ORIGINAL ARTICLES

Enhancement patterns of pancreatic adenocarcinoma on conventional dynamic multi-detector row CT: Correlation with angiogenesis and fibrosis

Yuki Hattori, Toshifumi Gabata, Osamu Matsui, Kentaro Mochizuki, Hirohisa Kitagawa, Masato Kayahara, Tetsuo Ohta, Yasuni Nakanuma

Yuki Hattori, Toshifumi Gabata, Osamu Matsui, Kentaro Mochizuki, Department of Radiology, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Japan

Hirohisa Kitagawa, Masato Kayahara, Tetsuo Ohta, Department of Gastroenterologic Surgery, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Japan

Yasuni Nakanuma, Department of Human Pathology, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8641, Japan

Author contributions: Hattori Y and Gabata T performed the majority of experiments; Hattori Y, Gabata T, Matsui O, Mochizuki K and Nakanuma Y designed the research; Hattori Y, Gabata T and Nakanuma Y performed the research; Kitagawa H, Kayahara M and Ohta T provided specimens; Hattori Y, Gabata T, Matsui O and Nakanuma Y analyzed the data; Hattori Y, Gabata T and Matsui O wrote the paper.

Correspondence to: Yuki Hattori, MD, Department of Radiology, Kanazawa University Graduate School of Medical Science,13-1 Takara-machi, Kanazawa 920-8641, Japan. hattori-ht2ryk@nifty.com
Telephone: +81-76-2652323   Fax: +81-76-2344256

Abstract

AIM: To evaluate retrospectively the correlation between enhancement patterns on dynamic computed tomography (CT) and angiogenesis and fibrosis in pancreatic adenocarcinoma.

METHODS: Twenty-three patients with pancreatic adenocarcinoma underwent dynamic CT and tumor resection. In addition to the absolute and relative enhanced value that was calculated by subtracting the attenuation value on pre-contrast from those on contrast-enhanced CT in each phase, we defined one parameter, "tumor-aorta enhancement ratio", which was calculated by dividing enhancement of pancreatic cancer by enhancement of abdominal aorta in each phase. These enhancement patterns were correlated with the level of vascular endothelial growth factor (VEGF), microvessel density (MVD), and extent of fibrosis.

RESULTS: The absolute enhanced value in the arterial phase correlated with the level of VEGF and MVD ($P = 0.047$, $P = 0.001$). The relative enhanced value in arterial phase and tumor-aorta enhancement ratio (arterial) correlated with MVD ($P = 0.003$, $P = 0.022$). Tumor-aorta enhancement ratio (arterial) correlated negatively with the extent of fibrosis ($P = 0.004$). The tumors with greater MVD and higher expression of VEGF tended to show high enhancement in the arterial dominant phase. On the other hand, the tumors with a larger amount of fibrosis showed a negative correlation with the grade of enhancement during the arterial phase.

CONCLUSION: Enhancement patterns on dynamic CT correlated with angiogenesis and may be modified by the extent of fibrosis.

Key words: Computed tomography; Contrast media; Pancreatic cancer; Angiogenesis

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INTRODUCTION

Pancreatic cancer is one of the leading causes of cancer-related death, with an overall 5-year survival rate of < 5%[1]. Surgical resection is still the only potentially curative treatment for pancreatic cancer. However, the resection rate is < 40%[2,3] because of the difficulty in achieving early detection. In addition, the results of other treatment methods including radiation therapy and chemotherapy are also poor.

Angiogenesis is the development of new blood vessels and is required for tumor growth. In the 1970s, Folkman reported that the development of neoplasms is angiogenesis-dependent[4,5], with this process induced by angiogenic factors such as vascular endothelial growth factor (VEGF). As a result, microvessel density (MVD) increases in neoplasms. Recently, it has been clarified that the grade of tumor angiogenesis is a useful prognostic marker in human cancers[6-9], including pancreatic cancer[10-14]. Generally, tumors with strong expression of angiogenesis show a poor prognosis. Therefore, anti-angiogenic treatment may be effective in improving the prognosis of patients with neoplasms including pancreatic cancer.

