Cancer Biomarkers 33 (2022) 503–512
DOI 10.3233/CBM-210198
IOS Press

Risk stratification of pancreatic cancer by a blood test for apolipoprotein A2-isoforms

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Received 6 April 2021
Accepted 16 June 2021

Abstract. Though pancreatic cancer is uncommon, with an age-adjusted annual incidence of 12.9 cases per 100,000 person-years, it is considered a refractory cancer due to the mortality of 11.0 per 100,000 person-years. To efficiently identify patients with potentially surgically-curable pancreatic cancer, high-risk individuals (HRIs) for pancreatic cancer should be identified by easily and minimally invasive methods from the general population. We have identified unique processing patterns in the C-terminal amino acids of apolipoprotein A2 homodimer in the blood of patients with pancreatic cancer and in HRIs, and we called them apoA2-isoforms (apoA2-i). We then established an enzyme-linked immunosorbent assay (ELISA) to measure circulating apoA2-i in the blood stream. The diagnostic accuracy of apoA2-i to distinguish pancreatic cancer HRIs was verified by several retrospective studies, blind testing with the National Cancer Institute (NCI) Early Detection Research Network (EDRN), a prospective study with prediagnostic samples organized by the European Prospective Investigation into Cancer and Nutrition (EPIC) study, and the prospective screening study of pancreatic cancer in Kobe.
The apoA2-i blood test is a potential biomarker to identify HRIs and the curative stage of pancreatic cancer in the general population.

Keywords: Biomarkers for risk stratification of pancreatic cancer, pancreatic cancer, apolipoprotein A2-isoforms (apoA2-i), intraductal papillary mucinous neoplasm (IPMN)

1. Introduction

The age-adjusted annual incidence of pancreatic ductal adenocarcinoma (PDAC) is 12.9 cases per 100,000 persons per year. On the other hand, the mortality is 11.0 deaths per 100,000 persons per year, with the incidence and mortality of PDAC being similar. In 2020, an estimated 42,700 patients were diagnosed with pancreatic cancer, and 36,700 patients died with pancreatic cancer in Japan. In the United States, the 5-year overall survival rate of pancreatic cancer patients was only 9% [1], and the overall survival rate of pancreatic cancer patients was 7.7% in Japan [2].

To improve the mortality and prognosis of pancreatic cancer, early detection of pancreatic cancer to provide the opportunity for curative surgical treatment is the most important issue, but since the incidence of pancreatic cancer is low, there is no efficient screening method for pancreatic cancer. In fact, the US Preventive Services Task Force (USPSTF) does not recommend screening for pancreatic cancer in an asymptomatic population (D recommendation), because the USPSTF found no evidence that screening for pancreatic cancer or treatment of screen-detected pancreatic cancer improves disease-specific morbidity or mortality [1].

The lifetime risk of pancreatic cancer in the general population is estimated to be approximately 1.6%, and it is considered that screening for pancreatic cancer with any imaging modalities is not feasible due to its low incidence. On the other hand, the International Can-

cer of the Pancreas Screening (CAPS) Consortium recommends targeted screening using magnetic resonance imaging (MRI) and endoscopic ultrasonography (EUS) for high-risk individuals (HRIs) for pancreatic cancer having either $>$ 5% lifetime risk or a 5-fold increased relative risk compared with the general population [3]. Individuals having two cases of pancreatic cancer in two first-degree relatives have a significantly high relative risk of 6.4-fold [95% confidence interval (CI) 1.8–16.4] compared with the general population. However, it is not sufficient to screen only the population with a family history [4], because about 90% of pancreatic cancers develop sporadically from the population without a family history [5].

Intraductal papillary mucinous neoplasm (IPMN), chronic pancreatitis, pancreatic cysts, diabetes mellitus, obesity, and smoking are also risk factors for pancreatic cancer [6]. In particular, it is considered that IPMNs are precancerous lesions of pancreatic cancer. Oyama et al. reported 5-year and 15-year incidence rates of developing PDAC of 3.3% and 15%, respectively, after diagnosis of branch duct (BD)-IPMN in a long-term follow-up study [7]. To establish an efficient screening method for pancreatic cancer, HRIs should be identified before imaging examinations from the asymptomatic population. The population with a family history, diabetes mellitus, obesity, or smoking can be identified by interviews. However, those with organic diseases such as IPMN, chronic pancreatitis, or pancreatic cysts cannot be identified from the asymptomatic population without imaging examinations. Carbohydrate antigen 19-9 (CA19-9) is the conventional biomarker for the detection of PDAC, and it is commonly used to monitor the therapeutic response in patients with PDAC [8]. However CA19-9 has some limitations, because it cannot detect small tumors in the still curable stage [9], and it cannot identify HRIs. Furthermore, CA19-9 is not expressed at all in individuals genetically expressing non-sialylated Lewis blood group antigens [10]. Biomarkers for risk stratification of pancreatic cancer patients and HRIs that can be obtained from blood tests are very important for identifying the subjects who should undergo imaging examinations to detect the organic diseases associated with pancreatic cancer risk.

In the last two decades, we identified unique alterations of processing patterns in C-terminal amino acids of circulating apoA2-homodimer [apoA2-isoforms (apoA2-i)] in patients with pancreatic cancer and in HRIs, and we have continued to develop efficient screening methods for pancreatic cancer with a blood test for apoA2-i [6,11,12]. The possibility of developing efficient pancreatic cancer screening with the apoA2-i blood test is reviewed in this report.

