Diagnostic value of NPTX2 (neuronal pentraxin II) methylation in patients with pancreatic cancer: meta-analysis.

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ABSTRACT

Purpose: Pancreatic cancer (PC) is a devasting disease of which mortality almost parallels its incidence. Pancreatic cancer tissue may express aberrantly methylated NPTX2, but it is unclear what the consequences of this are. The purpose of the present study was to assess the diagnostic performance of methylated NPTX2 in PC diagnosis. Methods: We conducted a comprehensive search of PubMed, Web of Science, Chinese National Knowledge Infrastructure (CNKI), and the Cochrane Library for published studies from inception to July 15, 2020. Using STATA 13.0, diagnostic OR (DOR) and AUC (Area Under the Curve of Receiver Operating Characteristic) were calculated to evaluate the diagnostic efficacy. Results: Nine studies were found eligible for the meta-analysis. The overall results of DOR and AUC were 11 (95%CI: 4-26) and 0.80, respectively. These data indicate that aberrantly methylated NPTX2 can correctly predict PC. Subgroup analysis revealed that quantitative real-time methylation-specific PCR (QMSP) had the highest diagnostic value for differentiating pancreatic cancer from chronic pancreatitis using a laboratory method. Furthermore, the detection of hypermethylated NPTX2 found in plasma was suggested to be a promising diagnostic biomarker, though a meta-analysis was not feasible due to the limited number of samples. The Deeks' funnel map revealed no obvious public bias in the literature. Conclusion: aberrantly methylated NPTX2 has high sensitivity and specificity for the diagnosis of pancreatic cancer. However, further research is required to validate the use of methylated NPTX2 as a biomarker in the clinical diagnosis of pancreatic cancer.

Key words: neuronal pentraxin II ; pancreatic cancer ; diagnostic

INTRODUCTION

Pancreatic cancer (PC) is one of the most severe malignant tumors in the world, with an estimated 227,000 deaths per year worldwide and is the fourth leading cause of cancer death in the USA. [1] Substantial progress has been made to our understanding of pancreatic cancer biology by developments in the detection and management of the disease. [2, 3] However, it still poses a major threat to human health due to poor diagnosis and prognosis. For patients with advanced PC, the overall five-year survival rate is about 6% (ranges from 2% to 9%) and the average median survival time is only six months. [4, 5] Only 10–20% of pancreatic cancers can be surgically resected with curative intent at the time of diagnosis, and many patients suffer from metastases and recurrence after surgical resection. [6] The main reason for diagnosis at the advanced stage is the lack of high sensitivity and high specificity biomarkers for early detection as well as a lack of overt clinical symptoms.

Endoscopic retrograde cholangiopancreatography (ERCP)-guided pancreatic duct brush cytology and percutaneous fine-needle aspiration (FNA) cytology or biopsy can provide pathologic confirmation for the diagnosis of PC. However, ERCP-guided pancreatic duct brush cytology and percutaneous FNA are invasive procedures, and it can be challenging to reach the mass in the pancreas anatomically due to surrounding major vessels. Furthermore, there is a risk of seeding cancer cells during surgery. Therefore, there is an urgent need to find biomarkers for early and accurate diagnosis of PC to improve the mortality rates and prognosis.

Evidence recently emerged that the formation of pancreatic cancer involves multiple processes, including epigenetic alterations and accumulation of gene changes. [7] Some of the most significant mechanisms of epigenetic regulations include DNA methylation, histone modification (methylation, acetylation, phosphorylation, ubiquitination, and sumoylation), chromatin remodeling, and non-coding ribonucleic acids (RNAs).[8] DNA methylation is one of the key mechanisms of epigenetic regulation. DNA methylation at gene promoter CpG islands blocks transcription initiation, whereas DNA methylation in the gene body may facilitate different splicing and transcription elongation.

Aberrant DNA methylation is one of the best-characterized epigenetic alteration mechanisms in cancer. [9] Epigenetic changes, such as hypermethylation, have been discovered in the early stages of many tumors. [9-11] During the development of pancreatic cancer, several genes, including SPARC, p53, p16, K-Ras, and NPTX2, can be aberrantly methylated and may provide early diagnostic value. [12-16] The neuronal pentraxin II gene (NPTX2) is a tumor suppressor that plays anti-tumoral roles through the promotion of G0-G1 arrest and cell apoptosis. [17] Methylated NPTX2 can be detected in over 90% of pancreatic cancer and is rarely found in healthy tissues. [17, 18] Therefore, methylated NPTX2 in pancreatic juice, plasma, or fine-needle aspirates (FNA), may serve as a reliable novel biomarker for pancreatic cancer. In this study, we, for the first time, performed a systematic review and meta-analysis to evaluate the diagnostic value of methylated NPTX2 in pancreatic

cancer. Our data may aid in clinical decision-making and the development of methylated NPTX2-based targeted therapies.

