Prognostic significance of peritumoral fibrosis after resection of pancreatic head cancer

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Abstract. Prognostic value of peritumoral fibrosis (PF) in pancreatic head cancer after resection was evaluated. A total of 143 pancreatic cancer patients who underwent tumor resection were enrolled. All patients underwent routine preoperative examination, including contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). Patients receiving preoperative chemoradiation were excluded because it affects the proportion of fibrosis and cancer cells. Histopathological confirmation and classification of pancreatic head cancer (PHC) was made according to the standards of World Health Organization and the American Joint Committee on Cancer (AJCC). The presence of fibrosis was assessed histologically, and correlated with the clinicopathological characteristics and overall survival using univariate Kaplan-Meier analysis and a stepwise multivariable Cox regression model. Vein resection, resection margin, grading, nodal status, preoperative CA19-9 levels and PF were significantly associated with overall survival. Multivariate analysis showed that all the aforementioned were independent predictive factors of survival. In addition, the survival of patients with PF was significantly worse compared to those without (HR 1.392; P=0.027). Tumor necrosis is a valuable prognostic tool that can be included in the routine post-resection histopathological evaluation of pancreatic head cancer patients.

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Introduction

Pancreatic cancer is the fifth most commonly diagnosed cancer and the fourth leading cause of cancer-related mortality worldwide (1). Because of its characterizations of aggressive and early dissemination, the overall 5-year survival rate of pancreatic cancer patients is a dismal 3-5%, which increases to 15-25% among those who undergo curative resection (2,3). However, the mortality rate remains high and has not shown any obvious improvement in the past few decades. A better understanding of the underlying molecular mechanisms of this cancer might contribute to demarcate the patients into different prognostic groups, as well as identify novel markers associated with prognosis.

Hypoxia is one of the common features of human cancers, and manifested histologically by necrosis and peritumoral fibrosis (PF) (4-6). Tumor necrosis has been identified as a marker of poor prognosis in renal, breast, lung, pancreatic and colorectal cancers (7-10), whereas PF affects the outcome and prognosis of inflammatory and hematopoietic disorders (11,12). No study so far has analyzed the formation of PF and its potential relationship to the clinicopathological parameters and prognosis of pancreatic head cancer (PHC). The present study evaluated the clinical significance and prognostic value of PF in PHC patients after resection.

Patients and methods

Patients and tumor samples. Total of 143 samples from patients with PHC resection between January 2007 and December 2011 at the Department of Hepatobiliary Surgery, Yi Ji Shan Hospital of Wannan Medical College were included in the present study. All patients with PHC received routine preoperative work-up including a contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). The contraindications for curative resection included metastases, complete occlusions of the superior-mesenteric/portal vein or arterial infiltration (>180˚ circumference), except tumor contact to the portal vein alone. All the patients with PHC underwent the Kausch-Whipple procedure and standard lymphadenectomy along the right side of the superior mesenteric artery, the hepatoduodenal ligament, and the celiac trunk/upper pancreatic margin. PHC was confirmed histopathologically and classified according to the

Table I. Relationship between clinicopathological characteristics and presence of peritumoral fibrosis.

Characteristics	No. of patients	Peritumoral fibrosis		P-value
		Presence	Absence	
Age (years)				0.404
<70	104	64	40	
≥70	39	21	18	
Sex				0.663
Female	61	43	18	
Male	82	55	27	
Intraoperative blood transfusion				0.918
Yes	42	30	12	
No	101	73	28	
Vein resection				0.820
Yes	25	14	11	
No	118	69	49	
Grading				0.814
G1/2	114	72	42	
G3/4	29	19	10	
T stage				0.067
T1/2	21	14	7	
T3/4	122	102	20	
Resection margin				0.140
Negative	105	72	33	
Positive	38	21	17	
Nodal status				0.678
Negative	82	51	31	
Positive	61	40	21	
Preoperative CA19-9 (U/ml)				0.842
<37	21	14	7	
≥37	122	84	38	
No. of examined nodes				0.327
<12	52	23	29	
≥12	91	48	43	
Complications				0.339
Yes	70	28	42	
No	73	35	38	

criteria of World Health Organization and the American Joint Committee on Cancer (AJCC) (13,14). Patients who underwent preoperative chemoradiation were excluded since it affects the ratio of fibrosis to cancer cells (15).