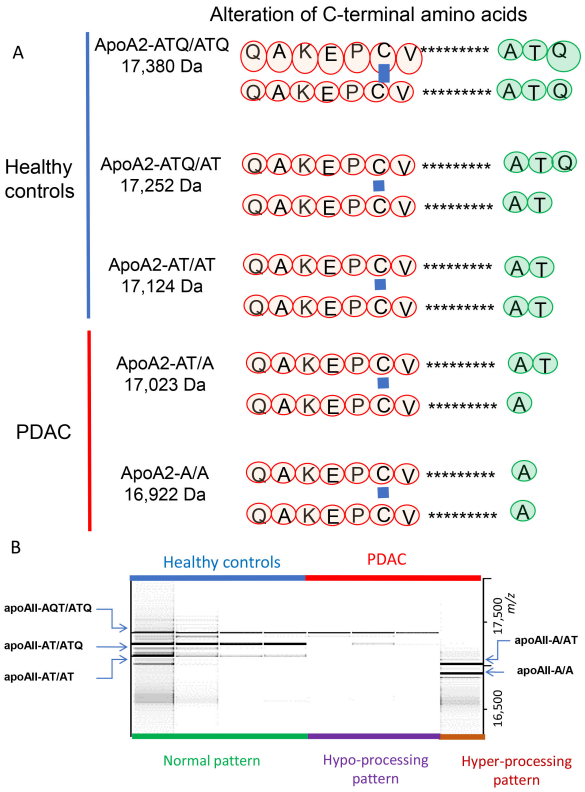

Fig. 1. Amino acid sequences of apolipoprotein A2 (apoA2) homodimers and several isoforms of apoA2-isoforms (apoA2-i) [12]. Apolipoprotein A2 (apoA2) is comprised of 77 amino acids and forms a homodimer via disulfide bonds. The circulating ApoA2-isoforms (apoA2-i) in the bloodstream consist of 5 isoforms that have different C-terminal amino acids. We named them apoA2-ATQ/ATQ (17,380 Da), apoA2-ATQ/AT (17252 Da), apoA2-AT/AT (17,124 Da), apoA2-AT/A (17, 023 Da), and apoA2-A/A (16,922 Da). ApoA2-ATQ/ATQ, -ATQ/AT, and -AT/AT are observed in healthy controls. In contrast, apoA2-AT/A and -A/A are observed only in pancreatic adenocarcinoma (PDAC).

2. Identification of apoA2-i as the biomarker for early detection of pancreatic cancer with top-down proteomics

To develop biomarkers for early detection of pancreatic cancer using a blood test, we investigated the comprehensive protein expression profiles in plasma samples that were obtained from healthy controls and patients with pancreatic cancer by top-down proteomics [13]. We identified several peptides that have different molecular weights to be able to clearly distinguish between pancreatic cancer and healthy controls. To confirm this phenomenon, we established a new measurement assay for protein and peptide profiling with matrix-assisted laser desorption/ionization

(MALDI) quadrupole time-of flight (QqTOF) mass spectrometry (MS) [14].

MALDI-QqTOF MS showed that the circulating apoA2-homodimers consist of 3 isoforms with different C-terminal amino acids in healthy controls, including the heaviest homodimer (ATQ/ATQ, 17380 m/z), the lightest homodimer (AT/AT, 17124 m/z), and the intermediate homodimer (ATQ/AT, 17252 m/z) [15]. Moreover, other C-terminal amino acids with lighter molecular weight than AT/AT are only identified from the plasma of patients with pancreatic cancer, such as AT/A (17023 m/z) and A/A (16922 m/z). We named each apoA2-isoform (apoA2-i): apoA2-ATQ/ATQ (17380 m/z), apoA2-ATQ/AT (17252 m/z), apoA2-AT/AT (17124 m/z), apoA2-AT/A (17023 m/z), and apoA2-A/A (16922 m/z) (Fig. 1). In particular, the expression profile of MALDI-QqTOF MS showed that apoA2-ATQ/AT of the intermediate type of apoA2-i was significantly decreased in pancreatic cancer patients compared with healthy controls in the Japanese multi-institutional study (Fig. 2) [14].

The area under the curve (AUC) of apoA2-ATQ/AT that was measured by MALDI-QqTOF MS to distinguish pancreatic cancer patients ($n = 249$) from healthy controls ($n = 128$) was 0.866 in the Japanese multi-institutional study. The high AUC for distinguishing pancreatic cancer patients was found not only in the Japanese multi-institutional study, but also in the German retrospective study; the AUC of apoA2-ATQ/AT in the German retrospective study was 0.958 [14].