MATERIALS AND METHODS

Search strategy:A systematic literature search was conducted according to PRISMA (preferred reporting items for systematic reviews and meta-analyses) and Cochrane guidelines. The systematic literature search was performed using four electronic databases (PubMed, Web of Science, the Chinese National Knowledge Infrastructure (CNKI), and the Cochrane Library) with restriction to language in English and Chinese. Searches were performed from inception to July 15, 2020, and were limited to publications with human subjects. Abstract data and completed studies were included. The citations within the identified articles were examined manually.

The following search terms were used: ("NPTX2" or "neuronal pentraxin II") and ("pancreatic cancer" or "pancreatic carcinoma" or "pancreatic tumor" or "pancreatic neoplasm") and ("methylation" or "methylated")

Inclusion and exclusion criteria:The inclusion criteria for this study were: (a) the diagnosis of PC was made based on the histopathological confirmation or any definite diagnostic, (b) case-control studies or cohort studies included standard references for the PC diagnosis, including patients with benign disease or healthy individuals as the control groups, (c) the study provided sufficient data, including samples, sensitivity, and specificity, (d) the level of NPTX2 in plasma, pancreatic juice, or cells was detected, and (e) the diagnostic value of hypermethylated NPTX2 in pancreatic cancer was assessed.

The exclusion criteria for this study were: (a) did not assess methylated NPTX2 or pancreatic cancer, (b) did not provide information on research samples or sensitivity or specificity, (c) animal studies, case reports, reviews, conference abstracts, letters, and expert opinions, (d) duplicated data, or (e) not available in full text.

Data extraction

Two reviewers (WQH and LFX) evaluated the eligible studies independently. The following data were extracted from each study: a) basic characteristics of the eligible studies, including the first author, year of publication, ethnicity, mean age, specimen, test method, and b) diagnostic value: the sensitivity, specificity, and cut-off value. Any disagreements were resolved by discussion or by consulting the other author (YQN).

The quality assessment of the included studies was independently conducted by two authors using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria checklist in the Rev Man software 5.0 (http://ims.cochrane.org/revman/download). The scale consists of four domains, including Patient Selection, Index Test, Reference Standard, Flow, and Timing. The estimations of each study, including the risk of bias and application concerns, were ranked "low risk", "unclear risk", and "high risk", which matched to the answer of each question "yes", "unclear", and "no". Besides, the applicability concern did not

apply in the flow and timing domain. [20] The answer “yes” meant a low risk of bias, while the answer “no” or “unclear” meant a high risk of bias. Any disagreements were resolved by discussion or by consulting the other author (YQN).

Statistical analysis

The software Stata 13.0 (Stata Corporation, College Station, TX, USA) was used to evaluate the pooled statistics (95% CI) of sensitivity, specificity, positive and negative likelihood ratios (PLR and NLR) [PLR = sensitivity/(1 - specificity), NLR = (1 - sensitivity)/specificity], diagnostic odds ratio (DOR), and area under the summary receiver operating characteristic curves (AUSROC) of each eligible study for assessing the diagnostic value of methylated NPTX2 for PC detection. PLR and NLR are the discriminatory properties of positive and negative test results, respectively. [21] DOR represents the positive odds of methylated NPTX2 expression in PC patients compared to the probability of benign disease controls. SROC values of 0.5-0.7, 0.7-0.9, and 0.9-1.0 were used to demonstrated low, moderate, and high diagnostic accuracy, respectively. A p-value <0.05 was considered statistically significant. Publication bias of diagnostic studies was assessed using the Deeks’ test. [22]

The Q Test and I2 Test were used to evaluate the heterogeneity among the included studies. A low heterogeneity was indicated by $P > 0.1$ and $I2 < 50\%$, for which a fixed-effects model (Mantel-Haenszel method) was chosen. Otherwise, when $P \leqslant 0.1$ and $I2 > 50\%$ (indicating higher heterogeneity), a random-effects model (DerSimonian and Laird) was chosen. Meta-regression analysis was used to explore the source of heterogeneity, and a subsequent subgroup analysis was performed to identify potential covariates. If there was no apparent methodological heterogeneity, a random effect model was used. [23, 24]