The study was approved by the Ethics Committee of The First Affiliated Hospital of Wannan Medical College (Wuhu, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients and/or the guardians.

Formalin-fixed and paraffin-embedded (FFPE) tumor tissue blocks were retrieved from the database of the Department of Pathology, Yijishan Hospital of Wannan Medical College, sectioned, and stained with hematoxylin and eosin (H&E) for histological analysis of the primary tumor stage and nodal status. The clinicopathological characteristics of the patients with pancreatic head cancer are summarized in Table I. The patients were followed up after the operation by telephone conversation and/or out-patient clinic interviews.

Classification of PF. The tumor specimens were classified into 3 categories according to the degree of PF: negative (<10% fibrotic change), moderate (11-30%) and severe (>30%).

Table II. Univariate survival analysis after resection of pancreatic head cancer.

Characteristics	No. of patients	HR	95% CI	P-value
Age (years)				
<70	104	0.923	0.611-1.394	0.704
≥70	39	1		
Sex				
Female	61	1.051	0.746-1.480	0.777
Male	82	1		
Intraoperative blood transfusion				
Yes	42	1.182	0.790-1.767	0.790
No	101	1		
Vein resection				
Yes	25	1.646	1.048-2.588	**0.031**
No	118	1		
Grading				
G1/2	114	1		
G3/4	29	1.843	1.135-2.993	**0.013**
T stage				
T1/2	21	0.683	0.414-1.128	0.136
T3/4	122	1		
Resection margin				
Negative	105	1		
Positive	38	1.542	1.022-2.324	**0.039**
Nodal status				
Negative	82	1		
Positive	61	1.790	1.257-2.556	**0.001**
Preoperative CA19-9 (U/ml)				
<37	21	1.783	1.070-2.972	**0.026**
≥37	122	1		
No. of examined nodes				
<12	52	0.883	0.575-1.178	0.288
≥12	91	1		
Peritumoral fibrosis				
Presence	85	3.079	1.975 -4.844	**<0.001**
Absence	58	1		
Complications				
Yes	70	0.885	0.624-1.255	0.493
No	73	1		

P-value in bold print indicates statistical significance.

Statistical analysis. All statistical analyses were performed using the R3.1.3 program (http://www.R-project.org). Pearson's Chi-square test and Fisher exact probability test were performed to analyze the correlation between different parameters. Univariate Kaplan-Meier analysis was performed to assess the prognostic factors for survival, as well as compared using the two-sided log-rank test. The Cox proportional hazard model (forward selection strategy using a likelihood ratio statistic; inclusion P=0.05) was performed by multivariate survival analysis, including hazard ratios and their 95% confidence interval. P-values <0.05 were considered statistically significant.

Results

Clinicopathological characteristics of the patients. The clinicopathological features of patients with PHC who underwent

Table III. Multivariate survival analysis after resection of pancreatic head cancer.

Characteristics	No. of patients	HR	95% CI	P-value
Vein resection				
Yes	25	2.251	1.348-3.758	**0.002**
No	118	1		
Grading				
G1/2	114	1		
G3/4	29	1.856	1.145-3.009	**0.012**
Resection margin				
Negative	105	1		
Positive	38	1.977	1.212-3.225	**0.006**
Nodal status				
Negative	82	1		
Positive	61	2.973	1.947-4.540	**<0.001**
Preoperative CA19-9 (U/ml)				
<37	21	2.398	1.166-4.926	**0.017**
≥37	122	1		
Peritumoral fibrosis				
Presence	85	1.392	1.038-1.869	**0.027**
Absence	58	1		

P-value in bold print indicates statistical significance.

