Int J Clin Exp Med 2014;7(10):3225-3234
www.ijcem.com /ISSN:1940-5901/IJCEM0001786

Original Article

Diagnostic value of combining CA 19-9 and K-ras gene mutation in pancreatic carcinoma: a meta-analysis

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Received August 8, 2014; Accepted September 15, 2014; Epub October 15, 2014; Published October 30, 2014

Abstract: Aims: To assess diagnostic value of Carbohydrate Antigen 19-9 (CA 19-9), combined CA 19-9 and K-ras mutation in plasma DNA in diagnosing patients with pancreatic carcinoma. Materials and methods: MEDLINE, EMBASE, the Cochrane Library, Sinomed, CNKI and other databases, from established to November, 2013, were searched for initial studies. All the studies published in English or Chinese relating to the diagnostic value of CA 19-9 and K-ras gene mutation for patients with pancreatic cancer were collected. Methodological quality was assessed. The statistic software called "Meta-disc" (version 1.4) was used for data analysis. Results: 10 studies were included in this meta-analysis. The pooled sensitivity estimate for CA 19-9 (78%) was significantly higher than K-ras mutation (65%). While, for the specificity estimate, K-ras mutation (93%) was significantly higher than CA 19-9 (77%). The pooled DOR estimate for K-ras mutation (21.82) was significantly higher than CA 19-9 (18.36). SROC curves for K-ras mutation showed better diagnostic accuracy than CA 19-9. For CA 19-9 measurement, its diagnostic value decreased in differentiating pancreatic cancer for patients with pancreatitis, especially chronic process. Conclusion: CA 19-9 was a high sensitive and K-ras was a high specific method in diagnosing patients with pancreatic cancer. These two modalities probably act different roles during different conditions in diagnosing pancreatic carcinoma.

Keywords: Carbohydrate antigen 19-9, K-ras mutation, pancreatic carcinoma, diagnosis, meta-analysis

Introduction

Pancreatic carcinoma (PC) is currently one of the most aggressive malignant tumors and a leading cause of cancer-related death. Despite the advances of diagnostic and therapeutic methods, PC remains distressing outcome with poor 5-year survival rate less than 5% [1, 2]. Radical resection at early stage is the only chance for patients to get long survival time [3]. Unfortunately, lack of early symptoms, biomarkers and its biological features, most were diagnosed either locally advanced or clinical evident metastases but only ~20% of patients with pancreatic cancer are considered suitable for surgery [4, 5]. Since PC is resistant to radiotherapy and chemotherapy, early diagnosis seems to be the sole option to improve survival rate.

Tumor-associated antigen CA 19-9 is a glycoprotein produced by gastric and pancreatobiliary tumors. In the past decades, CA 19-9 has been considered to be the most widely used marker in the management of carcinoma of pancreas [6]. However, CA 19-9 is usually evaluated in benign pancreatobiliary diseases, especially in chronic pancreatitis [7], so the specificity is not satisfied. Moreover, CA 19-9 is limited by the Lewis status [8].

K-ras is a common oncogene in human and it can bind guanine nucleotide involving growth factor signal transduction pathway, while pathological mutation of K-ras leads to cell proliferation [9]. Some scholars consider that K-ras mutation is an important and early event in tumorigenesis [10-12] and it could break by Lewis antigen limitation with better specificity.

So detecting K-ras mutation in plasma DNA could be used as a potential tumor marker in diagnosing patients with pancreatic carcinoma. However, some doctors hold an opinion that the relationship between K-ras mutation and pancreatic cancer is not positive correlation [13].

The aim of this meta-analysis is to evaluate and compare whether combined K-ras and CA 19-9 can improve the diagnostic value of pancreatic carcinoma, and find the best non-invasive diagnostic method for pancreatic carcinoma.

Materials and methods

Article search

A systematic article search was carried out to identify studies assessing the diagnostic value of CA 19-9 and K-ras gene mutation for human pancreatic carcinoma. The PubMed, Embase and Cochrane Library, from established to October 2013, were searched with the following words: (Antigens, Tumor-Associated, Carbohydrate OR CA 19-9 Antigen OR CA 19-9) AND (Genes, ras OR ras Gene OR K-ras Oncogene OR K-ras Gene OR ras Oncogene) AND (pancreatic neoplasm OR pancreas neoplasm OR pancreas cancer OR pancreatic cancer OR pancreatic carcinoma OR pancreas carcinoma). The SinoMed, CNKI and VIP databases were searched with the following keywords: (CA 19-9 AND K-ras) AND pancreatic carcinoma (in Chinese). Other databases, such as Web of Science, Elsevier were also checked with relevant articles with similar method.

