Multi-Biomarker Panel Prediction Model for Diagnosis of Pancreatic Cancer: a retrospective and multi-center cohort study

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Research

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Abstract

Background

Early diagnosis is paramount in increasing the survival rate of pancreatic ductal adenocarcinoma (PDAC). However, effective early diagnostic tools are lacking at present. The current study aimed to develop a prediction model using a multi-marker panel (LRG1, TTR, and CA19-9) as a diagnostic screening tool for PDAC.

Methods

A large multi-center cohort of 1,991 samples were collected from January 2011 to September 2019, of which 609 are normal (NL), 145 are other cancer (OC; colorectal, thyroid, and breast cancer), 314 are pancreatic benign disease (PB), and 923 are PDAC. The automated multi-biomarker Enzyme-Linked Immunosorbent Assay kit was developed using three potential biomarkers, LRG1, TTR, and CA 19 – 9. Using a logistic regression (LR) model trained on training data set, the predicted values for PDACs were obtained, and the result was classified into one of the three risk groups: low, intermediate, and high. The five covariates used to create the model were sex, age, and biomarkers TTR, CA 19 – 9, and LRG1.

Results

Participants were categorized into four groups as NL (n = 609, 30.6%), OC (n = 145, 7.3%), PB (n = 314, 15.7%), and PDAC (n = 923, 46.4%). The NL, OC, and PB groups were clubbed into the non-PDAC group (n = 1068, 53.6%). The positive and negative predictive value, sensitivity, and specificity were 94.12, 90.40, 93.81, and 90.86, respectively.

Conclusions

This study demonstrates a significant diagnostic performance of the multi-marker panel in distinguishing PDAC from normal and benign pancreatic disease states, as well as patients with other cancers. The study indicates that the introduced multi-marker panel prediction model for PDAC diagnosis can help guide medical decisions for patients, including patients with early stage PDAC or with normal levels of CA 19 – 9.

Background

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal gastrointestinal malignancies and is the seventh leading cause of cancer-related deaths [1, 2]. Despite improving perioperative outcomes, PDAC has a poor prognosis, with a 5-year survival rate of only 8–10% among patients [1–3]. Most patients are diagnosed in the advanced stages, and effective systemic therapies are lacking. Approximately 80% of patients with PDAC are detected at the unresectable or metastatic stage [1, 2].

Therefore, it is essential to improve early detection of PDAC to increase the survival rate. However, current imaging modalities, such as computed tomography (CT) or magnetic resonance imaging (MRI), are not suitable as screening tests for pancreatic cancer due to limitations of cost-effectiveness, time consumption, and capacity of inspection [4].

The only biomarker for pancreatic cancer that is clinically approved by the US Food and Drug Administration (FDA) is serum carbohydrate antigen 19 – 9 (CA 19 – 9) which has approximately 79% sensitivity and 82% specificity. However, CA 19 – 9 is ineffective for early diagnosis of PDAC in asymptomatic patients, and there is no individual marker that diagnoses PDAC with satisfactory sensitivity and specificity [5, 6]. Thus, there is an unmet need for a clinical method that can effectively differentiate pancreatic malignancy from normal, benign states, and other malignancies [6].

Increasing efforts to identify tumor-specific biomarkers have been made over the past few decades; however, the translation of these novel biomarkers into clinical practice has been very limited [7–9]. There are some assays that had been approved by the FDA for certain cancers, but none of these were introduced for pancreatic cancer except for CA 19 – 9 [10]. A microarray-based biomarker test (IMMray™ PanCan-d) for PDCA was introduced and approved by the FDA; however, owing to its high cost, it may not be practical to be used as a screening tool [11].

Recently, two studies reported a multi-biomarker microarray, externally validated in a large cohort, using the serum of patients for early detection of PDAC [6, 11]. However, some requirements for an ideal screening test, such as cost-effectiveness and simplified usage were still lacking.

Park et al. reported that a multi-marker panel using a triple-marker, validated in large-scale samples by multiple reaction monitoring-mass spectrometry (MRM-MS) and immunoassay, has clinical applicability in the early detection of PDAC [6]. The panel including leucine-rich alpha-2 glycoprotein (LRG1), transthyretin (TTR), and CA 19 – 9 had a sensitivity of 82.5% and a specificity of 92.1%. The triple-marker panel exceeded the

diagnostic performance of CA 19 – 9 by more than 10% (Area under ROC curve (AUC); $AUC_{CA19-9} = 0.826$, $AUC_{panel} = 0.931$, $p < 0.01$) in all PDAC samples and by more than 30% ($AUC_{CA19-9} = 0.520$, $AUC_{panel} = 0.830$, $p < 0.001$) in patients with a normal range of CA 19 – 9 [6].

For a diagnostic screening tool for PDAC, the multi-marker panel needs to be validated using cohorts from the normal population, benign pancreatic diseases, and other malignancies except PDAC. Hence, the current study aimed at developing a prediction model as a diagnostic screening tool for PDAC using a multi-marker panel (LRG1, TTR, and CA19-9) in a large cohort including multiple centers.