RESULTS

Search results:As presented in the flow diagram (Figure 1), 106 articles met our search strategy criteria after duplicates were removed. By evaluating the titles and abstracts, 68 articles were excluded: 54 did not cover NPTX2 or pancreatic cancer, 3 were animal experiments, and 11 were reviews. The remaining 38 articles were assessed by full-text reading. Then, 29 articles were excluded: 18 did not have relevant outcomes reported, and 11 were without sufficient data. Finally, nine articles were eligible to be included in the meta-analysis. [25-33]

Study characteristics and quality assessment:The baseline characteristics of the nine eligible studies are summarized in Table 1. Between 2003 and 2019, 512 patients with pancreatic cancer, 332 patients with benign pancreatic diseases, and 77 healthy controls were involved. Seven studies detected methylated NPTX2 in pancreatic juice, which is obtained using a duodenal endoscope, and two studies collected plasma data. Eight studies used both the real-time methylation-specific PCR (MSP) and quantitative real-time MSP (QMSP) methods to determine the content of NPTX2 in pancreatic cancer tissues, while one study used only the MSP method for detection. All included studies had selected chronic pancreatitis (CP) as the control group for

pancreatic cancer, and three studies assessed benign intraductal papillary mucinous neoplasms (IPMNs), two studies assessed benign biliary strictures with healthy pancreas, two studies evaluated malignant intraductal papillary mucinous neoplasms, and two studies included healthy individuals.

The quality assessment of included studies by QUADAS-2.0 indicated that none of the studies had significant shortcomings to exclude them from the meta-analysis. As shown in Figure 2A and 2B, more than half of the studies were considered to have potential bias and applicability concerns in the patient selection. Almost half of the studies were estimated that have an unclear risk of flow and timing domains.

Overall results of the diagnostic value of methylated NPTX2 :Nine eligible studies were included in the meta-analysis. Pancreatic cancer and benign pancreaticobiliary disease were chosen as the sample groups to calculate overall outcomes. Calculations were made using the bivariate mixed-effects model developed by von Houwelingen (von Houwelingen, 1993, 2001) for treatment trial meta-analysis, which were then modified for the synthesis of diagnostic test data (Reitsma, 2005; Riley, 2006), The forest plot of the sensitivity and specificity are depicted in Figure 3. It shows a significant heterogeneity in the summary sensitivity (I2 =72.93, $p < 0.001$) and specificity (I2 =78.13, $p < 0.001$). The summarized sensitivity was 0.67 (95% CI 0.58 – 0.76), the specificity 0.84 (95% CI 0.69 – 0.92), the PLR 4.1 (95% CI 2.1 – 8.2), the NLR 0.39 (95% CI 0.29 – 0.52), the DOR 11 (95%CI 4 – 26) and the AUC 0.80 (Figure 2). Furthermore, the summary receiver operator characteristic (SROC) curve and AUC was found to be 0.80 [95% CI 0.76 - 0.83]. The data points did not show a "shoulder arm" in the ROC plot, suggesting that the included studies might not have a threshold effect. There was no publication bias observed in the eligible studies according to the Deeks' test (P=0.39). The above results indicate that methylated NPTX2 might be a specific biomarker for pancreatic cancer. In addition, the calculated data also point out that there exists significant heterogeneity between the studies and further subgroup research is needed to find the sources of the heterogeneity.

Subgroup analysis of chronic pancreatitis:We performed a subgroup analysis including different controls: three benign intraductal papillary mucinous neoplasms (IPMNs) studies, nine chronic pancreatitis (CP) studies, two benign biliary strictures with normal pancreas studies, two malignant intraductal papillary mucinous neoplasm studies, and two healthy individual studies. Only the number of chronic pancreatic studies was enough to run the meta-regression. The result of results of that analysis were: sensitivity 0.67 (95% CI: 0.57-0.76), specificity 0.85 (95% CI: 0.73-0.93), PLR 4.6 (95% CI: 2.4-8.9), NLR 0.39 (95% CI: 0.29-0.50), DOR 12 (95% CI: 6-26), and AUC 0.82 (Figure 4). Compared to the analysis not dividing on benign disease, the specificity, PLR, DOR, and AUC were observed higher when separating chronic pancreatitis from pancreatic cancer.