Study extraction

Two investigators, who were blind to the journal, author, institution and date of publication, independently evaluated every article. According to a standardized data extraction form, we read all the abstracts in order to the potentially eligible articles and after that we manage to get full text to determine whether they are exactly eligible. Disagreements were resolved by discussion with the third investigator.

Studies were included if they (1) Articles were reported in English or Chinese. (2) Presented original data sufficient to calculate true-positive (TP), false-positive (FP), true-negative (TN) and false negative (FN). (3) Pancreatic carcinoma was diagnosed by at least one of the following: computerized tomography (CT), ERCP, laparoscopy/laparotomy and confirmed by histology. (4) Detecting CA 19-9 level and *K-ras* gene mutation for every pancreatic patient and control one. (5) Included more than 10 patients in the study. (6) The studies were based on per-patient statistics. (7) We just take the pancreatic ductal adenocarcinoma (PDAC) into our study. (8) Concerning to the quality of study design, only the article in which the number of the answer 'yes' for the 14 questions in QUADAS quality assessment tool [14] was more than 9 was included.

The excluded criteria were as follows: (1) Articles did not contain primary data such as reviews, case reports, comments, editorials, letters and congress. (2) Detecting CA 19-9 level or K-ras gene mutation in pancreatic specimen, pancreatic juice or stool instead of plasma. (3) Diagnosis of pancreatic carcinoma with other existing disease and could not be differentiated. (4) Other types of PC like Pancreatic cystic tumors or neuroendocrine tumors. (5) No comparison group. (6) Not in humans. (7) If the data duplicates, we just extract the latest one. (8) Golden diagnostic standard is not specified.

Quality assessment

A quality assessment tool for diagnostic accuracy studies, named "QUADAS", was used to assess the quality and extract relevant study design characteristics of included studies. This tool is an evidenced-based quality assessment tool developed for use in systematic reviews of studies of diagnostic accuracy and was fully described by Whiting [14].

Statistic analysis

We use statistic software "Meta-disc" (version 1.4) to analyze data for CA 19-9 and K-ras mutation. We calculate pooled sensitivity, specificity and diagnostic odds ratio (DOR) for each modality. We also calculated summary receiver operating characteristic curves (SROC) and *Q index. It is defined by the point where sensitivity and specificity are equal, which is the closest point to the ideal top-left corner of the SROC space.

The Galbraith plots (visual inspection of forest plots of accuracy estimates) and the I^2 value were used to assess heterogeneity among the studies included in the meta-analysis. A fixed-

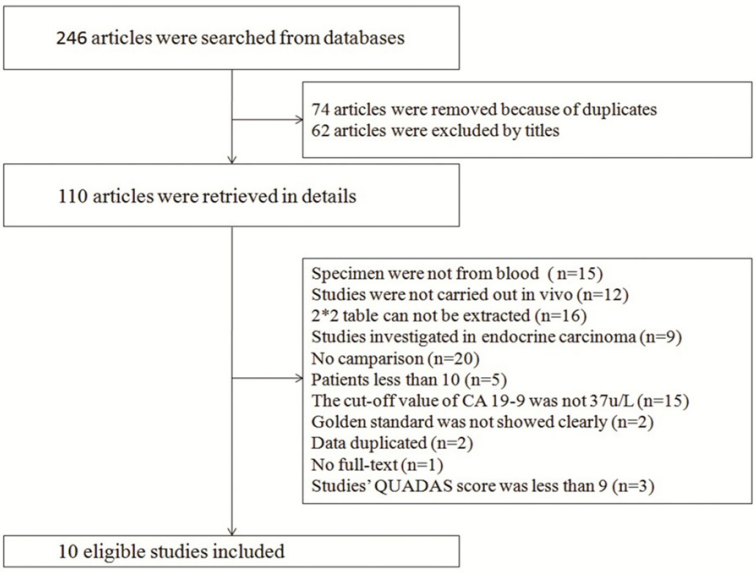

Figure 1. Studies evaluated for inclusion in this meta-analysis.

effects model was utilized if no homogeneity existed among different studies, while a random-effects model was used if heterogeneity existed.

