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Original Article

CT contrast enhancement correlates with pathological grade and microvessel density of pancreatic cancer tissues

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Abstract: Pancreatic cancer typically carries a poor prognosis, and new methods of diagnosis and treatment are needed to improve outcomes for the disease. Non-invasive imaging techniques that accurately predict disease severity may aid in the treatment of pancreatic cancer patients. This study sought to investigate the correlation between computed tomography (CT) contrast enhancement and the histopathological grades and intratumoral angiogenesis in pancreatic carcinoma. The study included 54 patients with pancreatic carcinoma who underwent surgical resection in our hospital. All participants received a CT scan with contrast enhancement before surgery. Pathological specimens obtained during surgery were paraffin embedded for immunohistochemistry to assess microvessel density (MVD; CD31 staining) and angiogenesis activity [vascular endothelial growth factor (VEGF) staining]. Results were analyzed using t tests and Spearman correlation. CT enhancement of pancreatic tumors was negatively correlated with the pathological grade (r_s=-0.784, P<0.05) and the MVD count in tumor hot spots (r_s=-0.790, P<0.05). Additionally, the pathological grade positively correlated with MVD count (r_s=0.516, P<0.05). However, there was no correlation between pathological grade and VEGF expression (r_s=-0.195, P>0.05). Finally, MVD was higher in individuals positive for VEGF expression than in those negative for VEGF expression (P<0.05). Thus, the extent of CT enhancement is related to the MVD in tumor hot spots and the malignant degree of pancreatic carcinoma. This suggests CT can be used to reflect the disease severity and extent.

Keywords: Pancreatic carcinoma, microvessel density, vascular endothelial growth factor, computed tomography

Introduction

Pancreatic cancer is a highly malignant tumor of the digestive tract that is difficult to diagnose and treat. Approximately 90% of cases originate from ductal adenocarcinoma of the gland duct epithelium [1]. Pancreatic cancer has been increasing in incidence and mortality in recent years, and the prognosis of pancreatic cancer patients is very poor: the 5-year survival is less than 1% [2]. The poor outcomes are related to the tendency of pancreatic cancer to invade and spread to surrounding tissues, a phenomenon closely correlated with angiogenesis [3].

Tumor angiogenesis, or the process of capillary formation in tumors and surrounding tissues, enables tumor metastasis [4]. A major contributor to this process is the vascular endothelial growth factor (VEGF). Indeed, analysis of VEGF expression can serve as a biomarker for the prognosis of malignant tumors [5]. The extent of angiogenesis of malignant tumors is evaluated currently by assessing microvessel density (MVD) using pathological approaches or in vivo imaging techniques like computed tomography (CT). Improved non-invasive imaging techniques could lead to more rapid diagnosis and better treatment. Here, CT contrast enhancement of the nidus where pancreatic cancer occurred was used to investigate the correlation between pathological grades of pancreatic cancer tissues and tumor angiogenesis. VEGF was used as an additional biomarker in the assessment. The findings provide a basis for imaging as a component of clinical evaluation of malignancy

and of a clinically rational selection of antiangiogenic treatments for pancreatic cancer.

Participants and methods

Participants

The study included 54 patients who underwent surgical resection for pancreatic cancer at Harbin Medical University Cancer Hospital between June 2010 and August 2014. All patients were confirmed cases of pancreatic cancer according to the definite contrast-enhanced CT reports, tumor histological specimens, surgical pathology reports, and had detailed clinical data; none received preoperative chemotherapy or radiotherapy. There were 37 (68.52%) males and 17 (31.48%) females; their mean age was 60.2 ± 10.9 years. The study was approved by the Ethics Committee of Harbin Medical University Cancer Hospital, and all participants signed the informed consent statements.

Contrast enhanced CT scan and evaluation

An upper abdominal CT scan was done for each patient before surgery. Then, 100 mL of an Ultravist® contrast agent were injected; at 35 s and 70 s after injection with the agent, a contrast-enhanced scan in the pancreatic parenchymal phase and a contrast-enhanced scan in the portal venous phase were done, respectively. Evaluation was performed by 2-3 imaging specialists. Classification of CT contrast enhancement was performed as follows: grade A showed isodensity enhancement, i.e., the enhancement degree of tumors was the same as that of surrounding normal pancreatic tissues; grade B, slightly low-density enhancement, i.e., the enhancement degree of tumors was slightly lower than that of surrounding normal pancreatic tissues, and there was presence of relatively less vascular area; grade C, slightly low-density enhancement accompanied by low-density cystic masses, i.e., there were small low-density cystic areas, and enhancement degree of tumors was slightly lower than that of surrounding normal pancreatic tissues; grade D, low-density cystic areas in the center of tumors that were accompanied by slightly low-density enhancement in surrounding areas of tumors, i.e., a necrosis was present in a majority of the central area of tumors, and areas surrounding the necrotic area in tumors showed slightly low-density enhancement.