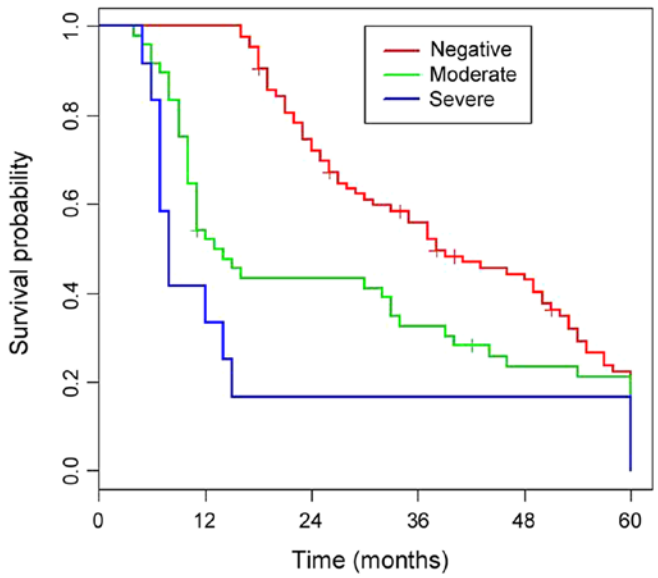

Figure 1. Correlation between post resection overall survival and the absence/presence of peritumoral fibrosis.

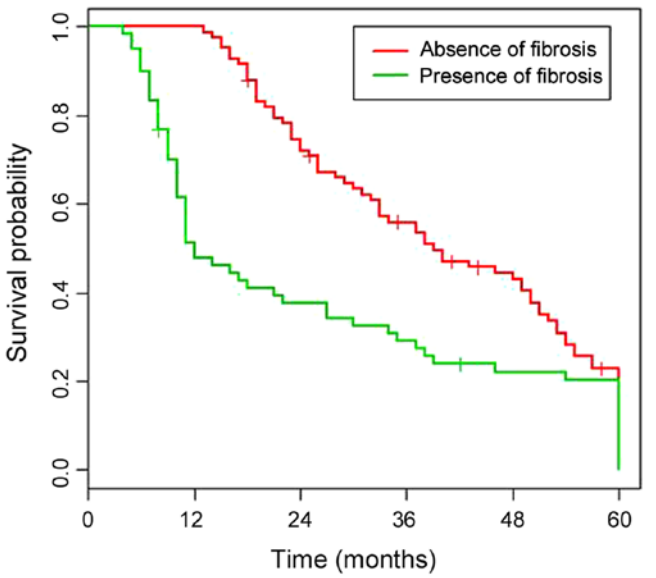

Figure 2. Correlation between post resection overall survival and the degree of peritumoral fibrosis.

pancreatic tumor resection are summarized in Table I. The median age of patients was 64 years (range 32-85 years). PF was not significantly correlated with any of the clinicopathological factors.

Univariate survival analysis. No patient died during the postoperative course. The median follow-up duration of the entire cohort was 28.7 months (range 5.3-60 months), and the median survival was 1.95 years. The cumulative 3- and 5-year survival rates were 31 and 19%, respectively. Univariate analysis (Table II) showed that vein resection, resection margin, grading, nodal status, preoperative values of CA19-9 and PF were significantly associated with survival. Patients with PF had significantly worse survival compared to those without

