—Original Article—

Independent risk factors for true malignancy in atypical cytologic diagnostic category in EUS-FNA/FNB of the pancreas: A novel prediction model

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ABSTRACT

Background and Objects: An atypical cytologic diagnosis arises from inflammation or early neoplastic process. It is commonly found in EUS-guided fine-needle aspiration/biopsy (EUS-FNA/FNB) tissue sampling of pancreatic malignancies. The aims of this study were to evaluate the diagnostic performance of EUS-FNA/FNB in patients with cytologic diagnosis of atypical cells and to develop a prediction model for malignant tumors of the pancreas in the atypical cytologic diagnostic category. **Methods:** Two hundred and twenty-six patients in the atypical cytologic diagnostic category were analyzed. Multivariate logistic regression analyses were performed to determine predictive factors for pancreatic malignancies. The final diagnoses were confirmed by repeat biopsy; surgical pathology, or clinical follow-up for at least 6 months. **Results:** The atypical cytologic diagnosis using EUS-FNA/FNB was associated with an absolute risk of malignancy (82.3%). Multivariate logistic regression analyses revealed that older age, long axis of the mass, and increased carbohydrate antigen 19-9 (CA19-9) were independent risk factors for true malignant pancreatic tumors among patients in the atypical cytologic diagnostic category. The calibration curve had a slope of 0.96, and a regression coefficient (R^2) of 0.91. The area under the receiver operating characteristic curve of the validation group was 0.803. **Conclusions:** Atypical lesions

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of EUS-FNA/FNB have a higher risk of malignancy. Older age, the long axis of the mass, and elevated serum CA19-9 level were identified as independent risk factors for true malignant pancreatic tumors among patients in the atypical cytologic diagnostic category.

Key words: atypical cytologic category, EUS-guided fine-needle biopsy, EUS-FNA, pancreas, pancreatic malignancy

INTRODUCTION

Pancreatic cancer is a deadly malignancy and ranks 10th among the most common cancers worldwide. In China, the incidence of pancreatic cancer was shown to be seven in 100,000 patients per year.[1] The prognosis of pancreatic cancer is extremely poor. Specifically, the 5-year survival rates in patients with localized pancreatic adenocarcinoma and distant metastasis were found to be 24% and 2%, respectively.[2]

Imaging technologies, including computed tomography (CT) and magnetic resonance imaging (MRI), allow for the evaluation of patients with suspected pancreaticobiliary tumors,[3,4] but it is challenging to distinguish malignant and non-malignant pancreatic masses (*e.g.*, autoimmune pancreatitis, benign tumors). EUS is superior to spiral CT or MRI for the detection of pancreatic tumors that are small and shows higher sensitivity to lymph node metastases or vascular tumor infiltration. EUS-guided fine-needle aspiration/biopsy (EUS-FNA/FNB) facilitates sampling of the pancreatic mass, and the sensitivity and specificity of EUS-FNA/FNB ranges from 54% to 95% and 71% to 100%, respectively.[5] Compared to obtaining pancreatic tissue specimens through surgery, acquisition of samples using EUS-FNA/FNB is markedly less invasive.

According to the Papanicolaou Society of Cytopathology, a cytologic diagnosis can be classified into one of the following six categories: not enough, negative, atypical, neoplastic, suspicious for malignancy, and positive.[6-8] Compared to numerous previous studies with a primary focus on benign or malignant lesions in the pancreas, less investigation has been conducted to assess the risk of pancreatic malignancy among patients diagnosed as "atypical."

Atypical cells may arise from benign inflammation or represent an early neoplastic process that can progress to pancreatic cancer. Therefore, in the clinical practice, it is challenging for physicians to predict the probability that cells classified as "atypical" in the diagnostic report would become malignant. The purposes of this study were mainly to clarify the probability of true pancreatic malignancies in the "atypical" diagnostic category, and then to identify independent risk factors for the prediction of pancreatic malignancies in patients with an atypical cytologic diagnosis.