Materials And Methods

Overview of study design

The automated multi-biomarker ELISA kit was developed using three potential biomarkers, LRG1, TTR, and CA 19-9, that were identified in a previous study using MRM-MS, and for which external validation was performed at multiple centers [6].

To validate our proposed predictive model using the multi-marker (TTR, CA19-9, and LRG1) Enzyme-Linked Immunosorbent Assay (ELISA) kit developed in a previous study, we collected a large amount of data including various types of data sets. Up to 1,991 samples were collected from various institutions. This included pancreatic benign disease (PB; chronic pancreatitis, intra-pancreatic mucinous neoplasm, neuroendocrine tumor, solid pseudopapillary neoplasm, mucinous cystic neoplasm, serous cystadenoma, pseudocyst, and pancreatolithiasis), other cancer (OC; breast, thyroid, and colorectal cancers), normal (NL), and PDAC. This was used for model development and verification of our proposed prediction model (Figure 1). The training data set was created by extracting 70% of NL and PDAC type data. The test data set was created by combining the remaining 30% of NL and PDAC, and all of the OC and PB data (Table 1). The NL and PDAC type data were randomly sampled at the same rate to prevent data skew. In addition, the values of the 3 markers used in the experiment were measured using the ELISA kit and log-transformed (Figure 1).

Table 1. The number of samples of training and test data set

		NL	OC	PB	PDAC	Total
Data set	Training	426	0	0	647	1,073
	Test	183	145	314	276	918
	Total	609	145	314	923	1,991

PDAC, pancreatic ductal adenocarcinoma; NL, normal; OC, other cancer; PB, pancreatic benign

Next, using the Logistic Regression (LR) method, a model developed on the training data set was used to obtain the predicted value for PDAC, and the result was classified into one of the three risk groups: low, intermediate, and high. To determine the risk groups, the predicted value for PDAC obtained through the model was divided using a combination of two thresholds. The optimal threshold was determined using four measures for the three risk groups: positive predictive values (PPV), negative predictive values (NPV), sensitivity (Sen), and specificity (Spe). The four measures were used as evaluation indicators for the predictive model. In this study, emphasis was placed on determining the cutoff value when the four measures attained a certain value at the same time.

Lastly, as the most important part of this study, it was confirmed that NL and PDAC data-based models showed a good performance in tests using OC and PB data. For this, model verification was conducted using large amount of data of various types including NL, PDAC, OC, and PB as test data sets. At this time, NL vs. PDAC, OC vs. PDAC, PB vs. PDAC, and non-PDAC (all test data set) vs. PDAC data set were each tested to compare and analyze the performance of the model for each type of data set.

Study Population

A total of 1,991 samples were collected between January 2011 and September 2019, of which 609 were NL, 145 were OC, 314 were PB, and 923 were PDAC. These data were collected from various centers in Korea including the Seoul National University Hospital (SNUH) or Seoul National University Hospital Healthcare System Gangnam Center (SNUH HSGC), National Cancer Center (NCC), Asan Medical Center (AMC), Samsung Medical Center (SMC), Yonsei Severance Hospital (YSH), and Ewha Womans University Medical Center (EUMC) (Table 2).

Table 2. The number of samples by Institute

Institute	NL	OC	PB	PC	All
SNUH	563	145	40	294	1,042
AMC	0	0	157	254	411
NCC	0	0	0	128	128
SMC	0	0	90	200	290
YMC	0	0	27	47	74
EUMC	46	0	0	0	46
Total	609	145	314	923	1,991

PDAC, pancreatic ductal adenocarcinoma; NL, normal; OC, other cancer; PB, pancreatic benign; SNUH, Seoul National University Hospital; NCC, National Cancer Center; AMC, Asan Medical Center; SMC, Samsung Medical Center; YMC, Yonsei Severance Hospital; EUMC, Ewha womans University Medical Center

This large-scale, retrospective, and multi-center study was approved by the institutional review boards of all participating institutions *(SNUH, H-1703-005-835, YSH; 4-2013-0725, NCC; NCCNCS13818, SMC; 2008-07-065, AMC; 2013-1061, EUMC; 2018-05-030).* Bio-specimens were collected from participants who provided informed consent.

The development of the predictive model was carried out using the LR method using age, sex, and the three ELISA markers, TTR, CA19-9, and LRG1 as covariates. The predicted values were classified into low-, intermediate-, and high-risk groups using a combination of two thresholds δ_1 and δ_2. The optimal threshold combination was the one that yielded the highest average for the four measures NPV, PPV, Sen, and Spe when all four measures exceeded the cutoff value simultaneously. The four measures were modified for the three risk groups by considering only the high- and low-risk groups for simplicity. The four modified measures were calculated without the intermediate group as follows:

$$NPV = \frac{n_{11}}{n_{11}+n_{21}}, PPV = \frac{n_{23}}{n_{13}+n_{23}}, Sen = \frac{n_{23}}{n_{21}+n_{23}}, Spe = \frac{n_{11}}{n_{11}+n_{13}}.$$

For NL, n_{11} represents the count of predicted probability smaller than δ_1, n_{12} the count between δ_1 and δ_2, and n_{13} the count larger than δ_2. For PDAC, n_{21}, n_{22}, and n_{23} represent the corresponding counts, respectively. Cutoff is defined as the value when all the four measures obtain a certain value, such as 90% at minimum. The mean of the four measures and the number of intermediates were determined by selecting a threshold combination. Since the four measures were calculated excluding the intermediate group, performance was highly dependent on the count of the intermediate group. In this study, the cutoff values ranging 85% to 95% were checked in increments of 1 unit following the various threshold combinations (Table 3).