Examination and observation of pathological specimens

Pancreatic tumor tissue was collected from the 54 patients who underwent surgical resection for pancreatic cancer. After hematoxylin and eosin staining of all pancreatic cancer specimens, the pathological grades of pancreatic cancer tissues were determined under a light microscope. The CD31 monoclonal antibodies (ZYMED Laboratories Inc., South San Francisco, CA, USA) were used to perform immunohistochemistrical staining of the vascular endothelial growth factor (VEGF) in all pathological tissues. Microvessel density (MVD) count was determined using the modified Weidner [6] method, and classified as follows: low MVD count (I), MVD count in tumor hot spots was 30 or less; moderate MVD count (II), MVD count in tumor hot spots ranged from >30 to 50; high MVD count (III), MVD count in tumor hot spots ranged from >50 to 60; abundant MVD count (IV), >60. The evaluation of VEGF expression in tumor tissues was performed using the scoring method proposed by Mattern [7]. In brief, two different scales were used, one for the staining degree or intensity, and the other for the percentage of cells positive for VEGF. The staining degree of tumor cells included 4 grades, as follows: grade 0, no staining; grade 1, weak staining; grade 2, moderate staining; and grade 3, strong staining. The percentage of positively stained cells included 4 grades, as follows: grade 0, 0%; grade 1, ≤25%; grade 2, 26%-50%; and grade 3, ≥50%. VEGF staining was considered negative when the sum of the two scales was 3 points or less; VEGF staining was considered positive when the sum of the 2 scales was more than 3 points.

Statistical analysis

In this study, double data entry was performed using EpiData version 3.1 to create a data bank, and logic checks were performed. SAS 9.2 (SAS Institute) was used to perform the treatment of data. The methods for analysis comprised a t test and Spearman rank sum test, and $P<0.05$ was considered to indicate a difference was statistically significant.

Table 1. Relationship between the degree and signal strength of CT enhancement and pathological level, the expression of VEGF, and MVD count in pancreatic carcinoma

Degree and signal strength of tumors	Pathological degree			Expression of VEGF		MVD count			
	High	Moderate	Low	+	-	I	II	III	IV
A (n=5)	3	2	0	2	3	2	3	0	0
B (n=20)	2	15	3	11	9	3	14	3	0
C (n=20)	0	5	15	14	6	0	5	15	0
D (n=9)	0	0	9	7	2	0	0	2	7
Total	5	22	27	34	20	5	22	20	7

Results

Pancreatic cancer pathology and immunohistochemistry

To determine the pathological status of each case of pancreatic cancer, the examination of pathological specimens from the 54 patients with pancreatic cancer detected papillary adenocarcinoma and tubular adenocarcinoma. In 29 cases, the tumor occurred in the head of the pancreas, in 7 the tumor was in the body of the pancreas, 5 cases had tumors in the head and neck of the pancreas, another 5 in the tail of the pancreas, 4 had tumors in the body and tail of the pancreas, and the remaining 4 cases in the uncinate process of the pancreas. Poorly differentiated adenocarcinoma was detected in 27 cases, moderately differentiated adenocarcinoma in 22, and well differentiated adenocarcinoma in 5. Additionally, the staining scoring analysis revealed that ~63% (34/54) of cases were positive for VEGF expression. Finally, the mean MVD count was 57.1 ± 20.4. The MVD count in well differentiated adenocarcinoma was 36.7 ± 8.1; the MVD count in moderately differentiated adenocarcinoma was 53.8 ± 5.0; and the MVD count in poorly differentiated adenocarcinoma was 67.5 ± 28.2.