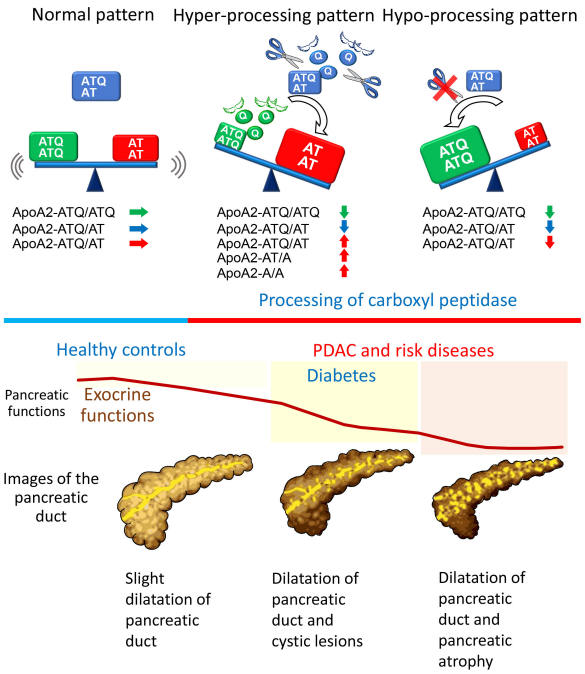

Fig. 2. Unique alteration of C-terminal amino acids in apoA2 homodimers according to the exocrine function of the pancreas [12,14,16]. A. Unique processing patterns of apoA2-isoforms are observed in PDAC and high-risk individuals (HRIs) of PDAC, such as: 1) hyper-processing pattern in which the heavy isoform (apoA2-ATQ/ATQ) is dominantly observed; 2) hypo-processing pattern in which the light isoforms (-AT/AT, AT/A, or A/A) are dominantly observed; and 3) middle processing pattern in which heavy and light isoforms are little processed. In any processing patterns, the intermediate isoform of apoA2-homodimers (apoA2-ATQ/AT) was significantly decreased in PDAC and HRIs of PDAC. It is considered that cleavage of C-terminal amino acids of apoA2-homodimers is involved in the latent exocrine function of the pancreas, which is correlated to exopeptidases including carboxypeptidase.

3. Establishment of enzyme-linked immunosorbent assay (ELISA) methods for measuring apoA2-isoforms and blind validation with the National Cancer Institute (NCI) Early Detection Research Network (EDRN)

To easily measure the concentration of circulating apoA2-i in the blood stream, we established two antibodies specifically reacting to apoA2-AT or apoA2-ATQ, and then we prepared the ELISA methods using these antibodies. The concentration of apoA2-ATQ/AT could be approximated by the formula with apoA2-AT and apoA2-ATQ, as $\sqrt{(apoA2\ AT * apoA2\ ATQ)}$.

We measured the concentration of apoA2-isoforms in the plasma samples that were collected in the Japanese multi-institutional study using this ELISA [16]. The AUCs of apoA2-ATQ/AT measured by ELISA in the Japanese cohort to distinguish patients with stage-I, -II, -III, and -IV pancreatic cancers from healthy controls were 0.939, 0.957, 0.926, and 0.946 respectively; all AUCs of apoA2-ATQ/AT for each stage were greater than of CA19-9 (Fig. 3A and B). In addition, the combination assay with CA19-9 and apoA2-isoforms increased the pancreatic cancer detection rate [16].

To confirm the clinical usefulness of apoA2-isoforms ELISA globally, we blindly measured the concentration of apoA2-isoforms in the serum samples that were obtained from the NCI EDRN as reference samples (https://edrn.nci.nih.gov/). The AUC of apoA2-ATQ/AT to distinguish patients with stage-I and stage-II pancreatic cancer was 0.809, greater than the AUC of CA19-9. The combination assay with apoA2-isoforms and CA19-9 significantly increased the AUC (0.879) to distinguish stage-I/-II pancreatic cancers by approximately 0.1 in comparison with measurement with only CA19-9 [12,16] (Fig. 3C–E).