Table 3. Cut-off values of measures, threshold change and number of risk groups from training data set

measures > cut-off	95%	94%	93%	92%	91%	**90%**	89%	88%	87%	86%	85%
	0.09	0.12	0.14	0.16	0.21	**0.22**	0.25	0.26	0.29	0.3	0.32
	0.96	0.94	0.92	0.89	0.89	**0.88**	0.87	0.86	0.82	0.8	0.79
PPV	97.7273	96.2766	96.6245	95.8478	95.8478	**94.1177**	92.6380	92.6346	91.9315	91.3753	91.1161
NPV	96.4706	94.0678	93.4307	92.1569	91.579	**90.4040**	89.2377	88.9381	87.0079	86.0902	85.5124
Sen	97.7273	96.2766	96.2185	95.8478	94.5393	**93.8111**	92.6380	92.8977	91.9315	91.3753	90.7030
Spe	96.4706	94.0678	94.1177	92.1569	93.5484	**90.8629**	89.2377	88.5463	87.0079	86.0902	86.1210
measures mean	97.0989	95.1722	95.09782	94.0023	93.8786	**92.2989**	90.9379	90.7542	89.4697	88.7328	88.3631
High	132	188	237	289	289	**306**	326	353	409	429	439
Intermediate	856	767	699	631	594	**569**	524	494	410	378	351
Low	85	118	137	153	190	**198**	223	226	254	266	283

Performance comparison by various cut-off values and verifying the performance of a predictive model using training data set. The first columns list the threshold values, four evaluation measures, and the number of patients in three risk groups. The other columns summarize the results for training dataset for the various cut-off values from 85 to 95%. PPV, positive predictive values; NPV, negative predictive values; Sen, sensitivity; Spe, specificity

Statistical analysis

The demographic analysis was performed using R ver. 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria). It was also used to create the graphical representations. The categorical variables of the non-PDAC (NL, OC, and PB) and PDAC data groups were compared using the chi-square test. Continuous variables were summarized using the means and standard deviations or median with range (25-percentile to 75-percentile) and compared using the F-test and Student's/Welch's t-test. Two-sided p-values < 0.05 were considered significant.

Results

Characteristics of the study population

Participants were categorized into four groups based on the diagnosis as NL (n=609, 30.6%), OC (n=145, 7.3%), PB (n=314, 15.7%), and PDAC (n=923, 46.4%). The NL, OC, and PB groups were together clubbed into the non-PDAC group (n=1068, 53.6%). Demographics of the participants in the non-PDAC and PDAC groups are summarized in Table 4. Significant differences were observed in age (Non-PDAC; 55.5 ± 12.0 versus PDAC; 63.1 ± 9.9 years, $p < 0.001$), sex ratio (females: n=474, 44.4% vs. males: n=561, 60.8%, $p < 0.001$), body mass index (23.6 ± 3.2 versus 22.9 ± 3.0 kg/m^2, $p = 0.001$), level of initial CA 19-9 (19.0 ± 98.6 versus 679.0 ± 1348.9 U/ml, $p < 0.001$), and level of LRG1, TTR, CA 19-9 in automated ELISA triple marker panel between the non-PDAC and PDAC groups. Levels of LRG1 and CA 19-9 were higher in the PDAC group than in the non-PDAC group, whereas the level of TTR was lower in the PDAC group than in the NL and OC groups.

Table 4. Demographics of study population

	Total	Non-PDAC			Non-PDAC	PDAC	*p*-value (Non-PDAC versus PDAC)
		NL	OC	PB			
Number	1991 (100.0)	609 (30.6)	145 (7.3)	314 (15.7)	1068 (53.6)	923 (46.4)	
Age, years	59.0 ± 11.7	56.4 ± 11.0	55.2 ± 11.5	53.9 ± 13.8	55.5 ± 12.0	63.1 ± 9.9	< 0.001
Sex							< 0.001
Male	1035 (52.0)	322 (52.9)	37 (25.5)	115 (36.6)	474 (44.4)	561 (60.8)	
Female	956 (48.0)	287 (47.1)	108 (74.5)	199 (63.4)	594 (55.6)	362 (39.2)	
BMI, kg/m^2	23.3 ± 3.1	23.8 ± 2.9	23.3 ± 3.3	23.2 ± 3.8	23.6 ± 3.2	22.9 ± 3.0	0.001
Initial CA 19-9, U/ml		11.7 ± 33.3	61.7 ± 261.9	14.6 ± 23.7	19.0 ± 98.6	679.0 ± 1348.9	< 0.001
Initial CEA, ng/ml		1.2 ± 0.8	26.6 ± 137.2	2.0 ± 3.2	7.4 ± 67.2	29.6 ± 231.5	0.087
Automated ELISA triple marker panel*							
LRG1, ng/ml		11324 (8348-16281)	11427 (8531-15321)	9351 (7341-12585)	10697 (7909-15138)	16836 (11182-25638)	< 0.001
TTR, ng/ml		286200 (154548-432460)	302800 (254720-342560)	151525 (127697-215568)	227910 (146600-365920)	150112 (123266-230000)	< 0.001
CA 19-9, U/ml		11.0 (6.5-18.8)	10.6 (7.9-17.3)	10.8 (5.3-21.1)	10.8 (6.6-19.2)	118.6 (26.5-537.9)	< 0.001