CT enhancement and tumor features

To understand the relationship between CT enhancement findings and tumor features, Spearman's rank correlation revealed an association between the enhancement degree and tumors formation in the pancreatic parenchymal phase and the pathological grades (r_s=-0.784, t=4.979, P<0.05). Spearman's rank correlation between the enhancement degree and form of tumors in the pancreatic parenchymal phase and the VEGF expression was r_s=-0.193 (t=1.257, P>0.05); and Spearman's rank correlation between the enhancement degree and form of tumors in the pancreatic parenchymal phase and the MVD count was r_s=-0.790 (t=6.218, P<0.05). See **Table 1** and **Figures 1-4**.

Pathology, VEGF expression and MVD

To know the association among the pathology, VEGF expression and MVD, Spearman's rank correlation between the pathological grade and VEFG expression in parenchymal cells of tumors was r_s=-0.195 (t=-0.991, P>0.05); and Spearman's rank correlation between the pathological grade and MVD count in parenchymal cells of tumors was r_s=0.516 (t=6.917, P<0.05). See **Table 2**.

VEGF expression and MVD count

The mean MVD count in patients negative for VEGF expression was 43.6 ± 12.3; in contrast, the count was significantly higher in those positive for VEGF expression (59.0 ± 11.8, t=-4.564, P<0.001).

Discussion

The pathogenesis of pancreatic cancer currently remains unclear. In recent years, studies have more often focused on tumor angiogenesis to understand disease progression. The ability of pancreatic cancer to invade the surrounding tissues was shown to be correlated with its abundant angiogenesis; histopathologically, this angiogenesis is manifested by an increase in the MVD [3]. Therefore, a rational evaluation of the degree of tumor angiogenesis is conducive to a preoperative evaluation of the malignant degree of pancreatic cancer and to a rational selection of antiangiogenic treatments of pancreatic cancer. Indeed, CT contrast enhancement of pancreatic cancer can indirectly reflect the status of angiogenesis of tumors in the living body [8].

In this study, the enhancement degree and tumors formation in the pancreatic parenchymal phase were negatively correlated with the

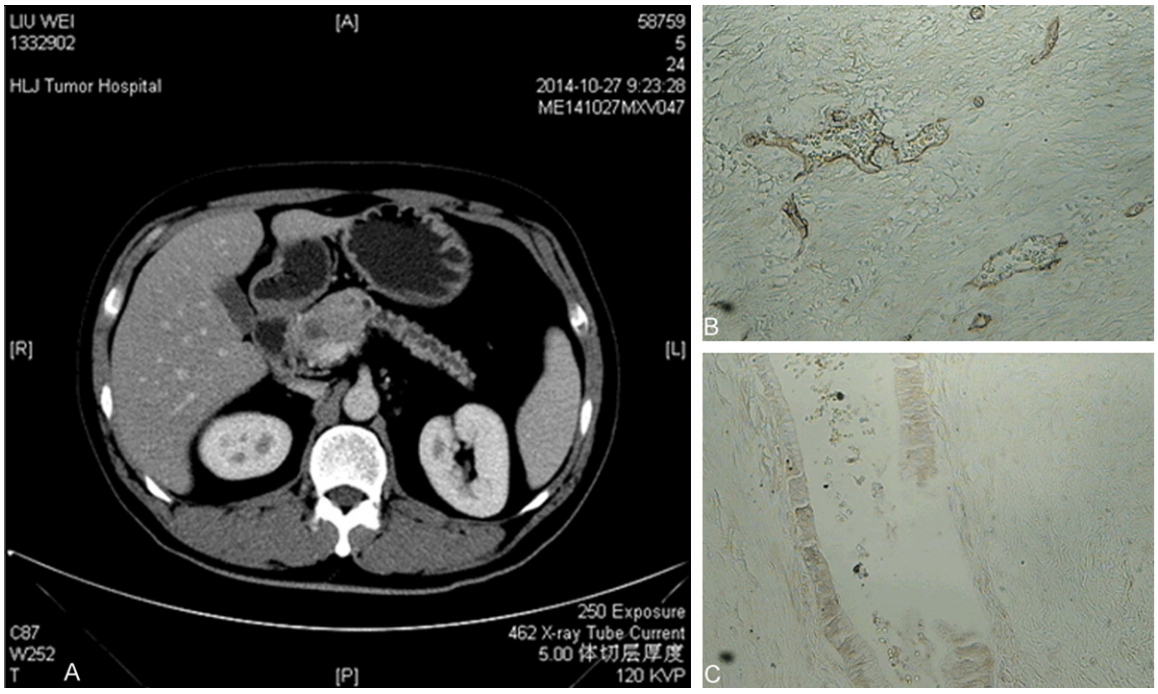

Figure 1. Well-differentiated adenocarcinoma of the pancreatic head. A. CT enhancement shows the degree of enhancement of the mass of the pancreatic head neck were the same as the normal pancreatic tissue. B. Tumor sections were stained with CD31 antibody (brown) and MVD in tumor cell "hot spots" was determined as low (ABC method, ×200). C. Tumor sections were stained with VEGF antibody and new microvascular endothelial cells were yellowish (ABC method, ×200).