METHODS

Patients

Two thousand and seventy-one patients with solid pancreatic neoplasms who underwent pancreatic EUS-FNA/FNB and had a cytologic or histologic diagnosis between January 2012 and December 2019 at Changhai Hospital (Shanghai, China). Data were retrospectively collected, reviewed, and analyzed. Patients with the following conditions were excluded from this study: (1) the FNA/FNB was obtained from multiple locations of the pancreas, (2) the diagnosis could not be confirmed with FNA, biopsy, or clinical follow-up, and (3) the diagnosis of non-solid mass with FNA/FNB or imaging. Finally, 226 patients in the atypical cytologic diagnostic category were further analyzed. The flow chart of patients included in this study as illustrated in Figure 1. The enrolled patients were randomly assigned at a ratio of 7:3 to one of

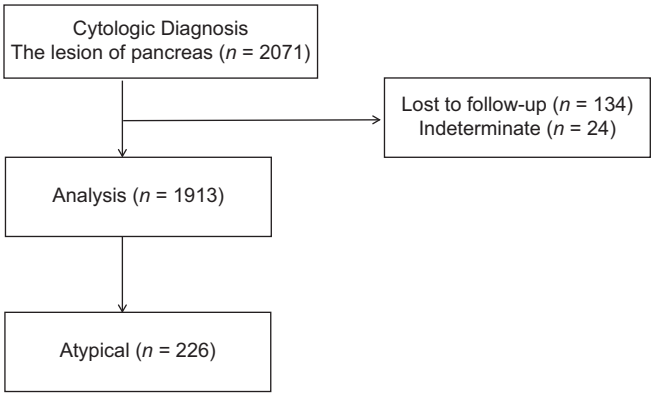

Figure 1. Flow chart of patients retrospectively screened and enrolled in this study. A total of 2071 patients underwent pancreatic EUS-guided fine needle aspiration/biopsy (EUS-FNA/FNB) between January 2012 and December 2019. Two hundred and twenty-six patients with a cytology diagnosis of "atypical" by EUS-FNA/FNB were included for further analysis in this study. 186 patients based on progression on imaging, surgery or repeated biopsy, included 28 patients with repeated FNA/FNB, 58 surgical patients, and 100 patients with clinical or imaging follow-up

two groups: the model group was used to establish a prediction model, and the validation group was used to validate the model.

This study was approved by the Ethics Committee of Changhai Hospital (Shanghai, China). Written informed consent was waived due to the retrospective nature of the study.

Pancreatic EUS-FNA/FNB procedure and specimen collection for cytology diagnosis

The pancreatic lesion region was found and carefully scanned to show its relationship with the surrounding vascular structures. The width of the lesion was measured as the maximum long-axis diameter. The optimal puncture tract was selected to avoid blood vessels and the pancreatic duct using Doppler flow. Three ultrasound endoscopy manufacturers were including OLYMPUS, FUJIFILM, and PENTAX. A wide variety FNA an FNB needles (Cook and Boston Scientific; including COOK EchoTip Procore, COOK EchoTip Ultra, Boston Expert; Specific model details: ECHO-22, ECHO-25, ECHO-HD-22-C, ECHO-HD-25-C and Expert-22, Expert-25) were used. During the EUS-FNA or FNB tissue acquisition, 3 different suction techniques were applied. A 10 ml negative pressure, without the use of negative pressure or a slow pull technique was used. The "fanning method" was used in all cases to allow a needle to traverse the target lesion for 10-20 times.

Pathology preparation and reporting

Two cytological methods were used: (1) For smear cytology method, the specimen was pushed onto a glass slide by inserting the stylet, then a second glass slide was pulled parallel over the first one to spread and distribute the specimen evenly. The slides were fixed in ethanol. Hematoxylin-eosin staining was carried out later; (2) After removing the stylet, saline solution flush was done to collect tissue into one single vial containing a BD CytoRich non-gyn fixatives (BD SurePath, Franklin Lakes, NJ, USA) for Sure Path processing. The slides derived from smear cytology preparation were stained by the Papanicolaou procedure. Tissue specimens obtained from each pass were fixed in formalin solution. Specimens were sent to the pathology laboratory for further staining and examination. Two pathologists with at least five years of experience in pathology practice were required to perform pathological examinations. When there was inconsistency of the diagnosis, another pathologist was asked to verify the results.