We performed a Z test to compare the areas under receiver operating characteristic curves for benign pancreatic disease and chronic pancreatitis. We ran the formula: Z =(S1-S2)/(SE1*SE1+SE2*SE2)^(0.5) using the MS Excel software package. S1 and

S2 represent the square of the receiver operating characteristic curves. SE1 and SE2 represent the standard error of the ROC, and the symbol ^ represents the root sign. If the average proportion of Z values was >2.0 or <-2.0， it would demonstrate a statistically significant difference.[35-37] We found that Z for this analysis was 0.7, and thus <2. Therefore, we found no statistical differences in the diagnostic value of methylated NPTX2 between PC and benign disease, as well as PC and CP. This suggests there was no difference in using methylated NPTX2 to detect patients with pancreatic cancers from chronic pancreatitis or benign pancreatic disease.

Comparison of the diagnostic value of different methods:Two lab methods were used to determine the rate of NPTX2 promoter hypermethylation: quantitative real-time MSP and MSP. In the meta-analysis assessing data obtained with the QMSP method, the specificity (0.89 (95% CI: 0.81-0.94), positive likelihood ratios (PLR) (5.7 (95% CI: 3.2-10.2)), diagnostic OR (DOR) (14 (95% CI: 6-30)), and the AUC (0.89) of QMSP in pancreatic juice collected from PC versus CP studies (Figure 5) was higher than those obtained with the MSP method (specificity: 0.79 (95% CI: 0.69-0.86), PLR: 3.2 (95% CI: 2.1-4.8), DOR: 8 (95% CI: 4-14), and AUC: 0.80) (Figure 6). The sensitivity forest plot (I2=63.4) for the QMSP method indicated a significant heterogeneity among the studies while the P-value of the specificity is 0.44, showing no statistical significance.. The diagnostic specificity of QMSP was higher than that of MSP, suggesting that this quantified method has a higher diagnostic value. After conducting the Z test, we found statistical significance between the different examinations in detecting NPTX2 (Z=-3.53 ＜ -2). This further confirmed the robustness of our conclusions.

DISCUSSION

Most patients with pancreatic ductal adenocarcinoma (PDAC) present with locally advanced or distant metastatic disease (80–85%), and only (15–20%) of these tumors are surgically resectable. [38,39] Several studies have indicated that patients with incidentally-discovered PDAC, especially those with sub-centimeter lesions, have higher survival rates, with a resectability rate of 99.0% and an operative mortality rate of 4%. [40-41] Therefore, early detection of patients with PC is essential to improve outcome. Many studies have attempted to identify useful diagnostic markers, as many genetic and epigenetic alterations occur during pancreatic tumorigenesis, such as mutant K-Ras, SPARC, p53, and p16, [12-16] which lack sufficient sensitivity and specificity for early PC diagnosis. [42-44] Universal screening tools, such as endoscopic examinations and biopsies, are invasive and inconvenient, leading to potential errors in PC detection. [45] Furthermore, there is a risk of seeding cancer cells during the surgery. Therefore, non-invasive biomarkers are urgently needed for the detection of PC, next to the development of a novel therapeutic regimen. Studies into novel tumor biomarkers have shown that DNA methylation may play a significant role in cancer. Variation has been observed in methylated NPTX2 expression levels between cancer patients and benign disease controls. [25-33] However, limited work has been done to perform meta-analyses on methylated NPTX2 as a diagnostic biomarker for pancreatic cancer.

This meta-analysis aimed to determine the diagnostic value of aberrantly methylated NPTX2 for pancreatic cancer detection. We collected nine eligible studies, including 512 patients with pancreatic cancer, 332 patients with benign pancreatic diseases, and 77 healthy controls, published between 2003 to 2019. The overall sensitivity was 0.67, the specificity was 0.84, and the AUC was 0.80 in pancreatic cancer as compared to the benign pancreatic disease group. The pooled DOR was 11 (95% CI: 4 – 26). A DOR value greater than 1 was acceptable, and the greater the value of DOR, the higher the diagnostic value. This showed that the aberrantly methylated NPTX2 gene might be a specific biomarker for differentiating pancreatic cancer from benign pancreatic disease. However, by pooling data in this manner, we found the sensitivity to be moderate. Therefore, we conjected that the diagnostic value would be inaccurate because of the significant heterogeneity and diagnostic threshold. Hence, the high heterogeneity should not be ignored, and statistical outcomes should not be interpreted blindly. Therefore, we next explored subgroup and regression analyses.