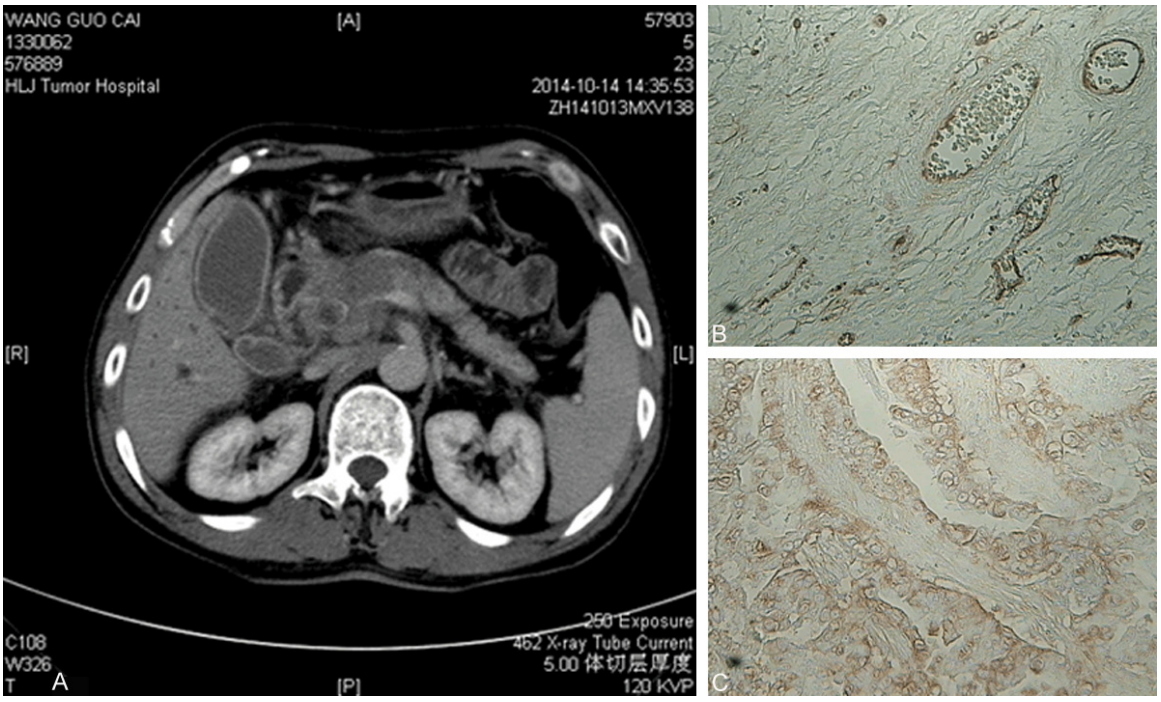

Figure 2. Moderately differentiated adenocarcinoma of the pancreatic head. A. CT enhancement showed the degree of enhancement of the mass of the pancreatic head neck and just below normal pancreatic tissue appeared some zones lacking vascularization. B. The MVD in the tumor cell "hot spots" was moderate by CD31 staining (brown) (ABC, ×200). C. VEGF stained new microvascular endothelial cells yellowish (ABC, ×200).