Results

Literature search and selection of the studies

The detailed procedure of article selection in the meta-analysis was showed in **Figure 1**. A total of 246 reference publications were identified by the predefined search strategy. We used "Endnote X6" to manage the articles. Finally, 11 articles fulfilled all the inclusion criteria and were selected for data extraction and data analysis. We got all the full text for the 10 [13, 15-23] eligible articles. One article can't be found because it was published long before. **Table 1** shows the basic characteristics of the 10 studies included the meta-analysis. CA 19-9 concentration and K-ras mutation were simultaneous measured. 37 u/L was considered as the most optimal cut-off value for positive diagnosis. Though Livia Theodor's study used the cut-off value as 100 u/L, it showed every patient's specific consequence so that we can calculate TP, FP, TN, FN by statistical evaluation. Radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) were used to gauge CA 19-9 concentration. Methods evaluating *K-ras* mutation were all based on PCR technique, including PCR-restriction fragment length polymorphism (PCR-RFLP), PCR-single strand conformational polymorphism (PCR-SSCP) and PNA mediated PCR clamping. According to clinical experience, pathological diagnosis was the golden standard, imagiological examination and clinical manifestation were also used as direct evidence to diagnose pancreatic carcinoma.

Methodological quality assessment

We used the "QUADAS" quality assessment tool to evaluate each study. All the eligible studies' score was more than 9 in the 14 questions, indicating good quality.

Summary estimates of sensitivity, specificity and diagnostic odds ratio

The pooled sensitivity for CA 19-9 and K-ras mutation were 78% (95% CI: 0.73, 0.82) and 65% (95% CI: 0.60, 0.70), respectively. And the pooled specificity for them were 77% (95% CI: 0.71, 0.82) and 93% (95% CI: 0.89, 0.95), respectively. Herein, for the sensitivity estimates, it was significantly higher for CA 19-9 than K-ras mutation ($p < 0.05$). For To estimate the specificity, K-ras mutation was significantly higher than CA 19-9 ($p < 0.05$). However, to the studies for CA 19-9, both of the specificity (heterogeneity I^2 = 75.5%) and specificity (I^2 = 77.3%) were highly heterogeneous, which affected the diagnostic value of CA 19-9. Compared with CA 19-9, the heterogeneity of studies about *K-ras* was much lower (sensitivity: heterogeneity I^2 = 44.5%, specificity: heterogeneity I^2 = 54.3%) The forest plots were showed in **Figures 2** and **3**.

The DOR expresses how much greater the odds of having the disease are for the people with a positive test result than for the people with a negative test result. The pooled DOR for CA 19-9 and K-ras mutation were 18.36 (95% CI: 6.82-49.41) and 21.82 (95% CI: 12.02-39.62). The DOR estimate for K-ras mutation is significantly higher than CA 19-9 ($p < 0.05$).

Heterogeneity assessing and subgroup analysis

Evaluated by plotting the sensitivity and specificity from these two studies on forest plots and

Table 1. Basic characteristics of the 10 studies included the meta-analysis

Reference	Standard	Cut-off value (CA 19-9)	Patients	Mean age	Method (CA199/K-ras)
Livia Theodor *et.al.*	CT/ERCP Pathology/laparoscopy	37 u/L*	20	70.6	ELISA‡ PCR-RFLP††
Changliang Wu *et al.*	CT/ERCP pathology	37 u/L	12	61.7	RIA† PCR-MASA
Dianxu Feng *et al.*	Pathology	37 u/L	90	61	RIA PCR-RFLP
Han Lin *et al.*	pathology	37 u/L	20	60.1	ELISA PCR-MASA/RFLP
Lijuan Xing *et al.*	CT/pathology	37 u/L	36	62.4	ELISA PCR-RFLP
Menghua Dai *et al.*	pathology	37 u/L	15	56	RIA PNA-PCR clamping
Rodolfo Marchese *et al.*	pathology	37 u/L	30	65.3	RIA PCR-RFLP
Lishu Xu *et al.*	CT/pathology clinical manifestation	37 u/L	50	58	RIA PCR-PFLP
F Marie *et al.*	CT/ERCP pathology	37 u/L	47	65	ELISA PCR-RFLP
Jan Dabritz *et al.*	CT/ERCP pathology	37 u/L	56	59.7	ELISA PNA-PCR clamping

†RIA: Radioimmunoassay; ‡ELISA: enzyme linked immunosorbent assay; ††PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.