Data from patients with a pathological diagnosis of "atypical, but otherwise non-specified" were collected. The final diagnoses were confirmed by postoperative pathological examinations or clinical and imaging follow-up for at least 6 months.

Construction and performance evaluation of a prediction model

A logistic regression model was constructed after considering important predictors and demographic characteristics (*e.g.*, gender, age). Predictive probability = exp(b0+b1x1+b2x2. bnxn/1+exp[b0+b1x1+b1x2. bnxn], in which x represents the significant predictor and b is the regression coefficient.

To evaluate the discriminant ability of the model, a receiver operating characteristic (ROC) curve was plotted to predict probability, and SPSS software was used to estimate the area under the curve (AUC). After determining the prediction probability of each case according to the above formula, the cases were classified into one of the two categories (malignant tumors, non-malignant lesions) according to the 95% confidence interval (CI) and treatment threshold of each category. For further performance evaluation, a calibration curve was plotted to examine the correlation between observed probabilities and predicted probabilities within the probability range (decile).

Study outcomes

The purposes of this study were mainly to clarify the probability of having true pancreatic malignancies in the "atypical" diagnostic category, and then to identify independent risk factors for the prediction of pancreatic malignancies in patients with an "atypical" cytologic diagnosis.

Statistical analysis

Statistical analysis was conducted using SPSS software version 22 (IBM Corp, Armonk, NY, USA). Student's *t*-test, a Chi-square test, or Fisher's exact test was used when appropriate to determine differences between the two groups. Predictive factors for malignant pancreatic tumors were analyzed using univariate and multivariate logistic regression analyses. A $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the study subjects

From a total of 2071 patients who underwent pancreatic EUS-FNA/FNB procedures during the period between January 2012 and December 2019, 226 patients were given the "atypical" cytologic diagnosis and they were further analyzed. One hundred and eighty-six patients were ultimately diagnosed with malignancies. Specifically, there were 28 patients with repeated FNA/FNB, 58 with surgeries, and 100 with clinical and imaging follow-up for at least 6 months who were ultimately diagnosed with malignancies.

Baseline characteristics of patients in the pathologically diagnosed cytologic "atypical" category

Two hundred and twenty-six patients with a cytology diagnosis of "atypical" were retrospectively enrolled in this study for further analysis, and their demographic and clinical characteristics are summarized in Table 1. The study subjects included 142 males (62.83%) and 84 females (37.17%).128 lesions (56.63%) were located in the head or uncinate of the pancreas. One hundred and ninety-seven patients (87.17%) had the long axis of the mass more than 2 cm. The final diagnoses were confirmed by postoperative pathological examinations or clinical and imaging follow-up for at least 6 months.

Table 1. Baseline characteristics of patients with pathologically diagnosed cytologic atypical category

Characteristics	Patients (*n*)
Age (years), mean±SD	60.32±11.14
Gender	
Male	142
Female	84
Location of lesion in the pancreas	
Head and uncinate	128
Neck and body and tail	98
Needle passes (median)	3
Long axis of the mass (cm)	
<2	29
2-4	143
>4	54

SD: Standard deviation

Table 2. Histological diagnostic in the cytologic atypical category

	Patients (*n*)
Benign	
Acute pancreatitis with/out pseudocyst	20
Chronic pancreatitis	11
Autoimmune pancreatitis	7
Inflammatory pseudotumor	2
Malignant	
Adenocarcinoma	180
Pancreatic neuroendocrine carcinoma	4
Adenosquamous carcinoma	2

Histological diagnosis in the cytologic "atypical" category

According to the histological diagnosis, as confirmed during follow-up, 226 patients who had a cytology diagnosis of "atypical" were categorized into benign, or malignant lesion categories [Table 2]. Notably, malignant tumors were confirmed in 186 cases (82.3%). Malignant tumors included pancreatic ductal adenocarcinoma, poorly differentiated neuroendocrine carcinoma, and adenosquamous carcinoma. The number of benign and malignant lesions in patients with a cytologic diagnosis of "atypical, NOS" is also listed in Table 2.