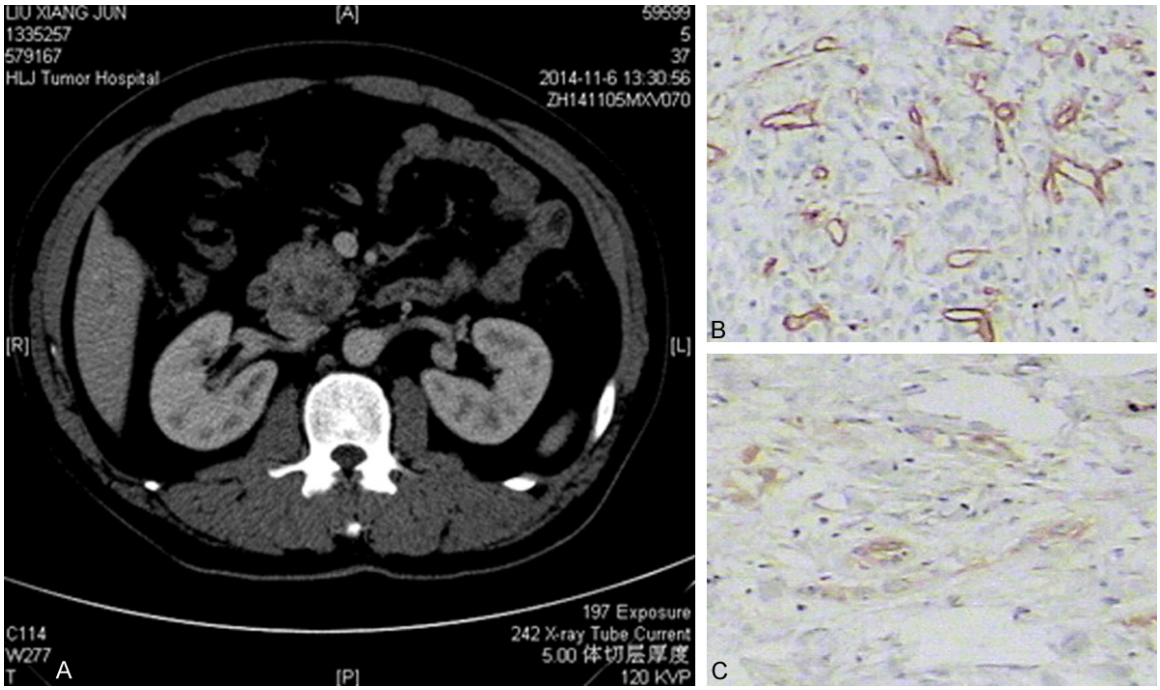

Figure 3. Moderate-to-poorly-differentiated adenocarcinoma of the pancreatic head. A. CT enhancement showed the degree of enhancement of the mass of the pancreatic head was low and contained low-density cystic necrosis. B. MVD in tumor cell "hot spots" was high by CD31 staining (brown) (ABC, ×200). C. VEGF stained new microvascular endothelial cells brown (ABC, ×200).

