Case Control Study in Diagnosis of Pancreatic Cancer
—Using multiple-logistic regression—

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ABSTRACT

We determined 13 markers of pancreatic cancer which are used presently and tried quantitative combination assay of pancreatic cancer. Case-control study using the logistic regression showed the possibility of pancreatic cancer quantitatively. When the level of CA19-9 is more than Mean + 2S.D. (40 u/ml), the possibility of pancreatic cancer is 15 times higher than when the level is less than Mean + S.D.. In the case that the high-level of CA19-9 and α_1-AT which are more than Mean + S.D., the possibility of pancreatic cancer is 30 times higher than the case that the level of CA19-9 and α_1-AT are less than Mean + S.D. when both levels of CA19-9 and α_1-AT are more than Mean + S.D..

The development of tumor markers has been extensive recently. We simultaneously determined various tumor markers in the diagnosis of pancreatic cancer and examined the mutual relation of markers using ACOS-4 computer previously. This time, we calculated the relative risk for pancreatic cancer compared with patients without gastrointestinal disease and those with chronic pancreatitis when each tumor marker was positive by the method of multiple-logistic regression.

SUBJECT AND METHODS

The subject is 60 cases with pancreatic cancer which are over T2 including 10 cases with liver metastasis diagnosed by ERCP, abdominal CT and operations (35 males 25 females; aged 30-88, mean 66.7), 30 cases with chronic pancreatitis I by criteria for diagnosis of chronic pancreati-

tis (23 males, 7 females; aged 40-76, mean 52.1), and 50 cases without gastrointestinal disease (29 males, 21 females; aged 18-86, mean 60.8). We gathered blood from the patients of these diseases when they are hungry in the early morning, and determined following markers:

Carbohydrate antigen 19-9 (CA19-9), CEA, ferritin, α_1-antitrypsin (α_1-AT), elastase 1, amylase, trypsin, tissue polypeptide antigen (TPA), β_2-microglobulin (β_2-mG), ribonuclease (RNase), pancreatic oncofetal antigen (POA), LDH, immunosuppresive acid protein (IAP), total bilirubin (T.B.) and creatinine. As normal control cases, we determined 49 cases for CEA, 261 cases for ferritin, 72 cases for TPA, 168 cases for β_2-mG, 70 cases for RNase, 70 cases for POA, 133 cases for LDH, 64 cases for α_1-AT, 117 cases for CA19-9, 114 cases for IAP, and 142 cases for trypsin.

Table 1. Mean, Mean ± S.D., and Mean ± 2S.D. of tumor markers among controls calculated based on log-normal distribution

Markers	Mean − 2S.D.	Mean − S.D.	Mean	Mean + S.D.	Mean + 2S.D.
CA19-9	2	3	7	17	40
CEA	0.4	0.7	1.3	2.4	4.2
Ferritin	6	17	47	132	368
α_1-AT	187	222	263	312	371
TPA	30	48	77	125	201
β_2-mG	0.7	1.0	1.4	1.9	2.7
RNase	42	66	103	160	250
POA	2.3	3.2	4.5	6.2	8.6
LDH	171	210	256	314	384
IAP	238	311	405	528	688
Elastase 1	102	153	229	343	514
Amylase	82	109	145	194	258
Trypsin	67	123	225	410	748

Concentration of CA19-9 was measured by a solid phase radioimmunoassay in accordance with the manufacture's accommodation (Centocor), CEA was assayed by CEA RIA kit (Dainabot), ferritin was by SPAC Ferritin kit, α_1-AT was by LASR-Antitrypsin kit (Haechist), elastase 1 was by ELASTASE 1 RIAKIT (Dainabot), trypsin was by RIA-Gnost Trypsin Kit (Haechist), and β_2-mG was by Phadebas β_2-micro Test Kit (Shionogi). RNase was measured using a poly(C) substrate and with the procedure described by Reddi[9], and POA was measured using rocket-immunoelectrophoresis by the method of Gelder[2]. Amylase was measured by the method of Blue Starch, and IAP was measured by the method of single radial immunodiffusion.

STATISTICAL METHODS

In order to examine the distribution of tumor markers, we previously[5] calculated skewness and kurtosis by the raw data of normal controls and the logarithmic data. And we determined Mean, Mean + S.D., and Mean + 2S.D. to permit a direct comparison of the diagnostic value of 13 tumor marker determinations. Using 5 tumor markers (CA19-9, CEA, α_1-AT, ferritin and elastase 1) we carried out Case-control study by the method of multiple-logistic regression[1]. Control is patients without gastrointestinal disease or with chronic pancreatitis, and Case is patients with pancreatic cancer.

We used BMDP[6] as a statistical package.

RESULTS

1) Distribution of tumor markers (Table 1).

We previously[5] decided the distribution of tumor markers based on log normal distribution, because log-transformed data approximate to skewness 0 and kurtosis 3 which show the normal distribution in all distribution of tumor markers. Table 1 shows the distribution of 13 tumor markers based on log-transformation.

2) Case-control study making patients without gastrointestinal disease as Control and patients with pancreatic cancer as Case (Table 2).