Univariate analysis of predictive factors for malignant tumors of the pancreas

Two hundred and twenty-six patients were randomly divided into the model group (70%) or the validation group (30%). In the model group (n = 150), it was noted that 22 (14.67%) were diabetic, 37 (24.67%) were jaundiced, 113 (75.3%) had abdominal pain, 64 (42.67%) had weight loss, and 22 (14.67%) had a history of pancreatitis. In the verification group (n = 76), it was found that 11 (14.47%) were diabetic, 15 (19.74%) were jaundiced, 60 (78.9%) had abdominal pain, 39 (51.32%) had weight loss, and 16 (21.05%) had a history of pancreatitis. Univariate analysis revealed that the length of the long axis of the mass, older age, and elevated serum levels of carbohydrate antigen 19-9 (CA19-9) were significantly associated with a diagnosis of malignant pancreatic tumors in the model group or the validation group [$P < 0.05$; Table 3].

Multivariate logistic regression analysis of independent predictive factors for malignant tumors of the pancreas

The factors significantly associated with malignant tumors of the pancreas were then used for multivariate logistic regression analysis. The resulting data showed that age, the long axis of the mass, and increased CA19-9 level (≥37U/mL) significantly correlated with a higher risk of malignant pancreatic ($P < 0.05$). Although a history of pancreatitis was previously reported to be associated with a lower risk of malignant tumors, there was no statistical significance in the multivariate logistic regression analysis of the current study [$P > 0.05$;

Table 3. Univariate analysis of predictive factors for malignant tumors of the pancreas

	Model group (150 patients)			Verification group (76 patients)		
	Nonmalignant lesions	Malignant tumors	*P*	Nonmalignant lesions	Malignant tumors	*P*
Patients, *n* (%)	27 (18)	123 (82)		13 (17.11)	63 (82.89)	
Age (years) (SD)	56.19±9.85	61.64±10.77	0.031	61.03±10.95	48.7±13.17	0.002
Male/female	19/8	72/51	0.25	9/4	42/21	0.85
Long axis of the mass (cm)						
<2	9	10	<0.001	4	6	0.03
≥2	18	113		9	57	
Smoking (yes/no)	6/21	33/90	0.62	5/8	41/22	0.07
Drinking (yes/no)	8/19	22/101	0.16	5/8	16/47	0.33
Abdominal pain (yes/no)	18/9	95/28	0.25	8/5	52/11	0.18
Poor appetite (yes/no)	9/18	38/85	0.80	5/8	21/42	0.97
Diabetes (yes/no)	6/21	16/104	0.38	3/10	8/55	0.59
Weight loss (yes/no)	9/18	55/68	0.28	5/8	34/29	0.31
Pancreatitis (yes/no)	5/18	17/106	0.33	5/8	11/52	0.18
Jaundice (yes/no)	5/22	32/91	0.41	3/10	12/51	0.96
CA19-9 increased (yes/no)	10/17	90/33	<0.001	5/8	49/14	0.012

SD: Standard deviation; CA19-9: Carbohydrate antigen.

Table 4. Multivariate logistic regression analysis of independent predictive factors for malignant tumors of the pancreas

	B-coefficient (SE)	Odd ratio (95% CI)	*P*
Age (years) (SD)	0.073 (0.029)	1.075 (1.016-1.138)	0.012
Long axis of the mass (cm)	1.263 (0.428)	3.537 (1.528-8.190)	0.003
Pancreatitis	0.199 (0.762)	1.718 (0.192-15.349)	0.628
Weight loss	0.535 (0.581)	1.708 (0.274-5.433)	0.794
CA19-9 increased	1.762 (0.578)	5.824 (1.875-18.093)	0.002
Constant	−6.117 (2.244)	0.002	0.006

SD: Standard deviation; CI: Confidence interval; SE: Standard error; CA19-9: Carbohydrate antigen.