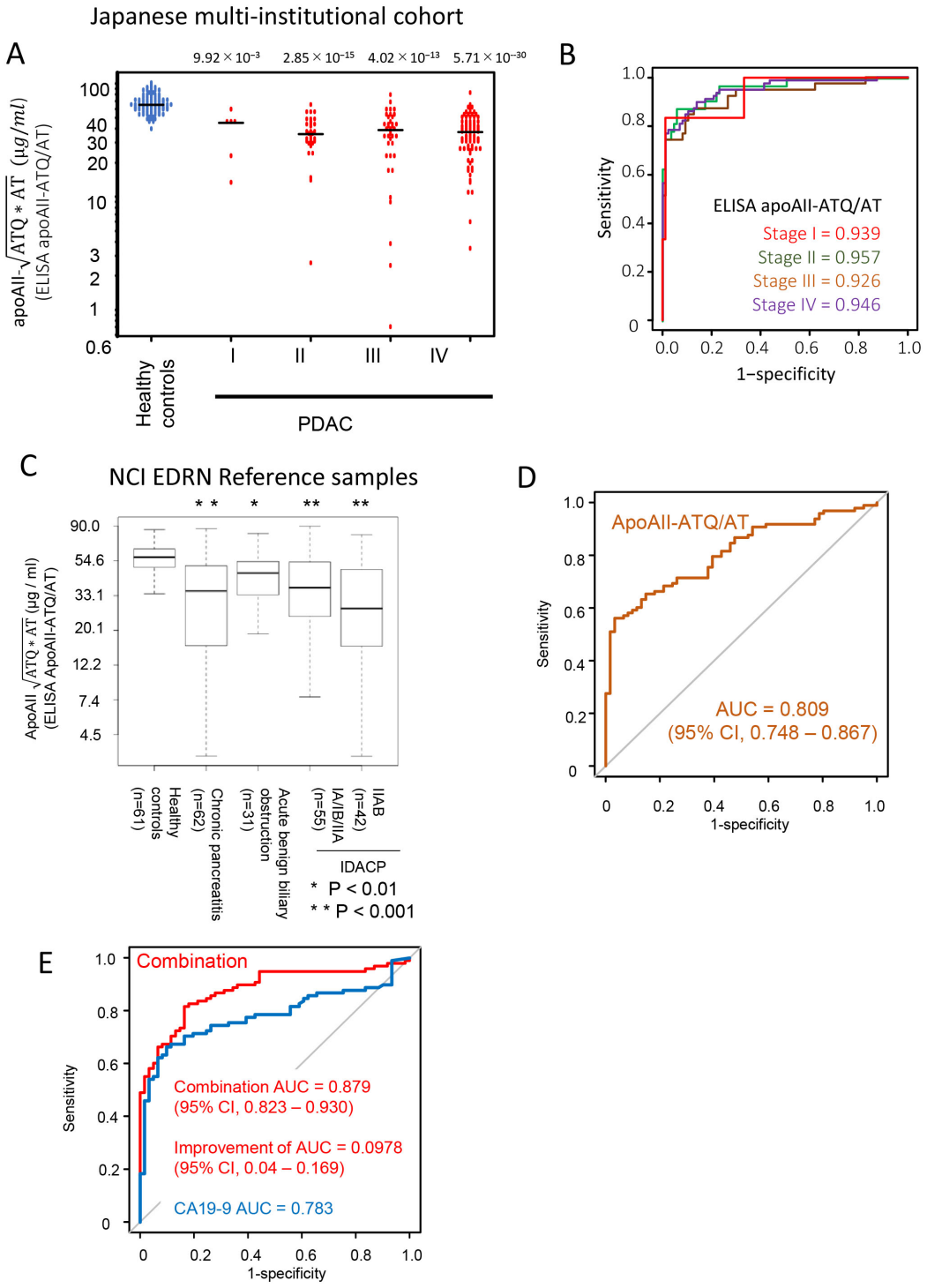

Fig. 3. Significant reduction of apoA2-ATQ/AT in PDAC on ELISA assay measurement [16]. Distribution and receiver operating characteristic (ROC) analysis of plasma apoA2-ATQ/AT (red line: stage-I, green line: stage-II, orange line: stage-III, and purple line: stage-IV) by ELISA assay for each stage of PDAC and healthy controls in the multi-Japanese institutional cohort (A and B). Distribution of serum apoA2-ATQ/AT (C) and ROC analysis (D) of plasma apoA2-ATQ/AT in the reference sample set of the National Cancer Institute (NCI) Early Detection Research Network (EDRN). ROC analyses of a combination assay with apoA2-ATQ/AT (red line) and CA19-9, and CA19-9 alone (blue line) (E).

4. High sensitivity of apoA2-i ELISA for detecting diseases associated with a high risk of pancreatic cancer

ApoA2-i has high sensitivity for detecting patients with not only pancreatic cancer, but also diseases associated with a high risk of pancreatic cancer, such as chronic pancreatitis and IPMNs. The result obtained from a Japanese multi-institutional study showed that AUCs to detect patients with endocrine neoplasms, IPMN, mucinous cystic neoplasm (MCN), serous cystic neoplasm (SCN), and chronic pancreatitis from healthy controls were 0.84, 0.92, 0.816, 0.983, and 0.992, respectively [16].

5. Aberrant alteration of unique processing of C-terminal amino acids of the apoA2-homodimer in pancreatic diseases, and its use as a potential biomarker for evaluating the exocrine function of the pancreas

Two-dimensional scatter plots with concentrations of apoA2-AT and apoA2-ATQ showed that there are three unique processing patterns in pancreatic cancer and pancreatic disorders: 1) a hyper-processing pattern in which light homodimers (apoA2-AT/AT) are mainly observed; 2) a hypo-processing pattern in which heavy homodimers (apoA2-ATQ/ATQ) are mainly observed; and 3) a middle processing pattern in which both homodimers (apoA2-AT/AT and apoA2-ATQ/ATQ) are slightly decreased. In pancreatic cancer patients, the concentration of intermediate homodimers (a poA2-ATQ/AT) is decreased in any processing pattern compared with healthy controls (Fig. 4).

Interestingly, the processing pattern was not recognized only in patients with pancreatic disorders including pancreatic cancer, and they were never recognized in any kind of cancers except pancreatic cancer. Carboxypeptidase A is an exopeptidase that cleaves amino acids from the C-terminus of a protein or peptide. This peptidase is a digestive enzyme that is primarily synthesized by the pancreas. It seems that the unique alteration of processing patterns of apoA2-i in pancreatic disorders is associated with the exocrine function of the pancreas. In fact, Kobayashi et al. recently reported that a significant increase of apoA2-ATQ/ATQ as a heavy homodimer was observed in autoimmune pancreatitis (AIP), in comparison with apoA2-AT/AT, and, in particular, many cases with extremely decreased apoA2-AT/AT were observed in AIP [17]. Thus, a hypo-processing pattern of apoA2-i is a unique finding in AIP, and a significant decrease of apoA2-AT/AT may be associated with the reduction of the exocrine function of the pancreas that occurs in AIP. In addition, Hayasaki et al. identified that apoA2-ATQ/ATQ was significantly increased in patients who underwent radiation for pancreatic cancer with neoadjuvant therapy before surgery, in comparison with the patients before undergoing radiation therapy [18]. This finding is also consistent with the reduction of the exocrine function of the pancreas with exposure to radiation.

These results suggest that apoA2-i ELISA is a potential in vitro diagnostic biomarker for evaluating the exocrine function of the pancreas.

6. Prospective evaluation of apoA2-isoforms and CA19-9 by the European Prospective Investigation into Cancer (EPIC) study

The study with NCI EDRN demonstrated a significant improvement of diagnostic accuracy for detecting pancreatic cancer by the combination assay of CA19-9 and apoA2-i compared with CA19-9 alone, but that study was a case-control comparison of patients already diagnosed with pancreatic cancer and cancer-free control subjects, and thus it did not allow any evaluation of the lead time by which the biomarkers may help anticipate the cancer diagnosis.