Data are expressed as n (%) or mean (standard deviation) unless indicated otherwise; *values are median (25 percentile to 75 percentile)

PDAC, pancreatic ductal adenocarcinoma; NL, normal; OC, other cancer; PB, pancreatic benign; BMI, body mass index; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; ELISA, enzyme-linked immunosorbent assay; LRG, leucine rich alpha 2 glycoprotein; TTR, transthyretin

Diagnostic model and determining cutoff value

An LR-based prediction model was created using NL and PDAC data from an automated multi-panel ELISA kit. The five covariates used to create the LR model were sex, age, and the three biomarkers TTR, CA 19-9, and LRG1. The fitted LR model is represented as follows:

$$\log\left(\frac{P(PDAC)}{1-P(PDAC)}\right) = 51.03 + 0.04Age + 1.19(Sex\ M),$$

$$-5.12\log(TTR) + 0.61\log(CA19-9) + 0.80\log(LRG1).$$

Through the model thus obtained, the predicted value of PDAC incidence for each sample of the training data was obtained and classified into low, intermediate, and high-risk groups using two thresholds (Figure 2a and b). In order to establish the feasibility for clinical use along with better performance, the optimal threshold combination was evaluated. We checked the cutoff values from 85% to 95%. Considering the mean of the four measures, and the sample numbers of low-, intermediate -, and high-risk groups, the threshold combination of 0.22 and 0.88 when all four measure values exceeded 90% were selected (Table 5). The mean of the four measures at this time was 92.2989, and the values of PPV, NPV, Sen, and Spe were 94.1177, 90.4040, 93.8111, and 90.8629, respectively. At threshold combinations of 0.22 and 0.88, the cutoff was 90%, and the number of samples in the high-, intermediate-, and low-risk groups were 306, 569, and 198, respectively.

Table 5. Comparison of measures and number of risk groups from various data set at cut-off 90%

Data set	Training	Test (NL vs PDAC)	Test (OC vs PDAC)	Test (PB vs PDAC)	Test (non-PDAC vs PDAC)
PPV	94.1177	93.9850	93.2836	88.6525	79.1139
NPV	90.4040	92.2222	91.5663	91.7647	97.1312
Sen	93.8111	94.6970	94.6970	94.6970	94.6970
Spe	90.8629	91.2088	89.4118	82.9787	87.7778
measures mean	92.2989	93.0282	92.2397	89.5232	89.680
High	306	133	134	141	158
Intermediate	569	236	204	364	516
Low	198	90	83	85	244

PDAC, pancreatic ductal adenocarcinoma; NL, normal; OC, other cancer; PB, pancreatic benign; PPV, positive predictive values; NPV, negative predictive values; Sen, sensitivity; Spe, specificity

Verification using various types of test data sets

To evaluate the performance of the predictive model derived from the training data set, it was verified using the test data. During this, by using the PB and OC types data in addition to NL and PDAC, it was demonstrated that the model had reliable performance prediction ability even when various types of data were provided. First, during modeling using training data, four measure values at various threshold combinations and sample numbers of low -, intermediate -, and high-risk groups were identified. It was found that at the threshold combination of 0.22 and 0.88, which was the optimal threshold combination, the cutoff value was 89.680. This was not significantly different from 92.1989 when training data was used (p = 0.573) (Table 6). The values of PPV, NPV, Sen, and Spe were 79.1139, 97.1312, 94.6970, and 87.7778, respectively, showing an overall good performance except for the PPV (Table 6).

Table 6. Cut-off values of measures, threshold change and number of risk groups from test data set

measures > cut-off	95%	94%	93%	92%	91%	**90%**	89%	88%	87%	86%	85%
	0.09	0.12	0.14	0.16	0.21	**0.22**	0.25	0.26	0.29	0.3	0.32
	0.96	0.94	0.92	0.89	0.89	**0.88**	0.87	0.86	0.82	0.8	0.79
PPV	84.2857	86.6667	83.6207	80.2817	80.2817	**79.1139**	76.7857	75.9777	72.9730	71.4876	70.2381
NPV	99.0741	98.6014	98.2456	98.4375	97.0086	**97.1312**	96.7626	96.7972	95.5128	95.6386	94.9704
Sen	98.3333	97.50	97.0	97.4359	94.2149	**94.697**	93.4783	93.7931	92.0455	92.5134	91.2371
Spe	90.6780	92.1569	89.8396	87.0968	89.0196	**87.7778**	87.3377	86.3492	83.2402	81.6489	81.0606
measures mean	93.0928	93.7312	92.1765	90.8130	90.1312	**89.680**	88.5911	88.2293	85.9429	85.3221	84.3766
High	70	90	116	142	142	**158**	168	179	222	242	252
Intermediate	740	685	631	584	542	**516**	472	458	384	355	328
Low	108	143	171	192	234	**244**	278	281	312	321	338

Performance comparison by various cut-off values and verifying the performance of a predictive model using all test data set.

