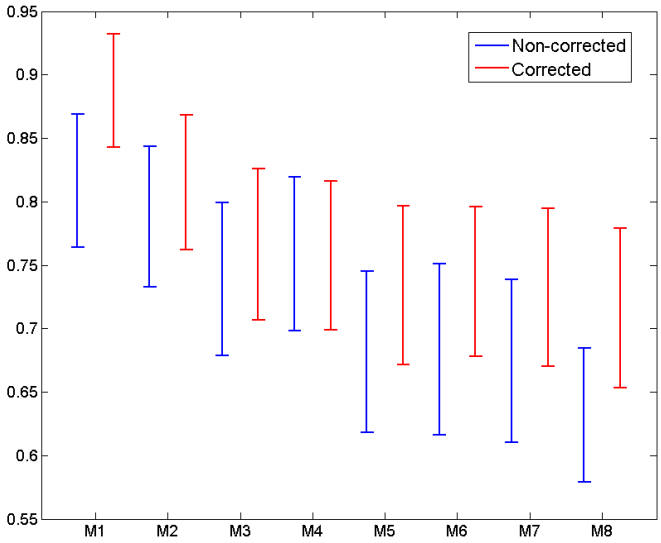


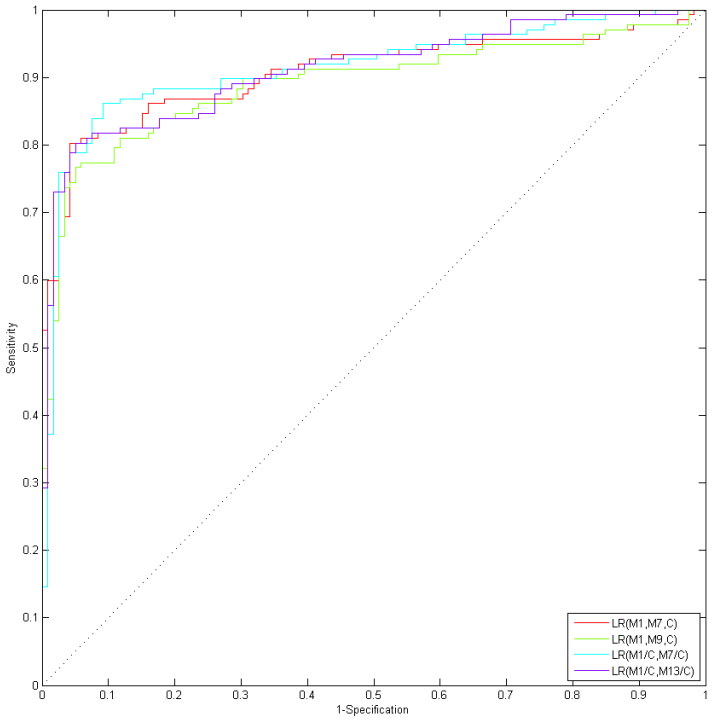
Figure 2. Graph of 95 confidence interval of top 8 markers

Table 2 shows the comparison of the performances of marker combinations when creatinine was combined equally to the other markers (columns 2 to 4) and when it was used only for calibration (columns 5 to 7). The top 20 combinations derived from the creatinine calibration group showed better performance compared to those from the uncalibrated group. In addition, the rankings of several of the marker combinations differed between the two groups. The marker M1 was included in the top 19 combinations in both groups, and thus appears to be a significant marker regardless of whether creatinine is used for calibration. The combinations that showed the best performance in both groups consisted of the same markers, although the 2nd ranking marker combination of the calibrated group slipped to 5th place when

not calibrated. In particular, the marker combination that ranked 3rd place in the calibrated group fell to 13th place when not calibrated. Because the selection of marker combinations cannot be determined by statistical criteria alone, the decision of whether or not to calibrate by creatinine seems to greatly affect the selection of marker combinations.

Table 2. Comparison of the performances of AUC values of the top 20 marker combinations

No	m1	m2	$AUC\left(LR\left(\mathrm{m1},\mathrm{m2},C\right)\right)$	m1	m2	$AUC\left(LR\left(\mathrm{m1}/C,\mathrm{m2}/C\right)\right)$
1	M1	M7	90.5	M1	M7	91.3
2	M1	M9	88.9	M1	M13	91.1
3	M1	M3	88.7	M1	M16	90.4
4	M1	M6	88.7	M1	M6	89.8
5	M1	M13	88.7	M1	M8	89.6
6	M1	M2	87.8	M1	M4	89.6
7	M1	M18	87.6	M1	M12	89.0
8	M1	M8	87.4	M1	M11	88.9
9	M1	M10	87.2	M1	M2	88.9
10	M1	M14	87.2	M1	M14	88.6
11	M1	M4	87.2	M1	M15	88.6
12	M1	M11	87.1	M1	M19	88.6
13	M1	M16	87.1	M1	M20	88.5
14	M1	M15	87.0	M1	M9	88.5
15	M1	M19	87.0	M1	M5	88.2
16	M1	M20	86.9	M1	M10	88.0
17	M1	M12	86.7	M1	M3	87.9
18	M1	M17	86.6	M1	M17	87.7
19	M1	M5	86.3	M1	M18	86.5
20	M2	M7	81.9	M2	M13	82.5