To evaluate the lead time, we analyzed apoA2-i ELISA and CA19-9 levels in pre-diagnostic serum samples of pancreatic cancer cases that were prospectively collected by the EPIC study.

The EPIC study is one of the largest cohort studies in the world, with more than 521,000 participants recruited across 10 European countries and followed for almost 15 years. EPIC was designed to investigate the relationships between diet, nutritional status, lifestyle, and environmental factors and the incidences of cancer and other chronic diseases [19,20]. We conducted a nested case-control study within the EPIC cohort, including all incident cases of invasive and exocrine pancreatic cancer clinically diagnosed within no more than 5 years after blood donation.

Combined models based on apoA2-ATQ/AT plus CA19-9 significantly improved discrimination $>$ 6–18 months before the diagnosis of pancreatic cancer (AUC 0.74 vs. 0.71 for CA19-9 alone, p=0.022). With specificity of 98%, for lag times of $\leqslant$ 6, 6–18, and $\leqslant$ 18 months, sensitivity was 57%, 36%, and 43% for CA19-9 combined with apoA2-ATQ/AT, respec-

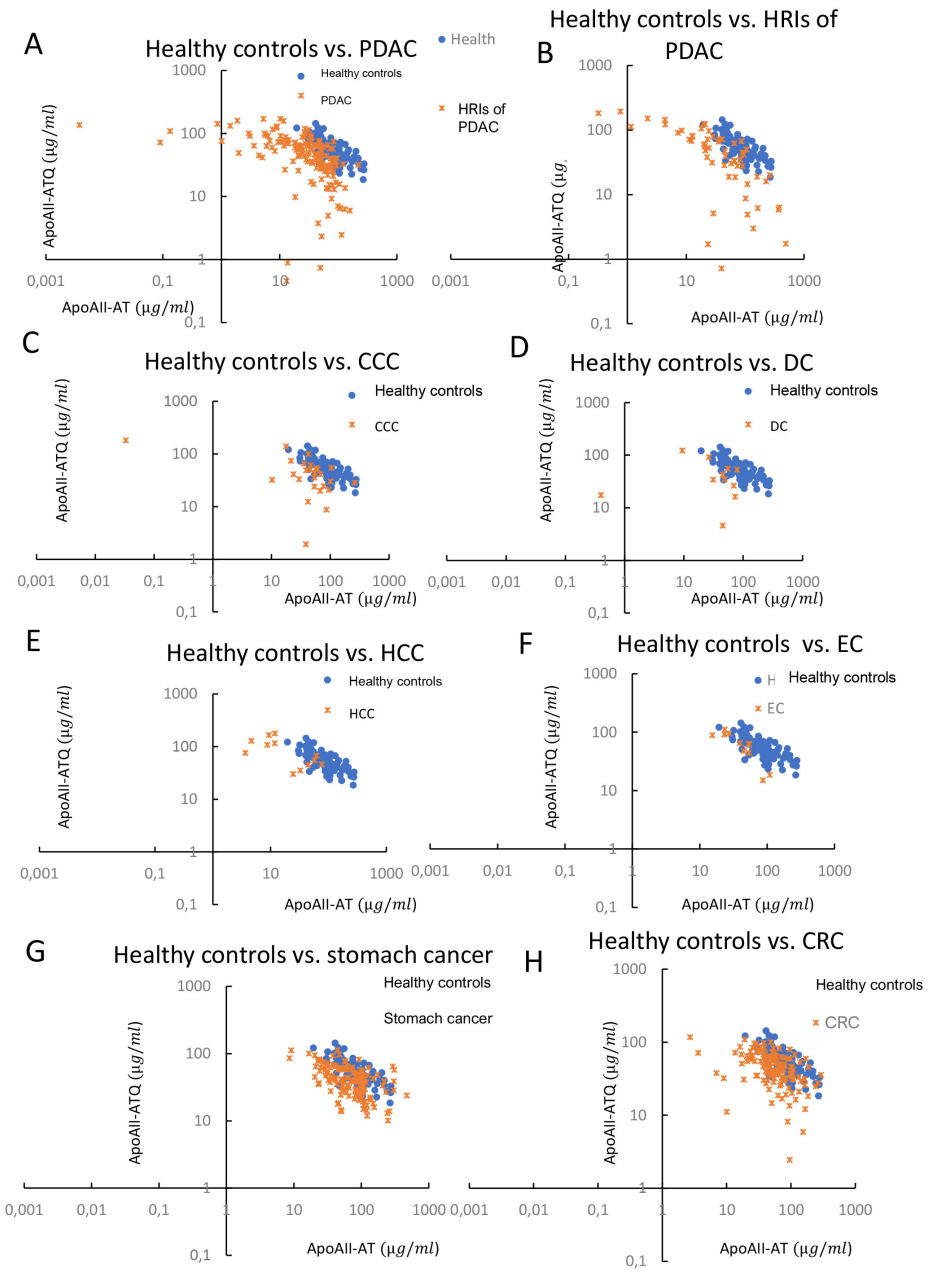

Fig. 4. Two-dimensional scatterplots of heavy (apoA2-ATQ/ATQ) and light (apoA2-AT/AT) apoA2 homodimers in the gastrointestinal (GI) cancers and HRIs that were enrolled in the multi-institutional Japanese study [14]. Healthy controls (blue circles) versus GI cancer and HRIs (Orange crosses). (A) Healthy controls versus PDAC, (B) healthy controls versus HRIs including IPMN and chronic pancreatitis, (C) healthy controls versus cholangiocellular carcinoma (CCC), (D) healthy controls versus duodenal cancer (DC), (E) healthy controls versus hepatocellular carcinoma (HCC), (F) healthy controls versus esophageal cancer (EC), (G) healthy controls versus stomach cancer, and (H) healthy controls versus colorectal cancer (CRC).