In 50 patients without gastrointestinal disease and 60 patients with pancreatic cancer, we calculated the relative risk of pancreatic cancer using odds ratio (adjusted by sex and age). In CA19-9, CEA, α_1-AT and ferritin, the range between more than Mean + S.D. and less than Mean + 2S.D. which was calculated by normal control case is taken for the slightly high-level, and the range more than Mean + 2S.D. is taken for the advanced high-level. As the low-level of elastase 1 is significant clinically, the range less than Mean − S.D. is taken for the low-level and the range more than Mean + S.D. is taken for the high-level.

Table 2 shows odds ratio and 95% confidence interval to each abnormality. Except for the advanced high-level of CEA and the low-level of elastase 1, it shows a significant odds ratio, and its ratio became higher with the advanced high-level. In CA19-9, the ratio of the slightly high-level is 8.9 times and that of the advanced high-level is 1.8×10^2 times compared with the case which is less than Mean + S.D., it shows high possibility of pancreatic cancer.

3) Case-control study making patients with

Table 2. Case-control study making cases without gastrointestinal disease as Control and cases with pancreatic cancer as Case*

Tumor Markers	Odds Ratio	95% Confidence Interval	
CA19-9			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	8.9	1.9	~ 4.3×10^1
≧ Mean + 2S.D.	1.8×10^2	3.4×10^1	~ 1.0×10^3
CEA			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	2.2×10^1	4.3	~ 1.1×10^2
≧ Mean + 2S.D.	1.3×10^5	0.0**	~ 5.3×10^2
α_1-AT			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	1.0×10^1	2.6	~ 3.8×10^1
≧ Mean + 2S.D.	7.8×10^1	1.6×10^1	~ 3.9×10^2
Ferritin			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	3.2	1.2	~ 8.4
≧ Mean + 2S.D.	1.4×10^1	4.1	~ 5.2×10^1
Elastase 1			
> Mean − S.D. and < Mean + S.D.	1		
≦ Mean − S.D.	1.9	0.6	~ 6.2
≧ Mean + S.D.	1.4×10^1	4.9	~ 3.9×10^1

* : logistic regression results adjusted by sex and age.
** : almost zero.

Table 3. Case-control study making cases with chronic pancreatitis as Control and cases with pancreatic cancer as Case*

Tumor Markers	Odds Ratio	95% Confidence Interval	
CA19-9			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	1.5	0.3	~ 7.6
≧ Mean + 2S.D.	1.9×10^1	4.0	~ 8.7×10^1
CEA			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	0.7	0.3	~ 1.4
≧ Mean + 2S.D.	2.2×10^1	8.8	~ 5.6×10^1
α_1-AT			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	1.2×10^1	2.0	~ 7.2×10^1
≧ Mean + 2S.D.	2.5×10^1	5.1	~ 1.2×10^2
Ferritin			
≦ Mean + S.D.	1		
> Mean + S.D. and < Mean + 2S.D.	4.4	2.1	~ 9.4
≧ Mean + 2S.D.	3.9×10^1	1.2×10^1	~ 1.2×10^2
Elastase 1			
> Mean − S.D. and < Mean + S.D.	1		
≦ Mean − S.D.	0.5	0.2	~ 1.2
≧ Mean + S.D.	2.2	1.1	~ 4.7

* : logistic regression results adjusted by sex and age.

chronic pancreatitis as Control and patients with pancreatic cancer as Case (Table 3). In 30 patients with chronic pancreatitis and 60 patients with pancreatic cancer, we calculated the relative risk of pancreatic cancer using odds ratio (adjusted by sex and age). The high-level of α_1-AT and ferritin and the advanced high-level of CA19-9 and CEA showed a significant odds ratio. In both α_1-AT and ferritin, the possibility of pancreatic cancer became higher with the

Table 4. Case-control study by the combination of CA19-9 and α_1-AT*

		Odds Ratio	95% Confidence Interval
[Pt. without G.I.**]			
CA19-9	α_1-AT		
0****	0	1	
1*****	0	6.7	0.7 ~ 6.4×10^1
0	1	4.9	0.6 ~ 4.1×10^1
2******	0	2.3×10^2	2.2×10^1 ~ 2.4×10^3
0	2	9.5×10^1	9.7 ~ 9.3×10^2
1	1	3.3×10^1	1.5 ~ 7.0×10^2
1	2	6.4×10^2	1.9 ~ 2.2×10^4
2	1	1.1×10^3	3.9 ~ 3.3×10^4
2	2	2.2×10^4	4.1×10^2 ~ 1.2×10^6
[Pt. with chr.Pa.***]			
CA19-9	α_1-AT		
0	0	1	
1	0	3.7	0.4 ~ 3.7×10^1
0	1	2.1×10^1	1.8 ~ 2.3×10^2
2	0	4.3×10^1	4.5 ~ 4.1×10^2
0	2	4.3×10^1	4.9 ~ 3.7×10^2
1	1	7.7×10^1	1.8 ~ 3.3×10^3
1	2	1.6×10^2	4.3 ~ 5.9×10^3
2	1	8.8×10^2	1.8×10^1 ~ 4.4×10^4
2	2	1.8×10^3	3.9×10^1 ~ 8.4×10^4

*   : logistic regression results adjusted by sex and age.
**   : patients without gastrointestinal disease.
***   : patients with chronic pancreatitis.
****   : $\leq$ Mean + S.D.
***** : > Mean + S.D. and < Mean + 2S.D.
****** : $\geq$ Mean + 2S.D.

advanced high-level.