PPV, positive predictive values; NPV, negative predictive values; Sen, sensitivity; Spe, specificity

Next, we checked the accuracy of classifying NL, OC, PB, and PDAC into one of the three risk groups (Table 7). When 0.22 and 0.88 were selected as the threshold combination, the number of samples belonging to the high-, intermediate-, and low-risk groups for NL type was 8, 92, and 83,

respectively. On the other hand, the corresponding numbers for PDAC type were 125, 144, and 7. Eight NL samples belonged to the high-risk group and the PDAC samples belonging to the low-risk group were seven. Therefore, we confirmed that our proposed prediction model has good prediction performance for PDAC. These results are demonstrated through the box plot and density plot (Figure 2c–f).

Table 7. Comparison of classified table of type into the predicted low, intermediate, and high risk groups

Group	Low	Inter	High	Low	Inter	High	Low	Inter	High	Low	Inter	High
Cut-off		0			95			94			93	
NL	21	160	2	42	138	3	52	127	4	63	115	5
OC	27	115	3	41	100	4	50	91	4	59	81	5
PB	12	302	0	24	286	4	39	271	4	46	259	9
PDAC	0	264	12	1	216	59	2	196	78	3	176	97
Cut-off		92			91			**90**			89	
NL	72	105	6	81	96	6	**83**	**92**	**8**	96	77	10
OC	63	74	8	72	65	8	**76**	**60**	**9**	82	53	10
PB	54	246	14	74	226	14	**78**	**220**	**16**	91	204	19
PDAC	3	159	114	7	155	114	**7**	**144**	**125**	9	138	129
Cut-off		88			87			86			85	
NL	97	75	11	105	65	13	108	59	16	113	54	16
OC	82	53	10	93	39	13	94	37	14	99	31	15
PB	93	199	22	100	180	34	105	170	39	109	161	44
PDAC	9	131	136	14	100	162	14	89	173	17	82	177

PDAC, pancreatic ductal adenocarcinoma; NL, normal; OC, other cancer; PB, pancreatic benign; Inter, intermediate

Lastly, we compared the mean of the four measures by using test data of each data type (Table 5). The prediction model had been trained on NL and PDAC data, but we intended to check if the model had effective performance in other data categories such as PB and OC. For this purpose, the test data of each type were compared in pairs, such as NL vs. PDAC, OC vs. PDAC, PB vs. PDAC, and non-PDAC (NL, OC, and PB) vs. PDAC. The mean of the four measures was highest at 93.0282 when verified with the test data set of NL and PDAC type combination. In contrast, the mean was the lowest at 89.5232 when PB and PDAC type data were used. In case of non-PDAC vs. PDAC the mean was 89.680, showing a moderate performance between NL vs. PDAC and PB vs. PDAC. This result indicates that PB has intermediate properties between NL and PDAC, as shown in the above table, density plot, and box plot. Based on these results, it was confirmed that OC has characteristics close to NL and that PB does not belong to either NL or PDAC.

Finally, even when different types of data including NL, PDAC, OC, and PB were tested, the proposed prediction model demonstrated satisfactory performance.

Discussion

In this large-scale, retrospective, and multi-center study, we introduced a prediction model for PDAC diagnosis using a multi-marker panel consisting of LRG1, TTR, and CA19-9 developed by conventional immunoassays and a high-throughput assay, followed by an advanced statistical machine-learning approach. The multi-marker panel was validated using bio-specimens of non-PDAC (NL, OC, PB), and PDAC in a large-scale cohort. Validated across various types of data including NL, OC, PB, and PDAC, the current proposed prediction model demonstrated satisfactory performance as a diagnostic screening tool for PDAC.

A multi-marker panel has been developed and used in the field of ovarian cancer prior to pancreatic cancer. Recently, several studies reported that a panel of biomarker signatures is a potential tool for prototype development in the future and may be used to develop other advanced approaches for early diagnosis of ovarian cancer that could be used as a screening tool to avoid false-diagnosis and excessive cost [12–15]. Ova1 is a multivariate index assay approved by the FDA, comprising a 5-serum protein biomarker panel for the triage of patients with pelvic mass into low- or high-risk ovarian cancer [15]. It is comprised of second-generation CA125-II, transferrin, beta-2 microglobulin, apolipoprotein A-1, and transthyretin [15]. The Ova1 performance score produced 96% Sen at 35% Spe in 590 women slated for resection of ovarian tumor [16, 17]. In

addition, the FDA approved the next generation of Ova1, the Overa in 2016, exhibiting 91% Sen and 69% Spe [16]. However, there are no similar approved screening tools for the diagnosis of PDAC in clinical practice.