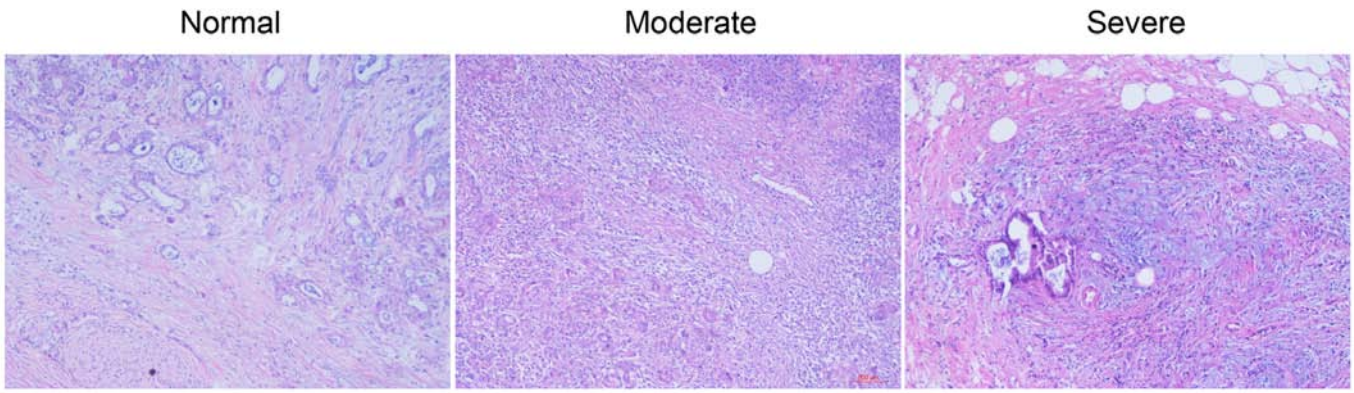

Figure 3. Histological analysis of a tumor specimen with peritumoral fibrosis (asterisk) (hematoxylin and eosin, x100).

(HR 3.079; P<0.001) (Fig. 1). The overall survival (OS) of patients with mild and severe PF is shown in Fig. 2 (P=0.02).

Multivariate survival analysis. Multivariate analysis (Table III) indicated that resection margin, vein resection, grading, preoperative values of CA19-9, nodal status and PF were all independent predictive factors of survival. The survival of patients with PF was significantly worse than those without (HR 1.392; P=0.027).

Discussion

Correlation between the presence of PF and various clinico-pathological parameters were evaluated in 143 pancreatic head cancer patients who underwent tumor resection. This is the first study to show the association between PF and poor post-resection overall survival in pancreatic cancer patients, and identify it as an independent negative prognostic factor (Fig. 3).

Scarce data is available on the association between PF and the clinico-pathological characteristics of pancreatic cancers. A recent study indicated the diagnostic importance of histological PF in PHCs (16). Consistent with this, we found that the presence of PF, as well as the severity of necrosis, was associated with significantly decreased OS. In addition, PF was also identified as independent prognostic factor of post-resection outcome. Based on our results, we hypothesize a diagnostic value of PF in evaluating the post-resection outcome in PHC patients. A rational clinical translation of these results suggests standardized utilization of PF as a prognostic tool in the scope of pathological evaluation of resected specimens from patients suffering from pancreatic head cancer.

The cause of fibrosis in PHC and the mechanisms underlying the poor clinical outcome in patients with PF remain largely unknown. One hypothesis is that inflammation, which has been recently described as the seventh hallmark of cancer (17,18), likely plays a role in the formation of fibrotic masses as well. Furthermore, there is evidence indicating that pancreatic stellate cells trigger fibrosis through various stromal interactions and allow wound healing, thereby promoting cancer cell invasion and dissemination (19-21). Despite recent advances in our understanding of the genetic and cellular basis of pancreatic head cancer progression, its diagnostic and prognostic evaluation is still mostly dependent on histopathological assessment. The histopathological parameters such as tumor grading and PF are easy to evaluate, and can allow individualized risk assessment and identify patients at high risk of poor outcome.

There are several limitations to our study, including those inherent to retrospective analyses. In addition, the surgical resection was performed by multiple surgeons, and reliable histological evaluation was only possible with the resected tumor specimens. This can be circumvented in future with high-resolution magnetic resonance imaging (MRI), which can allow non-invasive *in vivo* visualization at a 3D spatial resolution of up to 50 μm (22-24). Despite these limitations, our data suggest that PF is a simple diagnostic tool that can evaluate patients' outcome after resection of PHC.

In conclusion, PF is an independent prognostic factor of PHC and predictive of poor survival after resection. This indicates its potential role in pancreatic cancer progression as well as its diagnostic utility. Future prospective trials are needed to assess the value of PF as a criterion for adjuvant treatment.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

PC wrote the manuscript and was responsible for the collection and classification of tumor samples. YW and XF interpreted and analyzed the data. XW designed the study and performed the experiments. GW was responsible for the analysis and discussion of the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The First Affiliated Hospital of Wannan Medical College (Wuhu, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients and/or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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