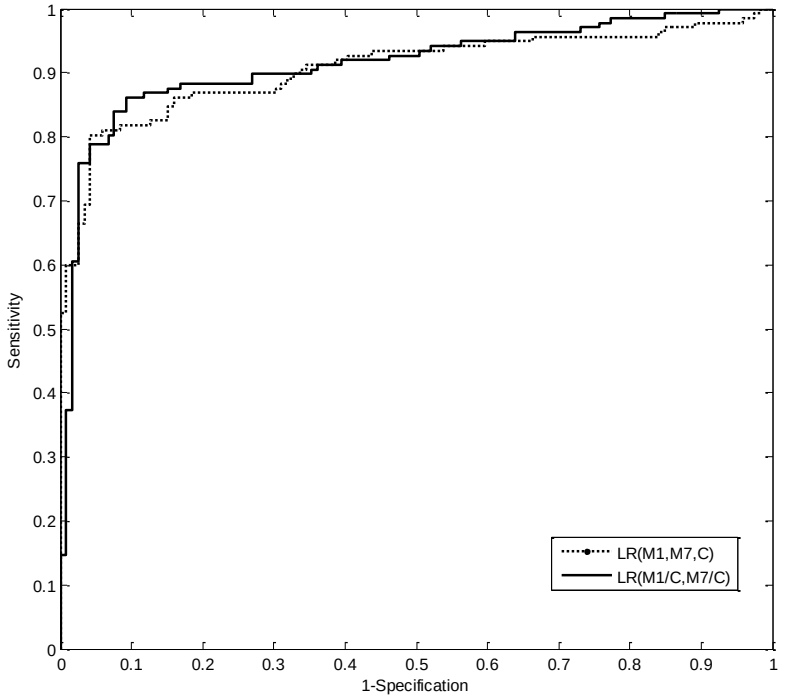


Figure 3. ROC curves showing the highest-ranked marker combinations in each combination group

Figure 3 shows the ROC curves regarding the highest ranked marker combinations in each group. The marker combination that was calibrated (solid line) clearly shows excellent sensitivity in a specificity range of 80–90%, but it is difficult to distinguish the best combinations with specificity above 90%. We also performed a Student's t-test on the top 10 markers of the two groups in order to determine whether there were differences in performance between the combinations before and after calibration. The results showed that there was indeed a significant difference in the combinations before after calibration ($p=0.002$), in which the calibrated combinations showed increased performance compared to the combinations before the calibration.

Based on the results above, we could not find any reason to believe that combining creatinine in an equivalent condition to the other markers would show any increased performance relative to calibrating by creatinine. Confirmation that creatinine can effectively calibrate the variation in the urine biomarker levels would indicate that creatinine could be used to calibrate other markers.

4. Conclusion

The present study compared the performances of combinations of urine biomarkers for diagnosing ovarian cancer when creatinine was combined equally to the other markers and when creatinine was used only to calibrate the other markers. The results of the experiment showed that using creatinine to calibrate the other markers yielded better performance in terms of the average AUC. The calibrated markers showed high diagnostic performance, and

both the maximum and minimum values of the confidence intervals of these markers were high. In addition, in the case of multiple markers, the average AUC was relatively high, and the results of the t-test of the top 10 combinations in the two groups showed that the combinations using creatinine for calibration performed significantly better. Furthermore, in the ROC comparison within the top-ranked combinations, we could not find any evidence to suggest that combining creatinine equivalently to the other markers was better than using creatinine only for calibration. In light of these results, we conclude that in multi-marker combinations, using creatinine as a urine biomarker to calibrate other markers is more desirable for diagnosing ovarian cancer as compared to using creatinine equivalently to the other markers.

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Authors

Eun-Suk Yang

She is now the B.S. student in Ubiquitous Computer Engineering of Hallym University. Her recent interests focus on biomedical system and bioinformatics.

Yu-Seop Kim

He received the Ph.D. degree in Computer Engineering from Seoul National University. He is currently a Professor in the Department of Ubiquitous Computing at Hallym University, South Korea. His research interests are in the areas of bioinformatics, computational intelligence and natural language processing.

Chan-Young Park

He received the B.S. and the M.S. from Seoul National University and the Ph.D. degree from Korea Advanced Institute of Science and Technology in 1995. From 1991 to 1999, he worked at Samsung Electronics. He is currently a Professor in the Department of Ubiquitous Computing of Hallym University, Korea. His research interests are in Bio-IT convergence, Intelligent Transportation System and sensor networks.

Hye-Jeong Song

She received the Ph.D. degree in Computer Engineering from Hallym University. She is a Professor in Department of Ubiquitous Computing, Hallym University. Her recent interests focus on biomedical system and bioinformatics

Jong-Dae Kim

He received the M.S. and the Ph.D. degrees in Electrical Engineering from Korea Advanced Institute of Science and Technology, Seoul, Korea, in 1984 and 1990, respectively. He worked for Samsung Electronics from 1988 to 2000 as an electrical engineer. He is a Professor in Department of Ubiquitous Computing, Hallym University. His recent interests focus on biomedical system and bioinformatics.