The current triple marker panel consists of TTR, LRG1, and CA 19 − 9. TTR is synthesized by pancreatic islets and is involved in pancreatic β-cell death and insulin release [18]. Moreover, TTR is decreased in type 1 diabetes mellitus, yet is highly abundant in pancreatic juice because the pancreatic islets are destroyed, allowing proteins to leak into the pancreatic ductal system [19, 20]. PDAC patients often experience malnutrition, lowering the levels of TTR, which is involved in energy intake, acute or chronic disease states, nutritional status, and inflammatory processes [21]. LRG1 levels are elevated in the blood of patients with non-small-cell lung cancer, ovarian cancer, colorectal cancer, and gliomas through transforming growth factor beta (TGF-β) signaling, which promotes endothelial cell proliferation and angiogenesis [22–26]. In addition, LRG1 is associated with endothelial dysfunction, arterial stiffness, and peripheral arterial disease in patients with type 2 diabetes [27]. LRG1 levels were elevated in the blood of patients with PDAC because of TGF-B signaling [28].

Recently, Mellby et al. reported a case-control study using a Scandinavian cohort, consisting of 16 patients with stage I, 132 patients with stage II, 65 patients with stage III, and 230 patients with stage IV PDAC, and 888 controls using an antibody microarray platform to identify the serum biomarker signature associated with early stage PDAC [11]. The biomarker signature could discriminate patients with stage I and II PDAC from controls in this independent patient cohort with an AUC of 0.96. However, the study had several limitations such as cost-effectiveness and lack of simplified usage. Moreover, the previous studies were not validated using patients with benign pancreatic disease and other malignancies excluding PDAC [11, 29]. In the current study, to ensure practical utility of biomarker panels, ELISA was used, making it minimally invasive and cost-effective as compared to multiple reaction monitoring mass spectometry (MRM-MS). Through the use of ELISA, the platform is standardized, and can be used universally at a low cost and easier quality assurance.

The current study used a diagnostic algorithm including age, sex, and triple markers to differentiate PDAC from non-PDAC. The algorithm for risk calculation provides a risk stratification to identify the actual likelihood of malignancy. At threshold combinations of 0.22 and 0.88, the cutoff was 90%, and the number of samples in the low -, intermediate -, and high-risk groups were 198, 569, and 306, respectively. Because of the existence of the intermediate group, the high-risk group and the low-risk group are more clearly distinguished, overcoming the shortcomings of a dichotomous classification, such as that using CA 19 − 9 alone. This model may better help the clinicians' judgment. Therefore, when this multi-marker panel is used in actual clinical practice as a diagnostic screening tool for PDAC, proper management guidelines for each group should be prepared. Additional strategies for targeting each group should be addressed in further studies. The 'Intermediate-risk group' may require re-evaluation using multi-marker panel and/or additional imaging studies including abdominal CT or MRI to confirm the presence of pancreatic neoplasm. If participants are categorized in the high-risk group, they are highly suspicious of having PDAC and thus require more precise examination or other interventions for confirming the diagnosis. The participants in the low-risk group may not need any further examinations.

The current prediction model had PPV, NPV, Sen, and Spe of 94.12, 90.40, 93.81, and 90.86, respectively, with the mean of the four measures being 92.29. Plasma LRG1 and TTR levels, with CA19-9, had a greater diagnostic value for PDAC than CA19-9 alone [5, 6]. Through this multi-marker panel, the ability to distinguish not only the NL group but also the OC and PB groups versus PDAC makes it more appropriate to be used for screening pancreatic cancer when compared to other studies [11, 29]. Although 10–15% of PDAC patients do not express CA19-9 due to the lack of Lewis A antigen [5], early stage pancreatic cancer can be distinguished by using the current multi-marker panel, thereby improving survival in patients with pancreatic cancer [6]. Finally, the multi-marker panel could lead to early diagnosis, reduce the costs of screening and treatment, and lengthen survival. It could also improve the quality of life of patients with PDAC because fewer invasive procedures would be performed and ineffective treatments would be withdrawn [6].

Conclusions

The current study is the first multi-center and large-scale corroboration for constructing a prediction model to be used as screening tool for PDAC diagnosis using a multi-marker panel (LRG1, TTR, and CA 19 − 9). This study demonstrates a significant diagnostic performance of the multi-marker panel in distinguishing PDAC from normal, benign pancreatic disease states, and patients with other cancers. The model satisfies the requirements of an ideal screening test, being simple to use, less expensive, and having a good diagnostic efficacy with NPV, PPV, Sen, and Spe, all greater than 90.0%. This model may be used for early diagnosis and can help prolong survival in PDCA patients, including in patients with early stage PDAC or patients with normal values of CA 19 − 9.