Only the number of chronic pancreatic studies was enough to run the meta-regression. For chronic pancreatitis controls, the specificity was 0.85 (95% CI: 0.73-0.93), the PLR was 4.6 (95%CI: 2.4-8.9), the DOR was 12 (95% CI: 6-26), and the AUC was 0.82, which were all higher than the group without stratification. However, the Z test value was 0.78, showing no statistical significance of the diagnostic value of methylated NPTX2 between PC and benign disease, as well as PC and CP. There was no difference in using methylated NPTX2 to detect patients with pancreatic cancers from chronic pancreatitis or benign pancreatic disease.

Furthermore, compared with the normal MSP, the quantitative MSP resulted in higher DOR and specificity in detecting methylated NPTX2 (specificity: 0.89 (95% CI: 0.81-0.94), PLR: 5.7 (95% CI: 3.2-10.2), DOR: 14 (95% CI: 6-30), and AUC: 0.89). The Z test value indicated that there existed statistical significance between the two methods of detecting NPTX2 ($Z=-3.53<-2$). The Z value ensured the robustness of the meta-regression conclusions. It suggested that the QMSP test could be a discriminating method in separating benign pancreatic diseases from pancreatic cancer. Parsi *et al*. [50] indicated that in those patients with suspected pancreaticobiliary disease, the collection of DNA methylation alterations in endoscopic retrograde cholangiopancreatography brush samples using QMSP had a significantly higher overall diagnostic accuracy than both pathological examination of cytology samples and the MSP technique.

Integrated layered results: The whole groups' DOR is higher than 8, in particular, the chronic pancreatitis group analyzed by using the lab method QMSP was found to be of the highest diagnostic value (14).

The aberrant methylation of genes can be detected in pancreatic juice, cytology, or even plasma samples. Therefore, it provides easier to reach and less invasive sample sources for the early detection of pancreatic cancer. There are very few studies using blood samples to obtain plasma DNA of pancreatic cancer patients to analyze the hypermethylation status of the genes. [46-49] In this meta-analysis, only two studies chose plasma as the sample source [29, 31] to detect methylated NPTX2. The

number of studies limited subgroup meta-regression analyses, decreasing the robustness of the diagnostic value. However, in those two studies, the results were promising, suggesting that NPTX2 hypermethylation status was statistically significant in pancreatic cancer as compared to chronic pancreatitis (P = 0.016), and the sensitivity and specificity were 80% and 76%, respectively. Therefore, further research to compare the diagnostic value of different sample sources is required as it might improve clinical diagnosis.

The present meta-analysis had several limitations. Firstly, the number of studies included was small and most of the sample sizes in the included studies were relatively small. Secondly, some eligible studies did not provide a cut-off value limiting the subgroup analyses based on those cut-off values, which could significantly influence the final diagnostic value. Thirdly, the eligible studies did not evaluate the effects of other risk factors, such as age, sex, smoking, and diet in PC, which might increase the robustness of the outcomes. Fourthly, the analysis only reviewed full-text studies in the English and Chinese language, which may have caused a selection bias. Fifthly, the number of studies that studied benign pancreatic diseases, except for chronic pancreatitis, was too small to perform a subgroup meta-regression, which might also decrease the robustness of the diagnostic value of methylated NPTX2 in PC.

CONCLUSION

This study revealed that the quantitative analysis of NPTX2 methylation could serve as a promising molecular biomarker for pancreatic cancer diagnosis, showing the highest diagnostic value in differentiating pancreatic cancer from chronic pancreatitis with the lab method QMSP. However, the number of plasma samples was too small to run a meta-regression. There is a need to increase experiments on methylated NPTX2 in plasma in the future. Further large-scale prospective studies are required to validate the use of methylated NPTX2 as a biomarker for the clinical diagnosis of pancreatic cancer.

Acknowledgments

None declared.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

W.q.H., L.f.X., and Y.q.N. conceived and designed the research. W.q.H., L.f.X., J.X., and H.m.X. searched the literature and analyzed the data. W.q.H., L.f.X. wrote the manuscript. J.X. and H.l.Z. proofread the manuscript. Y.q.N. finally revised the manuscript. W.q.H., L.f.X. contributed equally to this work as first authors.

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