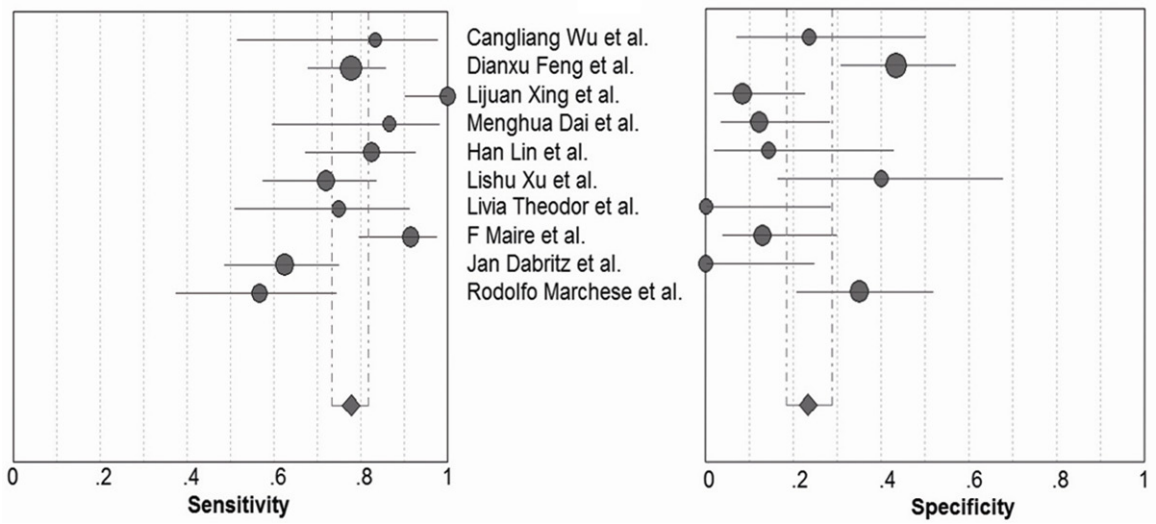

Figure 2. Pooled sensitivity and specificity of CA 19-9 in diagnosis of pancreatic carcinoma.

calculating the heterogeneity I^2 value, significant heterogeneity was found among study results of CA 19-9.

Threshold was one important extra source of variation in meta-analysis. If this effect appears, combining study results in these cases involves fitting an ROC curve would be better than pooling sensitivities and specificities. To decide whether the threshold effect existed or not, the Spearman correlation effect (-0.169) and *p*-value (0.620) were applied to test. Besides, the ROC plane doesn't show a curvilinear pattern. These two results showed no threshold effect in this meta-analysis.

Meta-regression analysis was used to explore other sources of heterogeneity in the studies for CA 19-9. We used the multi-variable regression model, with a backward stepwise algorithm. Variables evaluated included published year, detection method, sample size and region. Finally, detection method of CA 19-9, including enzyme immunosorbent assay (ELISA) and radioimmunoassay (RIA), was the source of heterogeneity.

The results of subgroup analysis of CA 19-9 (ELISA vs. RIA) were also presented in **Table 2**. For CA 19-9, the sensitivity, specificity, DOR and *Q index for studies that used ELISA to detect CA 19-9 were higher than those used RIA method ($p < 0.05$).