Lisf Of Abbreviations

PDAC: Pancreatic ductal adenocarcinoma, CT: computed tomography, MRI: magnetic resonance imaging, FDA: Food and Drug Administration, CA 19 − 9: carbohydrate antigen 19 − 9, MRM-MS: multiple reaction monitoring-mass spectrometry, LRG1: leucine-rich alpha-2 glycoprotein, TTR: transthyretin, AUC: Area under ROC curve, ELISA: Enzyme-Linked Immunosorbent Assay, PB: pancreatic benign disease, OC: other cancer, NL: normal, LR: logistic regression, PPV: positive predictive values, NPV: negative predictive values, Sen: sensitivity, Spe: specificity, SNUH: Seoul

National University Hospital, SNUH HSGC: Seoul National University Hospital Healthcare System Gangnam Center, NCC: National Cancer Center, AMC: Asan Medical Center, SMC: Samsung Medical Center, YSH: Yonsei Severance Hospital, EUMC: Ewha Womans University Medical Center

Declarations

Ethics approval and consent to participate

This large-scale, retrospective, and multi-center study was approved by the institutional review boards of all participating institutions *(SNUH, H-1703-005-835, YSH; 4-2013-0725, NCC; NCCNCS13818, SMC; 2008-07-065, AMC; 2013-1061, EUMC; 2018-05-030)*. Bio-specimens were collected from participants who provided informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

Conception and design: JYJ and TP.

Acquisition of data: AL, YH, YB, JSK, HK, WK, YAS, JSH, IWH, JOP, JKP, SCK, EJ, CMK, WJL, HKL, HL, SYJ, KEL, and WH.

Analysis and interpretation of data: DHL, WY, TP and SL.

Contributed analysis tools: YC, JN, SH, and SGY.

Drafting the article: DHL and WY.

Revising the manuscript: DHL, WY, JYJ and TP.

Study supervision: JYJ and TP.

All authors read and approved the final manuscript.