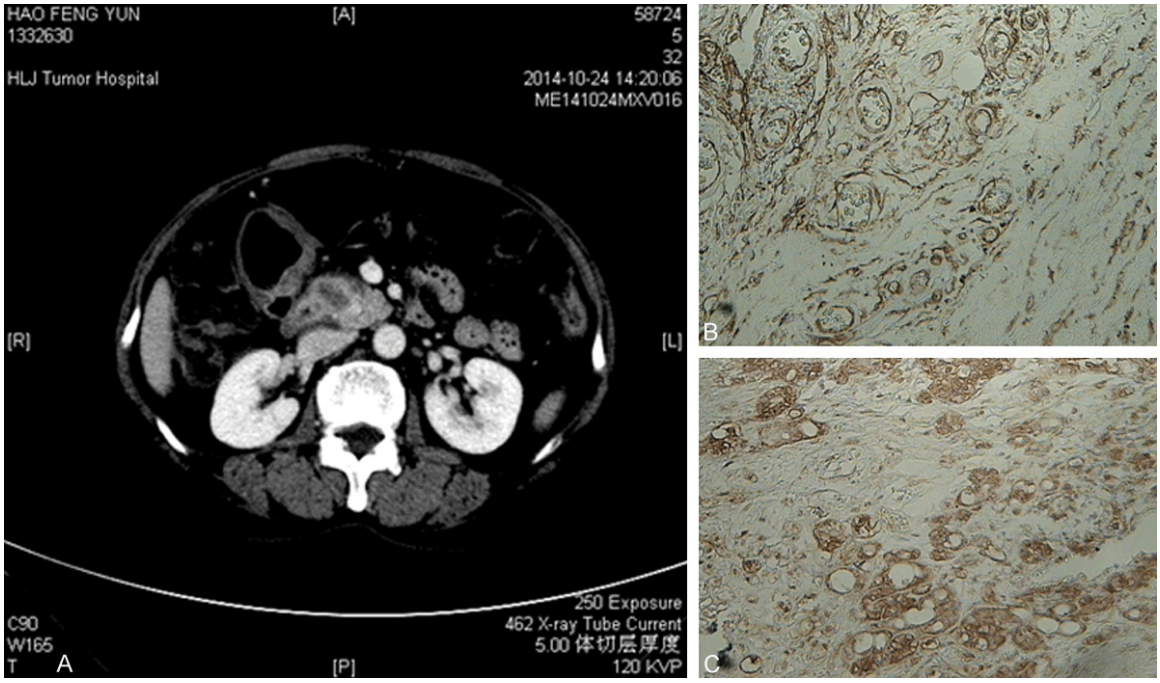

Figure 4. Poorly-differentiated adenocarcinoma of the pancreatic head. A. CT enhancement showed the pancreatic cystic mass in the central necrosis of the tumor area was not strengthened, the tissue surrounding the tumor showed mild enhancement. B. The MVD in tumor cell "hot spots" was very high and the lumen was bulky; new microvessels were stained brown by CD31 (ABC, ×200). C. VEGF stained new microvascular endothelial cells sepia (ABC, ×200).

Table 2. Tumor pathology, VEGF expression and MVD count in pancreatic carcinoma

Pathological grade	VEGF expression		MVD count			
	+	-	I	II	III	IV
High (n=5)	2	3	2	3	0	0
Moderate (n=22)	13	9	2	11	9	0
Low (n=27)	19	8	1	8	11	7
Sum (n=54)	34	20	5	22	20	7

pathological grade, or malignant degree, of tumors. Further, the grade and form were also negatively correlated with the MVD, which suggests that CT contrast enhancement can reflect the status of microvascular formation inside tumors and accurately evaluate the malignant degree of pancreatic cancer, which are consistent with the findings of Wong et al. [9]. Early enhancement of tumors was showed to mainly depend on the density of new vessels [10]. Wan et al. [11] showed that the enhancement of tumors was correlated with not only the MVD but also other factors, including the microvascular structure, the size of the extracellular space, and the vascular permeability of tumors. Therefore, tumor necrosis is an important factor that influences the degree and form of CT contrast enhancement.

In this study, the tumor grade was positively correlated with the MVD count in parenchymal cells of tumors, which suggests that the MVD is associated with the malignancy or progression of pancreatic cancer. In well differentiated adenocarcinoma, the main component was residual pancreatic tissue, and there was very little necrosis inside the tumors. The MVD count in parenchymal cells of tumors was low, but the MVD count was high in the residual pancreatic tissue. In moderately and poorly differentiated adenocarcinoma, as the malignancy degree rose, the presence of residual pancreatic tissue decreased, and the parenchymal cell volume of tumors increased. As a result, the MVD count decreased in the residual pancreatic tissue and increased inside the tumor parenchyma, but necrotic tissues increased markedly. Therefore, the overall MVD count inside tumors decreased compared with that of well differentiated adenocarcinoma, and the degree of CT contrast enhancement of well differentiated adenocarcinoma was higher than that of moderately or poorly differentiated adenocarcinoma.

This study found no correlation between the enhancement degree and form of tumors in the pancreatic parenchymal phase and VEGF expression; i.e., CT contrast enhancement did not reflect the VEGF expression level. Further, there was no correlation between the tumor grade and VEGF expression in parenchymal cells, which was consistent with the findings of Chen [12]. However, patients positive for VEGF expression had a higher MVD count than patients negative for VEGF expression. VEGF is known to promote the angiogenesis of pancreatic cancer, and tumor cells can secrete VEGF family members during disease progression. VEGFs are proteins that strongly and directly activate angiogenesis; in addition, VEGFs can increase vascular permeability, lead to plasma protein extravasation, and provide good foundations for the nascent capillary network of tumor cells [12].

In summary, the CT enhancement degree and form of tumors in the pancreatic parenchymal phase was negatively correlated with the degree of tumor malignancy as well as with the MVD count. The degree of tumor malignancy was positively correlated with the MVD count in parenchymal cells of tumors, and patients with positive VEGF expression had a higher MVD count than patients without VEGF expression. Therefore, the degree and form of CT contrast enhancement of cancer in the pancreatic parenchymal phase could be used to evaluate the degree of microvessels of tumors, reflect the malignancy of pancreatic cancer, and provide a basis for clinical treatment and prediction of prognosis.

Disclosure of conflict of interest

None.

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