*Summary ROC curves and the *Q index*

The SROC curve and the *Q index for CA 19-9 and *K-ras* mutation were showed in **Figures 4**, **5**. Because of the heterogeneity and no threshold effect, we chose the random-effect model

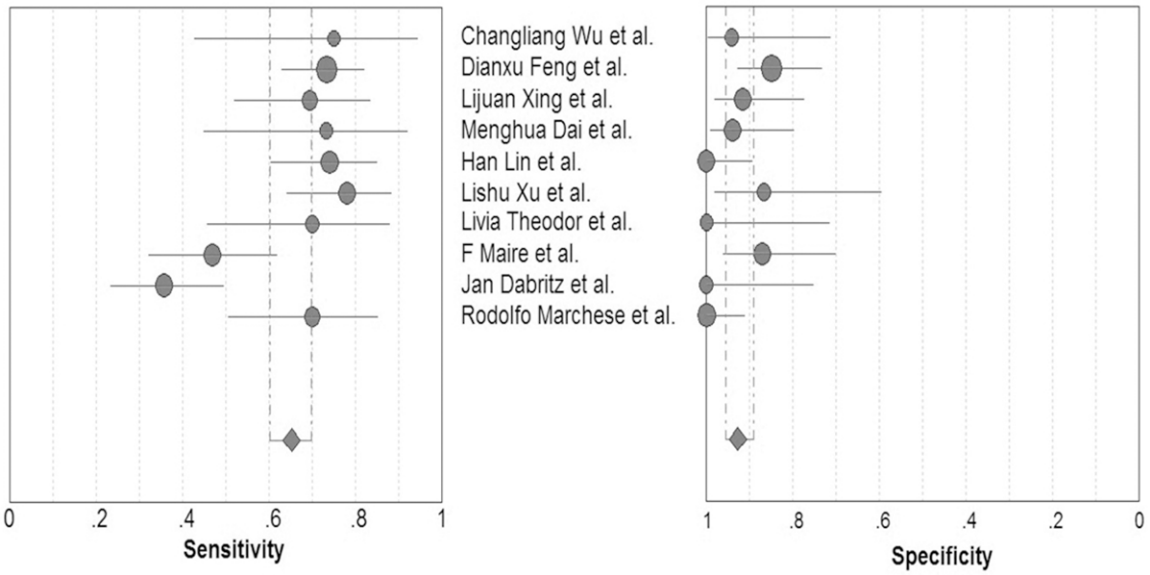

Figure 3. Pooled sensitivity and specificity of K-ras gene mutation in diagnosis of pancreatic carcinoma.

Table 2. subgroup analysis of CA19-9

Tumor markers	Pooled sensitivity	Pooled specificity	DOR	*Q
CA 19-9				
overall	0.78 (0.73-0.82)	0.77 (0.71-0.82)	18.36 (6.82-49.41)	0.8144
RIA	0.76 (0.70-0.81)	0.69 (0.61-0.75)	7.66 (3.29-17.84)	0.7545
ELISA	0.81 (0.74-0.87)	0.92 (0.85-0.97)	93.98 (29.72-297.18)	0.9075
K-ras				
overall	0.65 (0.60-0.70)	0.93 (0.89-0.95)	21.82 (12.02-39.62)	0.7811

to synthesize the ROC curves for CA 19-9. While the fixed-effect model was applied for *K-ras* mutation. The AUC of CA 19-9 and K-ras mutation were 0.8839 and 0.8500 respectively. The *Q index estimates for them were 0.8144 and 0.7811, respectively.

Discussion

Although the biopsy like endoscopic ultrasound guided fine needle aspiration (EUS-FNA) is the most accurate method, due to its invasive characteristic, few people would accept it, especially for health screening. The importance of tumor markers can't be overstressed. Detection of blood-based tumor markers was shown to be a promising and non-invasive assay [24]. Various kinds of tumor markers were expanding nowadays, but clinical use of these markers lags behind. For pancreatic ductal adenocarcinoma, this is most pronounced [25, 26]. CA 19-9 is mainly synthesized by pancreatic duct, as well as epithelium cells of gastrointestinal tract and biliary system. At the same time, it is the sole FDA approved tumor marker suggested for use in management of PDAC patients. It is widely used for diagnosing and evaluating prognostic survival rate. In recent years, an increasing number of scholars have been arguing its role in utilizing in the field of PDAC. Low specificity [27] and limiting by Lewis status [28] restricted its range of application.