tively, vs. 50%, 29%, and 36%, respectively, for CA19-9 alone [21]. These data suggested that the combination assay with CA19-9 and apoA2-i could improve the detection of pancreatic cancer up to 18 months prior to diagnosis under usual care, and that it may provide a useful first measure for pancreatic cancer detection prior to imaging examinations. In addition, it seems that almost all double-positive cases of apoA2-ATQ/AT and CA19-9 were diagnosed with PDAC within 5 years (Fig. 5).

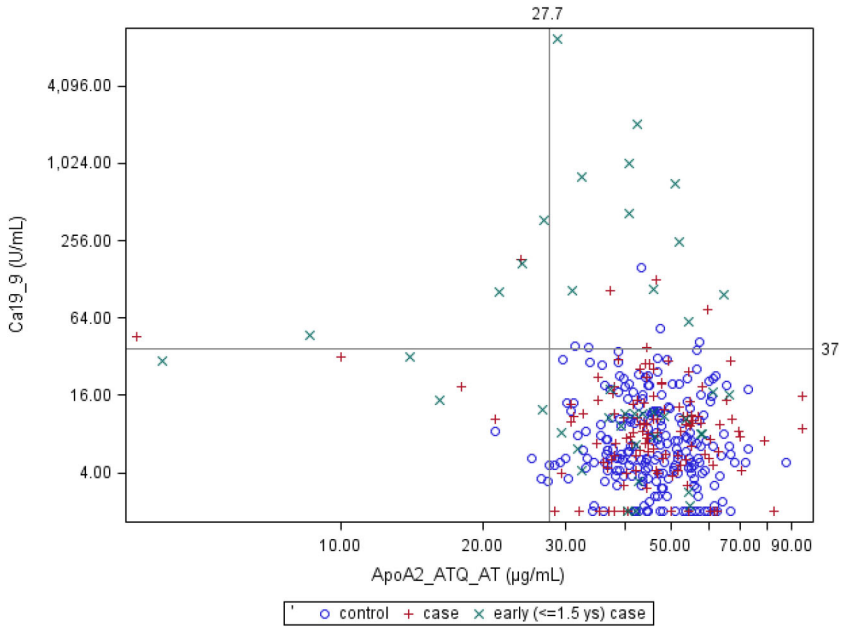

Fig. 5. Two-dimensional scatterplots of apoA2-ATQ/AT and CA19-9 in pre-diagnosis serum samples of PDAC that were enrolled by the European Prospective Investigation into Cancer (EPIC) study. Green crosses represent cases diagnosed within $\leqslant$ 18 months after blood was drawn, red crosses represent those diagnosed after longer time intervals (18–60 months). Blue circles represent those never diagnosed with PDAC [21].

7. Experimental screening for pancreatic cancer using the apoA2-i blood test in the Kobe Cancer Screening Network

In the previous retrospective and prospective studies, we showed that apoA2-i is a potential biomarker for detecting pancreatic cancer and HRIs. Then, we prospectively carried out experimental screening for pancreatic cancer in the general population in the Kobe Cancer Screening Network Japan. A total of 5120 asymptomatic subjects, older than 20 years, were enrolled in this study. The cut-off value of apoA2-ATQ/AT was defined as $\leqslant$ 35 μg/mL. Of the 5120 subjects, 84 (1.6%) were positive with values less than the cut-off values, and then 54 subjects underwent second screening with imaging modalities such as contrast-enhanced computed tomography (CECT), magnetic resonance cholangiopancreatography (MRCP), and/or EUS. Figure 6 shows that 1 PDAC, 9 IPMN, 3 chronic pancreatitis, 1 neuroendocrine tumor, 1 AIP, 4 pancreatic cysts, and 6 pancreatic abnormalities on imaging, such as suspected cases of early pancreatitis, focal fat invasion of the pancreas, etc., were detected in the 54 subjects who were judged as positive cases on apoA2-ATQ/AT experimental screening. The positive predictive value of abnormality including PDAC and HRIs by imaging was 48.1% (26/54 cases) (Fig. 6).

The comparison of background characteristics between apoA2-ATQ/AT-positive and -negative groups in the experimental screening study showed that age (years) [odds ratio (OR) 1.37, 95% CI 1.01–1.85], white blood cells (OR 1.32, 95% CI 1.11–1.56), total cholesterol (OR 0.94, 95% CI 0.92–0.97), low-density lipoprotein (OR 1.05, 95% CI 1.01–1.08), and pancreatic lesions detected by ultrasonography (%) (OR 3.04, 95% CI 1.01–9.14) remained significant on the multivariate logistic regression analyses (Table 1) [22]. Interestingly, the most strongly associated factor in the comparison of background characteristics was pancreatic lesions detected by ultrasonography.

This study has two limitations: 1) the enrolled age was too young at $\geqslant$ 20 years; and the 2) total number was too small to detect patients with pancreatic cancer.