Table 4]. As such, older age, longer axis of the mass, and elevated levels of CA19-9 were identified as independent predictors for malignant tumors of the pancreas in patients who underwent EUS-FNA/FNB with atypical cytologic diagnostic category.

Performance evaluation and validation of the logistic regression model for predicting malignant tumors of the pancreas

Based on the independent risk factors (age, long axis of the mass, and CA19-9), a logistic regression model was constructed to predict the malignant potential of pancreatic tumors. To evaluate the discrimination and calibration of the prediction model, calibration and ROC curves were plotted. As shown in Figure 2a, the constructed calibration curve had a slope of 0.96 and regression coefficient (R^2) of 0.91, suggesting strong correlation of predicted rates with observed probabilities. The ability of the model to discriminate between malignant and non-malignant pancreatic tumors or predict the malignancy of pancreatic tumors in patients with a cytology diagnosis of "atypical" following EUS-FNA/FNB was evaluated using an AUC analysis. As presented in Figure 2b, the AUC value for the prediction of malignant tumors of the pancreas was 0.831 in the model group (95% [95% CI], 0.736–0.926), the Chi-square value was 3.65, and the *P* value was 0.887, exhibiting good discrimination ability. Furthermore, as illustrated in Figure 2c, the performance of the prediction model was internally validated by the validation group, where results indicated that the AUC value was 0.803 (95% CI, 0.650–0.957), the Chi-square value was 8.196, and $P = 0.415$. These data suggested a high diagnostic accuracy of the model [Figure 2c].

DISCUSSION

Currently, there is no unified standard to define the "atypical" classification of pancreatic tissues. The criteria are lacking to explain the diverse results ranging from benign to malignant lesions. As such, it is challenging for clinicians to predict the probability of a true malignant tumor in the "atypical" category. Thus far, this is the study with the largest sample size of "atypical" cytological diagnoses. Our results revealed that the diagnosis of "atypical" was associated with an absolute risk of pancreatic malignancy of 82.3%. Older age, the long axis of the mass, and elevated CA19-9 level were identified as independent risk factors for having true malignant tumors of the pancreas among patients in the "atypical" diagnostic category.