Ras family are most common oncogene mediating signal transduction pathways, which involves inflammation and cells proliferation [29]. *K-ras* mutational activation is an early event in tumorigenesis [30]. Reports have shown that *K-ras* mutation in PDAC up to 47%-100% and most often in 12 [31-33]. With the development of molecular biology, detecting of *K-ras* mutation in serum via PCR based technique is possible and efficient [33]. Since its high mutation rates in many kings of cancers, some scholars regard it as a potential tumor marker.

In this meta-analysis, we found CA 19-9 was more sensitive and K-ras mutation was a more specific modality for patients suspected to

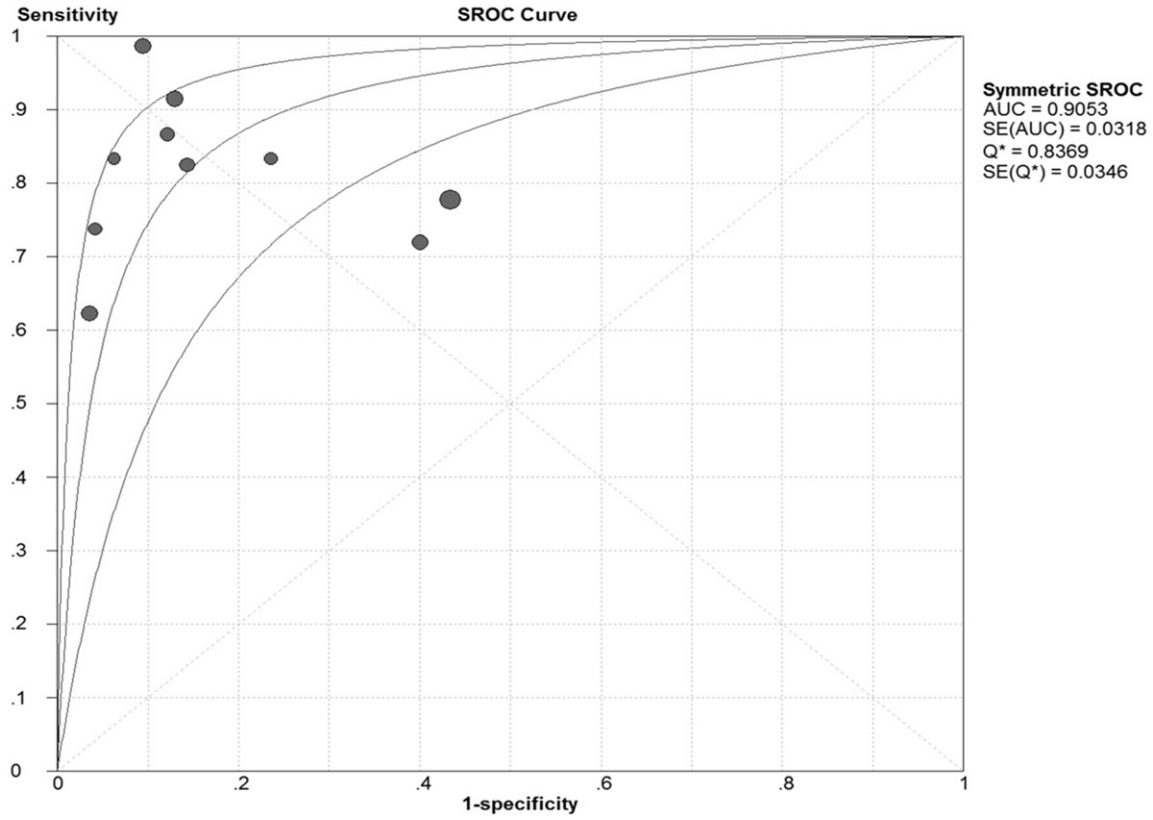

Figure 4. Summary receiver-operating characteristic (SROC) curves of CA 19-9 for assessment of pancreatic carcinoma.

have pancreatic cancer. These results indicate that CA 19-9 and K-ras mutation would play different roles in diagnosing PDAC. But the diagnostic value of CA 19-9 is restricted by its high heterogeneity. In order to explore the sources of heterogeneity in the studies for CA 19-9, threshold analysis and meta-regression were performed. Since according to the Spearman correlation effect and *p*-value, no threshold effect was found in these studies, the heterogeneity for CA 19-9 was caused by other factors including published year, sample size, detection assay *etc*. The results of meta-regression analysis indicate that the detection assay is the most important characteristic, which significantly influenced its diagnostic accuracy. Subgroup analysis showed that detecting CA 19-9 concentration by RIA assay would decrease its diagnostic value. This is perhaps these two methods- ELISA and RIA- have different characteristics, in terms of a specific solely antigen, ELISA has more advantage than RIA-higher throughout put [34].