To confirm the feasibility of pancreatic cancer screening with apoA2-i ELISA, we started expanding the experimental screening for pancreatic cancer to older than 50 years of age with the apoA2-i blood test in Kagoshima, Hyogo, and Hokkaido prefectures in Japan.

Future perspective

Calanzani et al. recently reported a systematic review identifying novel biomarkers ready for evaluation in low-prevalence populations for the early detection of

Table 1
Comparison of background characteristics between the apoA2-ATQ/AT-positive and -negative groups in the experimental screening for pancreatic cancer in the Kobe Cancer Screening Network [22]

Covariate	Negative group (apoA2-ATQ/AT: >35 μg/mL) ($n=5036$)			Positive group (apoA2-ATQ/AT: $\leqslant 35$ μg/mL) ($n=84$)			Univariate analysis ($n=5120$)				Multivariate analysis ($n=3540$)			
	n	Values		n	Values		OR	95% CI		P	OR	95% CI		P
Age (y)*	5024	52.0	(22–89)	84	61.0	(30–88)	1.76	1.46	2.11	<0.001	1.37	1.01	1.85	0.041
Males (%)**	5024	2607	(51.9)	84	55	(65.5)	0.57	0.36	0.89	0.015				
Height (cm)	4419	164.9	(158.3–171.5)	67	166.8	(158.6–171.3)	1.01	0.98	1.04	0.515				
Weight (kg)	4427	61.9	(53.2–70.8)	68	66.4	(54.3–75.7)	1.02	1.00	1.04	0.017				
BMI (kg/m^2)	4419	22.5	(20.5–24.8)	67	23.5	(21.1–26.4)	1.09	1.02	1.16	0.010				
Smoking (%)**	3979	1526	(38.4)	42	22	(52.4)	1.77	0.96	3.25	0.067				
Alcohol consumption (%)**	3610			41										
Daily		831	(23.0)		4	(9.8)	0.46	0.11	1.84	0.271				
A few times a week		1347	(37.3)		16	(39.0)	1.13	0.38	3.40	0.830				
Once a month		1052	(29.1)		17	(41.5)	1.54	0.51	4.59	0.443				
Never		380	(10.5)		4	(9.8)	1.00	–	–	–				
Diabetes (%)**	5036	267	(5.3)	84	19	(22.6)	5.22	3.09	8.83	<0.001	2.00	0.74	5.44	0.174
History of pancreatic disease (%)**	4689	57	(1.2)	77	5	(6.5)	5.64	2.2	14.5	<0.001	2.91	0.73	11.57	0.130
WBC ($\times 10^3/\mu$L)	5018	4.8	(4.1–5.8)	84	5.8	(4.8–7.0)	1.38	1.25	1.52	<0.001	1.32	1.11	1.56	0.001
RBC ($\times 10^4/\mu$L)	5017	458.0	(427.0–489.0)	84	444.0	(409.5–489.0)	1.00	0.99	1.00	0.077				
Hb (g/mL)	5017	14.1	(13.1–15.1)	84	13.9	(12.5–14.9)	0.88	0.77	1.01	0.080				
Ht (%)	5017	42.3	(39.7–45.1)	84	41.5	(38.0–45.5)	0.95	0.9	1.00	0.047				
Plt (/μL)	4980	23.2	(20.1–27.0)	84	21.9	(18.6–25.9)	0.97	0.93	1.01	0.180				
TP (g/dL)	4633	7.2	(7.0–7.4)	83	7.1	(6.7–7.4)	0.80	0.5	1.29	0.358				
ALB (g/dL)	4170	4.4	(4.2–4.6)	70	4.3	(4.0–4.5)	0.70	0.42	1.16	0.165				
AST (U/L)	5015	21.0	(18.0–25.0)	84	21.0	(17.0–25.0)	1.00	0.99	1.02	0.758				
ALT (U/L)	5016	19.0	(14.0–26.0)	84	18.0	(14.0–27.0)	1.00	0.99	1.01	0.972				
ALP (U/L)	4588	185.0	(153.0–224.0)	82	198.0	(158.0–243.0)	1.00	1.00	1.01	0.029				
γ – GTP (U/L)	4960	25.0	(17.0–41.0)	83	22.0	(15.0–39.0)	1.00	1.00	1.01	0.253				
T.BIL (mg/dL)	4383	0.8	(0.7–1.1)	74	0.8	(0.6–1.0)	0.27	0.14	0.54	<0.001	0.44	0.18	1.07	0.071
UA (mg/dL)	5008	5.3	(4.3–6.3)	84	5.4	(4.3–6.2)	1.01	0.86	1.17	0.945				
BUN (mg/dL)	4557	13.0	(11.0–15.0)	82	15.0	(12.0–18.0)	1.09	1.05	1.14	<0.001	1.04	0.96	1.13	0.287
Cre (mg/dL)	5009	0.74	(0.63–0.86)	84	0.76	(0.66–0.87)	2.23	1.18	4.21	0.014				
Na (mmol/L)	4050	140.0	(139.0–142.0)	78	141.0	(140.0–142.0)	1.10	0.97	1.24	0.144				
K (mmol/L)	4050	4.1	(3.9–4.3)	78	4.1	(3.8–4.3)	0.86	0.42	1.76	0.675				
CL (mmol/L)	4050	104.0	(102.0–105.0)	78	104.0	(102.0–106.0)	0.96	0.87	1.07	0.468				
Glucose (mg/dL)	5015	96.0	(90.0–103.0)	84	100.0	(91.5–113.5)	1.02	1.01	1.03	<0.001	0.99	0.97	1.01	0.397
HbA1c (%)	4501	5.5	(5.3–5.7)	67	5.6	(5.3–6.4)	1.82	1.46	2.28	<0.001	1.12	0.65	1.94	0.684
TC (mg/dL)	4937	206.0	(185.0–229.0)	84	184.5	(160.5–211.0)	0.98	0.97	0.99	<0.001	0.94	0.92	0.97	<0.001
LDL (mg/dL)	5016	123.0	(104.0–144.0)	84	118.0	(90.5–133.0)	0.99	0.98	0.99	<0.001	1.05	1.01	1.08	0.006
HDL (mg/dL)	5016	66.0	(55.0–79.0)	84	54.0	(47.5–67.0)	0.96	0.94	0.97	<0.001	1.00	0.97	1.03	0.805
TG (mg/dL)	5016	85.0	(60.0–123.0)	84	82.0	(58.5–111.5)	1.00	0.99	1.00	0.300				
Amy (U/L)	4822	72.0	(58.0–89.0)	81	67.0	(52.0–87.0)	1.00	0.99	1.01	0.544				
Pancreatic lesions detected by ultrasonography (%)**	4600	141	(3.1)	75	10	(13.3)	4.87	2.45	9.67	<0.001	3.04	1.01	9.14	0.047