4) Case-control study by the combination of CA19-9 and α_1-AT (Table 4).

We combined CA19-9 and α_1-AT and calculated the relative risk of pancreatic cancer. With the result of the experiment of the patients without gastrointestinal disease, except for the combination of slightly high-level of CA19-9 or α_1-AT and the other normal, all abnormal combinations showed significant odds ratios. The possibility of pancreatic cancer in the case where CA19-9 and α_1-AT are the advanced high-level is 2.2×10^4 times compared with when the level is normal. With the result of the experiment of patients with chronic pancreatitis, except for the combination of the slightly high-level of CA19-9 and normal level of α_1-AT, all abnormal combinations showed significant odds ratios. The possibility of pancreatic cancer in the case where CA19-9 and α_1-AT are the advanced high-level is 2.2×10^3 times compared with the case which the level is normal.

DISCUSSION

With the progress of recent sonographic measurement, the progress of pancreatic cancer diagnosis has been remarkable, and this diagnosis has been carried out relatively easily. The attention has been paid to the diagnosis of smaller pancreatic cancer nowadays, however, early diagnosis and early treatment of pancreatic cancer is still insufficient even with various examination methods. As the reasons for this;

1) There is no obvious symptom in an early pancreatic cancer.

2) Expect for jaundice, early symptoms are non-specific, and other gastrointestinal diseases should be excepted.

3) ERCP, angiography and pancreatic biopsy for the decision of pancreatic cancer are so invasive that they cannot be used for all suspected patients, so, the more non-invasive screening method should be carried out.

Accordingly, the study of sera and immunological method chiefly tumor markers, has been often carried out[8]. However, in spite of the

progress of many immunological diagnoses, the methods which are useful clinically are very few. So we tried choosing the best marker for the diagnosis of pancreatic cancer and estimating the possibility of pancreatic cancer quantitatively.

It is necessary to set a normal range first, but each hospital set it so variously that it is difficult for the comparison between hospitals. Various methods of calculation for the normal range of level of clinical examination were proposed[3], but the method of Mean + S.D. which assumed a normal distribution or a log-normal distribution and the method of probability paper are often used as the method of calculation for the normal range. However, even with a health group, there are many examination items which are not suitable to the normal distribution of the log-normal distribution, and sometimes an outlier can be obtained. Whether the outlier is significant or not can be changed by the assumed distribution form. However, up to now, the method of setting up a normal range has not been established yet, and the normal distribution or the log-normal distribution should be assumed as for simplicity[7]. As the results of the calculation of skewness and kurtosis of normal control cases from my own experiments, it is considered to assume the log-normal distribution of all markers. And then we calculated Mean + S.D. and Mean + 2S.D.. Mean − S.D. is suitable for the cut-off line as for elastase 1, amylase and trypsin because the low level of these markers is significant clinically. As for ferritin, the normal level generally should be determined classifying by male and female (low level on female), but we set up 132 ng/ml which is a middle level as a cut-off.

Using these cut-off levels, we calculated relative risk with logistic regression. As for CA19-9, in the patients without gastrointestinal disease as Control, the possibility of pancreatic cancer is 8.9 times in the slightly high-level that is less than Mean + 2S.D., and 1.8×10^2 times in the advanced high-level that is more than Mean + 2S.D., and the possibility of pancreatic cancer is significantly high compared with the case where the level is normal. However, in the cases with chronic pancreatitis as Control, it is 1.5 times (not significant) in the slightly high-level and it is impossible to distinguish between

chronic pancreatitis and pancreatic cancer definitely. Cut-off level more than Mean + 2S.D. is needed to distinguish between chronic pancreatitis and pancreatic cancer. Difficulty to distinguish between chronic pancreatitis and pancreatic cancer often happened[4], and it gives us an important suggestion when judging an application of more invasive examinations. As for elastase 1, in the both patients without gastrointestinal disease as Control and with chronic pancreatitis as Control (including patients with pancreatic insufficiency) the low cut-off line which is Mean − S.D. is difficult to distinguish from pancreatic cancer, but the high cut-off line which is Mean + S.D. is significant. This will be one of reference findings on suspecting pancreatic cancer. As a combination of the minimum number of markers, we made a combination assay of CA19-9 and α_1-AT. In the case that the high-level of CA19-9 and α_1-AT which are more than Mean + S.D., the possibility of pancreatic cancer is 30 times higher than the case that the level of CA19-9 and α_1-AT are less than Mean + S.D., and it is extremely useful as screening. Economically, it is preferable to do screening of pancreatic cancer by a minimum examination, but the determination of singular markers alone is insufficient. So, it is necessary to make a combination in the minimum markers. And then we must do an invasive examination such as ERCP and angiography for the patients who are suspected as pancreatic cancer.

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