Overall, PDAC portends a very poor prognosis, even for the minority of patients diagnosed at early stage who are amenable to surgical resection. Currently, clinicians usually take advantage of pathologic features, imageological examination and CA 19-9 levels to guide therapeutic strategies [35]. Even the arguments of CA 19-9 in diagnosing PDAC is increasing, it still remains the most widely used tumor markers in the management of PDAC patients [36]. Nevertheless, because of lower levels in early stage pancreatic cancer, CA 19-9 is unsatisfied for screening healthy people or small tumors [37]. Since the biomarkers for PDAC falls behind other solid tumors, this field has been expanding in recent years which includes other canbonhydrate antigens [38-40] (CA242, CA50, CA125), carcinoembryonie antigen (CEA), Dupan-2 [41], Span-1 [42], osteopontin, tissue inhibitor of metalloproteinase I [43] (TIMP I), matrix metalloproteinase 7 [43] (MMP-7) and genetic diagnosis (*K-ras* and *p53*). Among these tumor markers, we chose *K-ras* to com-

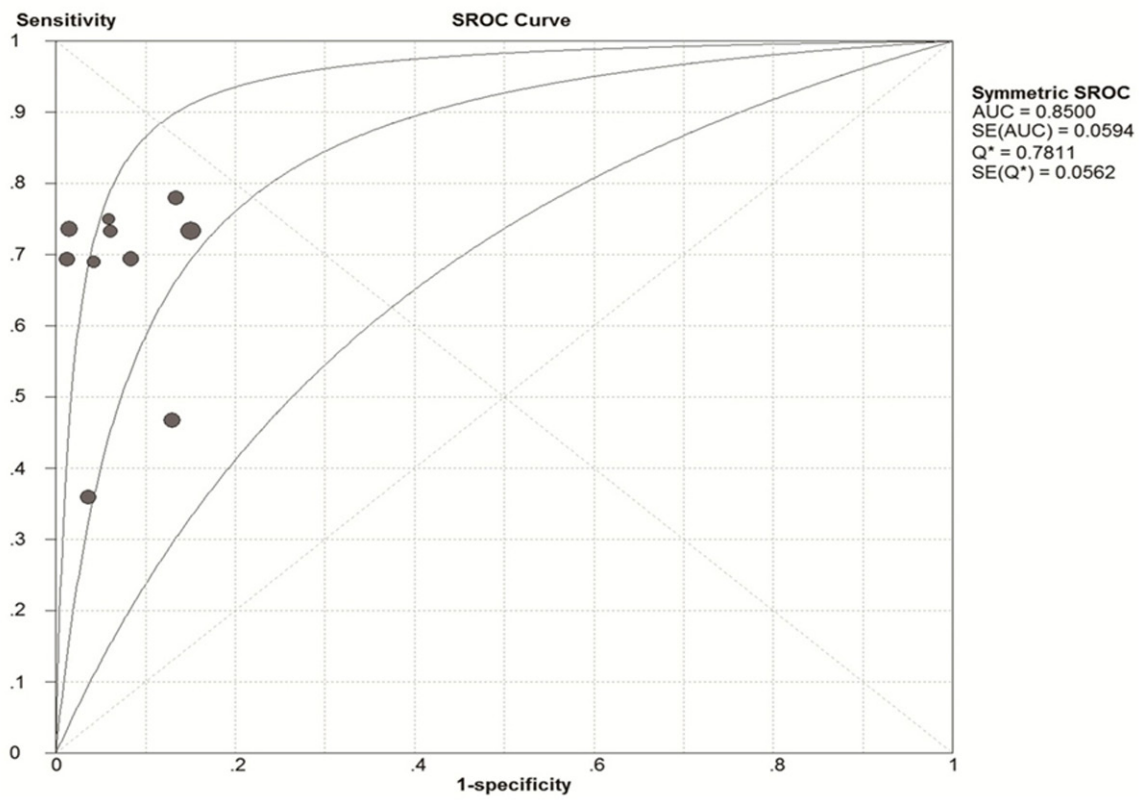

Figure 5. Summary receiver-operating characteristic (SROC) curves of k-ras gene mutation for assessment of pancreatic carcinoma.