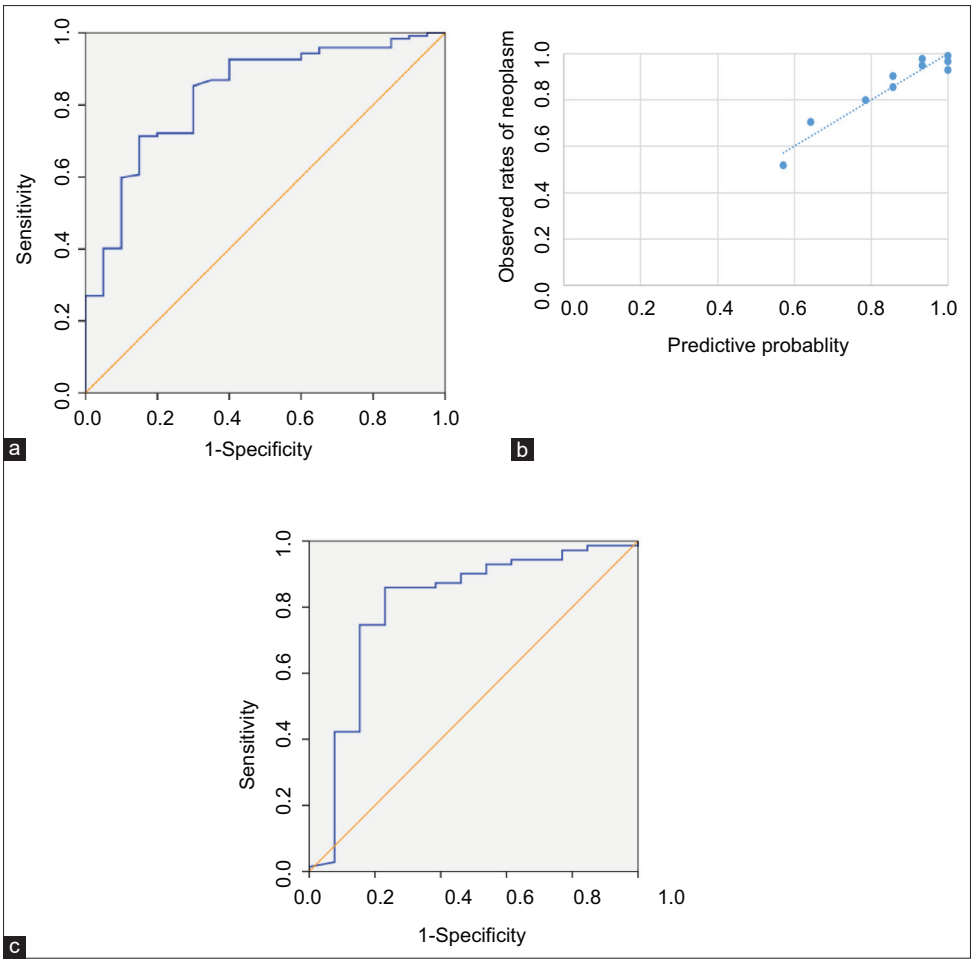

Figure 2. Discrimination, calibration, and validation of the prediction model using receiver operating characteristics and calibration curves. Based on the independent risk factors (age, long axis of the mass, and carbohydrate antigen 19-9), a logistic regression model was constructed to predict the malignant potential of pancreatic tumors. (a) Calibration curve of the prediction model. The slope was 0.96, and the regression coefficient (R^2) was 0.91, suggesting a strong correlation between predicted and observed probabilities. (b) Receiver operating characteristic (ROC) curve of the prediction model. The area under the ROC curve (AUC) value for prediction of malignant tumors of the pancreas was 0.831 (95% confidence interval, 0.736–0.926), indicating good correlation between predicted and observed probabilities. (c) ROC curve for the validation group. The AUC value was 0.803 (95% confidence interval, 0.650–0.957), suggesting good prediction of malignant tumors in the pancreatic "atypical" category

Compared to imaging or laboratory indicators in clinical practice, EUS is considered a useful tool to obtain tissue and cytological specimens.[9,10] According to the Papanicolaou Society of Cytopathology, a cytologic diagnosis can be classified into six categories. The "atypical" category accounted for 14% of cases, on average. In our center, approximately 9.7% of patients were diagnosed in the "atypical" category. Since the risk of developing a malignancy does not have clear indications among patients diagnosed as "atypical", usually a repeat FNA or a tissue biopsy/resection, or at least close follow-up was conducted clinically. However, the risk of the lesion becoming pancreatic cancer is considerably high among patients in the "atypical" category and relying on clinical follow-up may lead to delays in early diagnosis and intervention. The accuracy of repeat EUS-FNA/FNB tests ranged from 61% to 92.9%;[11] meanwhile, patients could be at risk of procedure-related complications and false-negative results. Surgical pancreatic tissue biopsy/resection is of high risk and very complex compared to a surgical biopsy/resection in a superficial organ, such as the breast or thyroid.[12-14] Therefore, the follow-up should rely on the balance of advantages and disadvantages in clinical practice.

Risk stratification is a key to determining the follow-up strategy, guiding therapy, and better estimating the prognosis.[15] Pancreatitis, older age, male sex, and the presence of diabetes have been reported as risk factors related to pancreatic adenocarcinoma.[16] Our results showed that older age, the long axis of the mass, and increased CA19-9 levels significantly correlated with a higher risk of developing malignant pancreatic tumors.