Evaluation of the grade of angiogenesis is important as a prognostic marker and is necessary for deciding the indications and evaluating the effect of anti-angiogenic treatment. For this, biopsy is necessary. However, because repeated biopsy is often difficult and invasive, and the specimen obtained does not always reflect the entire tumor, to establish the grade of tumor angiogenesis by non-invasive imaging may be important clinically. There have been several reports evaluating the correlation between angiogenesis and imaging findings in several types of cancers[15-18], but only a few such reports on pancreatic adenocarcinoma[19,20]. Recently, perfusion computed tomography (CT) has been used to measure the hemodynamic characteristics of various tumors, and many authors have reported the results of perfusion CT in this context[21-23]. The correlation of perfusion CT findings and MVD in lung cancer[24,25] and the evaluation of the effect of anti-angiogenic therapy by perfusion CT[26,27] have been described. However, this method requires an additional procedure for conventional CT examination and a special CT machine or software. In addition, its usefulness for pancreatic cancer is now under investigation. For the present, anti-angiogenesis agents are still not approved for the treatment of pancreatic cancer. However, as a preliminary investigation for future clinical application, to predict the grade of angiogenesis by conventional dynamic multidetector CT (MDCT), most commonly performed for the diagnosis of pancreatic cancer, would be useful clinically.

The purpose of the present study was to evaluate the validity of conventional dynamic MDCT findings to predict angiogenesis in pancreatic cancer. We analyzed retrospectively the correlation between the enhancement on CT and the histopathological findings, including the grade of tumor angiogenesis, with special reference to MVD and expression of VEGF, and the extent of fibrosis in surgically resected pancreatic adenocarcinoma.

MATERIALS AND METHODS

Patients
Thirty-six patients with pancreatic cancer underwent surgical resection between January 2003 and October 2004. Among them, 10 patients did not receive dynamic CT examination and were excluded from the study.

Additionally, two with adenosquamous carcinoma and one with mucinous carcinoma were excluded. Finally, 23 patients (15 men and eight women; age range, 34-79 years; mean age, 62.6 years) with tubular adenocarcinoma of the pancreas were evaluated. All patients underwent dynamic CT, surgical resection, and histopathological examination. The range of tumor sizes was 20-48 mm and the mean was 40.5 mm.

Our institutional review board approved this retrospective study and informed consent for the use of medical records was obtained from the patients.

CT imaging
CT images were obtained using a multi-detector row CT scanner (LightSpeed Ultra 16; GE Medical Systems, Milwaukee, WI, USA). The scanning parameters were 2.5 mm section thickness, pitch of 1.5, 120 kV, and auto mA. After pre-contrast CT scans, arterial dominant phase images of dynamic CT were obtained starting 30 s after the beginning of the intravenous bolus injection (3 mL/s) of 100 mL of iodized contrast medium at 300 mg/mL. The pancreatic phase and the late phase (near equilibrium phase) were also obtained, starting at 50 and 90 s after injection, respectively.

Imaging analysis
Two radiologists (Y.H., T.G., one with > 7 years and the other with > 23 years of experience in pancreatic imaging) evaluated retrospectively all images and determined a decision with consensus circular regions of interest (ROI) were decided in the most enhanced area of the tumor in the pancreatic phase, excluding cystic or necrotic areas and adjacent pancreatic parenchyma, and in the abdominal aorta of the same slice, and ROIs in the other phases were drawn on the same site. Attenuation values were measured in Hounsfield units (HU) (absolute enhanced value). The relative enhanced value was calculated by subtracting the attenuation value on pre-contrast CT from those on contrast-enhanced CT in each phase. Furthermore, we defined one parameter as follows. The "tumor-aorta enhancement ratio" was calculated by dividing the attenuation value (HU) of pancreatic cancer by that of the abdominal aorta in each phase of contrast-enhanced CT, as a parameter of the grade of tumor enhancement. The ratio in arterial dominant phase was defined as the "tumor-aorta enhancement ratio (arterial)", and in the pancreatic phase "tumor-aorta enhancement ratio (pancreatic)" and in late phase "tumor-aorta enhancement ratio (late)", respectively.