pare with CA 19-9 for diagnosing pancreatic cancer. Although the sensitivity for K-ras mutation is lower than CA 19-9, its specificity is better in our study. Perhaps this is because K-ras mutation does not increase in pancreatitis and it often occurred in the early stage of tumorigenesis. This will be right for high volume screening. While CA 19-9, and many kinds of tumor markers, usually elevate in biliary tract diseases [44] including biliary calculi, common bile duct carcinoma and biliary inflammation [33, 45]. This case will significantly influence the diagnostic value. Gemmel C et al. [46] showed that combination of blood-based markers, genetic markers and imaging methods might be the future for pancreatic screening. Continued efforts are needed to clarify efficient testing panel to identify patients without hereditary risk factors who will benefit from screening protocol. Traditional ultrosonography represents the first and convenient imaging assay for the study of PDAC. High resolution CT is regarded as the golden standard in the study of pancreatic lesions and tumor stage, contrast-enhanced CT has significantly improved the diagnostic accuracy of this assay. MR including MRCP supplies better study of pancreatic cystic lesions and pancreatic ductal system. Overall, final confirmation of a lesion relies on image-guided fine needle aspiration (FNA). Other assays like PET/CT remains the techniques employed as third-line examinations.

Ideal tumor markers should have 100% sensitivity and organ-specificity, while we search for the ideal markers, increasing reports [47-49] suggest that the studies also need to extend beyond the analysis of blood proteins and include molecules like (mi)RNA [50] and DNA. Sample related to PDAC such as pancreatic juice, cystic fluid and FNA specimens are likely to provide the clues of new molecules that will form the foundation for improved method in the near future. Sadly to say, therapeutic assays are very limited because of the late-stage diagnosis, which stresses the importance of the assessment of new molecular markers to improve the early diagnostic rate [51]. Additional studies are needed before applying these markers into routine clinical use for diagnosing

PDAC. To realization of screening panels currently seems to be the only way to reduce the mortality of PDAC, since the paucity of options for treatment beyond surgical.

In our study, bias was considered. For selection bias, besides the PubMed and EMBASE databases, the Cochrane library, Sinomed, CNKI were searched for eligible articles. Not only original studies but also academic dissertations were included. To decrease bias, two reviewers were blinded to the author, journal, institution and published date, independently chose articles on the basis of inclusion criteria. QUADAS scores ensure the quality of articles. Only the answer for the 14 questions in QUADAS assessment was over 9, was selected.

Nevertheless, some limitations still exist in our study. Since only the articles published in English and Chinese were selected in this meta-analysis. This would rise to inevitable bias. But this bias has little impact because most studies of high quality were published in English. We found that the quality of English article have always favored Chinese ones. We tried to analyze publication bias by evaluating of whether the size of studies and published year were associated with the results for diagnostic accuracy. While sample size as well as published year had no relationship with diagnostic accuracy. Confirmation bias was also taken into consideration. In these studies, references standard ranges from pathological analysis to clinical manifestation and imaging method. We tried to reveal that whether the type of assays influences diagnostic accuracy, while the results showed that no significant difference can be found. However, some drawbacks still exist. The major problem is that no perspective trials of high quality could be searched in the above databases. But for the trails in terms of tumor markers, most trails move a retrospective approach. The other problem is that the control group included various diseases including acute pancreatitis, chronic pancreatitis, non-gastrointestinal malignant diseases and healthy controls, if we can explore the role of CA 19-9 and K-ras mutation in different groups, these would be a promising study and that is also our future plan.

Conclusions

CA 19-9 and K-ras mutation could play different roles in diagnosing PDAC. Though the sensitivity of CA 19-9 is high, while its specificity is lower than K-ras mutation.

Acknowledgements

The author thanks the information resource of Shanghai Jiao Tong University, School of Medicine. Professor Xiaochun Qiu offered great assistance to our work.

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