Although patients with a history of pancreatitis had a lower risk of pancreatic malignancies in the current study, it was not a statistically significant factor for the development of malignancies in the pancreas ($P > 0.05$). It has been shown that obstruction of bile flow and alcohol abuse is more likely to cause pancreatitis and increase the risk for ductal carcinoma. Our study did not find that the obstruction of bile flow or alcohol abuse affected the neoplastic lesion, which was consistent with previous results that pancreatitis does not lead to malignant tumors. In a previous study, the bootstrap method was used to analyze the risk factors of pancreatic malignancies, and results suggested that the bootstrap method behaves erratically with numbers less than 200.[17] In this study, we attempted to develop a more accurate and reliable logistic regression-based prediction model. The enrolled patients were randomly assigned at a ratio of 7:3 to one of two groups: the model group was used to establish a prediction model, and the validation group was used to validate the model. Due to the relatively small number of patients after grouping, the identified risk factors for malignant pancreatic lesions were not consistent with those reported previously.[15] The current results revealed that the logistic regression model yielded an AUC value of 0.831 in the model group, and the model was also supported by a close correlation between predicted and observed probabilities in the calibration curve (slope, 0.96; R^2, 0.91). The performance of the prediction model for malignant tumors in the pancreas was validated with an AUC value for the validation group of 0.803 (95% CI, 0.650–0.957), as well as a close correlation between predicted and observed probabilities with calibration curve analysis. The AUC values were high in both the model and validation groups, suggesting good performance of the model to predict the risk of developing a neoplastic lesion in this study.

The current study showed that the "atypical" lesions were mainly located in the head/uncinate. One possible reason for this finding could be the difficulty in EUS needle puncturing this position which may lead to lack of cellularity. This was consistent with a previous report that pancreatic body/tail lesions were associated with higher diagnostic sensitivity with liquid-based cytology compared to lesions in the pancreatic head/uncinate.[18] A meta-analysis revealed that the sensitivity and specificity of EUS-FNA/FNB for the diagnosis of pancreatic solid masses were 86.8% and 95.8%, respectively.[19] In this study, the risk of malignancy was as high as 82.3% among the patients in the "atypical" category. However, there were still some misdiagnosed cases in our center. One possible explanation is the lack of a unified standard for classification that may lead to differences between various cytologists, and another reason was the limited amounts of the specimens. The cytological diagnosis is not as accurate as histopathology. Therefore, cytologists should formulate unified standards, while endoscopists should advance FNA/FNB puncture technology to increase the number of puncture cells collected and thereby improve the diagnostic accuracy in the "atypical" category.

This study has limitations. First, this is a retrospective single-center study, and we do not have on-site cytopathology, which could affect the result of final diagnosis. Secondly, the two experienced pathologists at our tertiary center in this study were responsible for cytological diagnosis, the diagnostic accuracy of a malignant tumor is higher compared to other centers. As such, the results of predictive models are difficult to exceed the accuracy of a clinical diagnosis. Third, considering that "atypical" classifications vary greatly across centers, the findings in this study may not represent the research results of other centers, and a future prospective multicenter study is required for further verification of the model.

In summary, this study has demonstrated that older age, the long axis of the mass, and increased CA19-9 level are independent risk factors for the development of malignant pancreatic tumors among patients with a cytologic diagnosis of being "atypical". The diagnostic accuracy of the prediction model based on the above factors is consistent with the "atypical" calculation results in EUS-FNA/FNB tests. Therefore, the prediction model as constructed in this study has a good ability to predict pancreatic malignancies, and thereby holds promise as a novel tool in future clinical practice.

Author contributions

Wang KX proposed the study concept and provided administrative support; Jin ZD conducted EUS procedure and study supervision; Zhang PP and Wang T prepared the samples and drafted the manuscript; Li SY and Li L critically revised the important contents of the manuscript; Su XJ analyzed and Li F interpreted the data; Qian YP and Gao L interpreted and graded the pathological samples; Li F performed proofreading, editing and syntax correction, and Gu PY collected the data.

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Conflicts of interest
Zhen-Dong Jin is an Associate Editor of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of these editors and their research groups.

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