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Figures

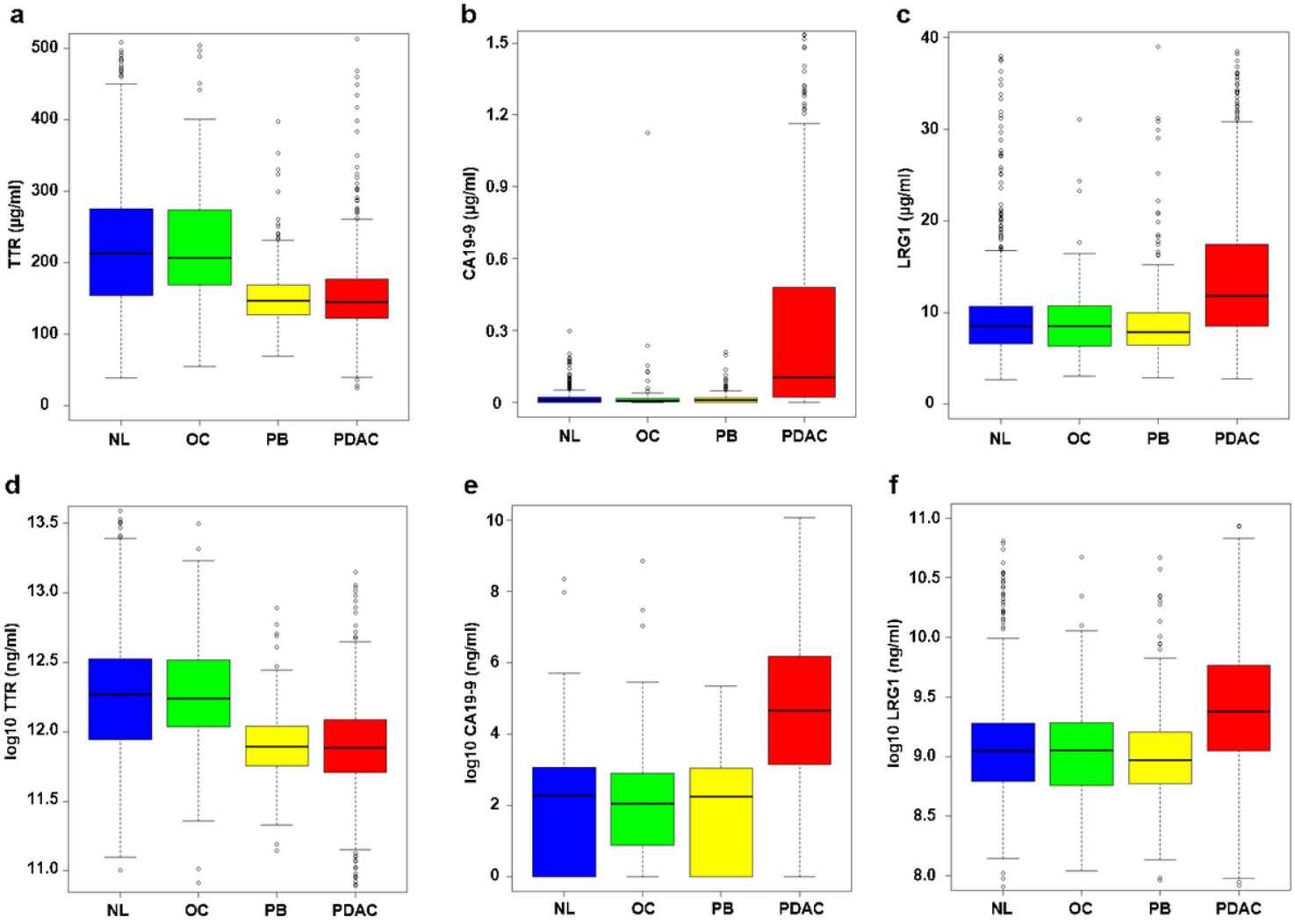

Figure 1

Box plots for expressions of LRG1, TTR, and CA19-9. Comparison of (a) LRG1, (b) CA19-9 and (c) TTR from ELISA kit by various types. The levels of log-transformed of (d) LRG1, (e) CA19-9 and (f) TTR are also shown.

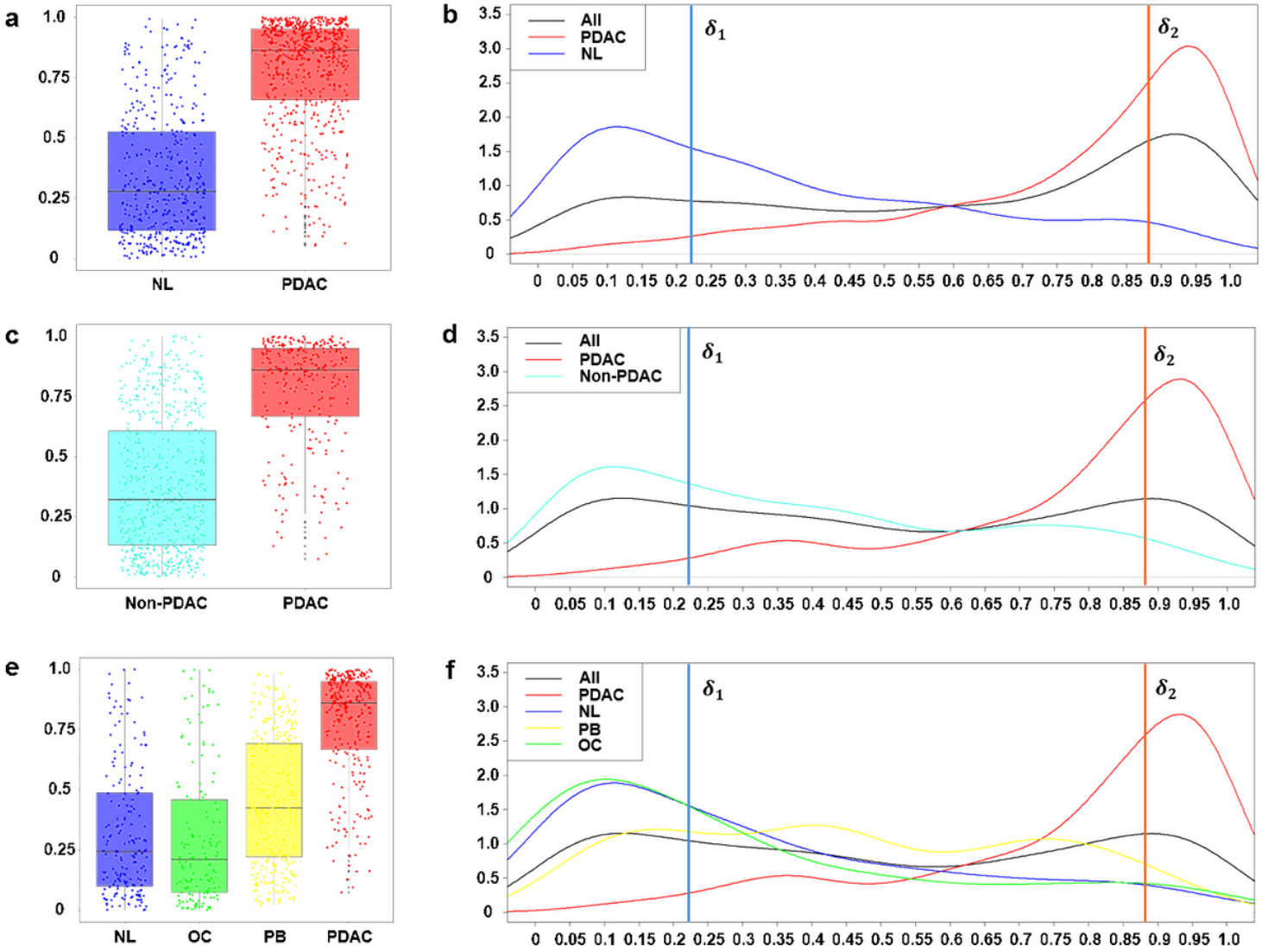

Figure 2

Predicted value from training and test data set. The box plot (a), and density plot (b) shows that the predicted values from prediction model using training data set. The predicted value using data set by all types (c and d) and each type (e and f) are also shown. Sky blue and orange line indicate threshold value (δ_(1,) δ_2)=(0.22, 0.88) at cut-off 90%.