On univariate logistic regression analyses, patients' characteristics were compared using Fisher's exact test for categorical variables and Welch's t test or the Wilcoxon rank-sum test for continuous variables. Factors that were significant on univariate analyses were included in the multivariate logistic regression analysis. Significance was defined as a value of $p<0.05$. Data are shown as median and interquartile range values. *Data are shown as median and range values, and odds ratios (ORs) per 10 years are estimated. **Data are shown as absolute values and percentages. OR, odds ratio; 95% CI, 95% confidence interval; p, p-value.

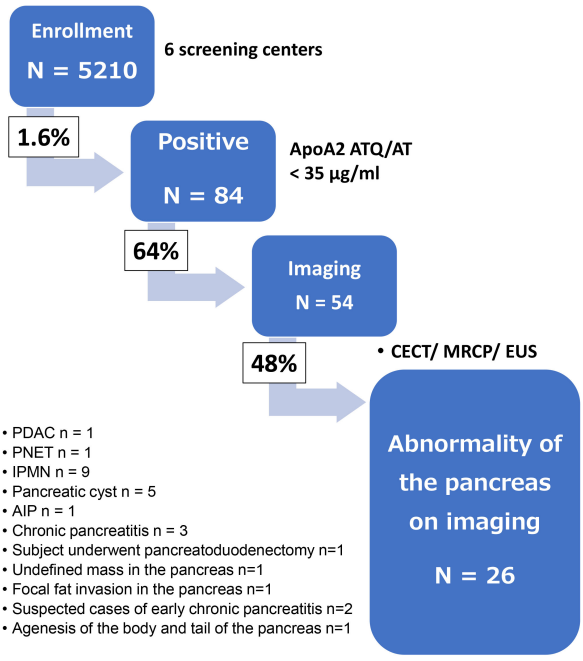

Fig. 6. Flow diagram of the experimental screening of pancreatic cancer using the apoA2-i blood test in the Kobe Cancer Screening Network [22]. CECT, contrast-enhanced computed tomography; MRCP, magnetic resonance cholangiopancreatography; EUS, endoscopic ultrasonography; PDAC, pancreatic adenocarcinoma cancer; IPMN, intraductal papillary mucinous neoplasms; CP, chronic pancreatitis; NET, pancreatic neuroendocrine tumors; AIP, autoimmune pancreatitis.

upper gastrointestinal cancers. This study highlights the need for more biomarker studies that consider the primary care/community setting as their intended population. It suggested that apoA2-ATQ/AT and pepsinogens (PGI and PGII) were the most promising biomarkers for pancreatic cancer and gastric cancer, respectively [23].

ApoA2-isoforms could detect patients with not only PDAC, but also HRIs, including pancreatic cysts. Although this is a strong point for identifying early-stage PDAC, on the other hand, it is a potential weak point for PDAC screening. Zerboni et al. reported that the rate of incidentally detected pancreatic cystic lesions was 8% in their meta-analysis including 17 studies with 48,860 patients. Given the very high incidental prevalence of these conditions, which increases with age, this could be an important limitation to its clinical use as a general screening tool for the entire population because it would lead to many unnecessary imaging procedures [24]. In fact, apoA2-ATQ/AT was significantly associated with the age factor in *Kobe's study*. If PDAC screening with apoA2-isoforms is combined with clinical information about factors that increase the risk, such as smoking, *overweight*, and diabetes mellitus, efficient screening methods for PDAC may be established.

The National Cancer Institute is leading a project to create a cohort of people who are newly diagnosed with diabetes mellitus in the hope that this group, who are at increased risk of developing pancreatic cancer, will provide the clues in their blood and tissues to unravel some of the unknowns about this highly fatal cancer. In the future, a screening study with the apoA2-i blood test should also be considered to identify the population with new-onset diabetes with a high risk of pancreatic cancer.

Acknowledgments

These studies were supported by grants from the Project for Cancer Research and Therapeutic Evaluation (P-CREATE) (20cm0106403h0005), the Practical Research for Innovative Cancer Control Program (19ck0106280h0003 and 21ck0106661h0001), and AMED CREST (19gm0710013h0306) from the Japan Agency for Medical Research and Development (AMED).

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