Immunohistochemical staining
Surgical specimens of 5 mm thickness were cut in the axial plane for pancreatic head cancer and the sagittal plane for pancreatic body and tail cancer. Formalin-fixed, paraffin-embedded tissues were sectioned at 4 μm thickness. The sections were stained with hematoxylin and eosin. Immunohistochemical and elastica van Gieson (EVG) staining was performed in the section that corresponded to the particular CT slices employed for the evaluation of enhancement pattern of the tumor. Immunohistochemical

staining was performed using the dextran polymer system (EnVision+ System; DAKO, Glostrup, Denmark). Color development was performed using 3,3'-diaminobenzidine tetrahydrochloride (DAKO), followed by hematoxylin counterstaining. For the detection of VEGF, which is an angiogenic factor, we used rabbit polyclonal anti-VEGF antibodies (A-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:100. The sections were heated in citrate buffer (pH 6.0; 10 mmol/L) using microwaves at 95℃ for 20 min, and incubated at 4℃ overnight in humid chambers with primary antibodies. For the detection of CD34 expressed on small-vessel endothelial cells, we used mouse monoclonal anti-CD34 antibodies (clone GBEnd/10; IMMUNOTECH, Marseilles, France) at a dilution of 1:200. The sections were incubated at room temperature for 1 h with primary antibodies. Islets of Langerhans and endothelium of arterial branches were used as internal positive controls for VEGF and CD34, respectively.

Histopathological analysis

One author (Y.H.) evaluated the anonymous histological specimens without any information about the radiological images under assistance of one pathologist (Y.N., with > 30 years of experience). The level of VEGF staining was scored in comparison with that in the islets of Langerhans as a positive control: score 1, extremely weak; score 2, weak; score 3, mildly weak; score 4, almost equal. Each CD34-stained slide was scanned at a low magnification (× 40) to determine five "hot spot" areas of the largest number of microvessels. MVD was determined according to the mean number of microvessels counted in the five hot spots at high magnification (× 200). The extent of fibrosis was scored according to the ratio of fibrosis in the tumor with EVG staining in which elastic fibers were stained dark brown and collagen fibers were stained pink, with a score of 1, 0%-25%; 2, 25%-50%; and 3, 50%-100%.

Statistical analysis

Statistical software (Dr. SPSS Ⅱ for windows; SPSS, Chicago, IL, USA) was used for statistical analysis. The extent and dynamics of enhancement on dynamic CT were correlated with the level of VEGF, MVD and extent of fibrosis, to analyze whether the dynamic CT parameters defined above reflect the histopathological findings, including tumor angiogenesis. In addition, we also analyzed the correlation among the expression of VEGF, MVD and fibrosis. For these analyses, Spearman's rank correlation test was used. $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Correlation between absolute and relative enhanced values and histopathological findings

Table 1 shows the averages of the absolute attenuation value (HU) of the tumor and abdominal aorta in each phase of dynamic CT, and Table 2 shows the averages of the relative enhanced value (HU).

Table 1 Absolute attenuation value (HU)

	Pre-contrast	Arterial phase	Pancreatic phase	Late phase
Pancreatic cancer				
mean ± SD	41 ± 4	61 ± 13	80 ± 13	86 ± 11
Median	41	56	81	85
Range	32-48	41-93	60-104	64-106
Abdominal aorta				
mean ± SD	45 ± 4	284 ± 50	185 ± 40	139 ± 21
Median	45	295	171	136
Range	38-51	205-409	127-307	107-208

Table 2 Relatively enhanced value (HU)

	Arterial phase	Pancreatic phase	Late phase
Pancreatic cancer			
mean ± SD	20 ± 14	39 ± 15	45 ± 13
Median	19	38	43
Range	6-54	15-70	23-74
Abdominal aorta			
mean ± SD	239 ± 51	140 ± 40	94 ± 22
Median	248	125	91
Range	157-365	86-269	69-171

Table 3 Tumor-aorta enhancement ratio

|	mean ± SD	Median	Range
Tumor-aorta enhancement ratio (arterial)	0.835 ± 0.053	0.066	0.029-0.216
Tumor-aorta enhancement ratio (pancreatic)	0.291 ± 0.12	0.244	0.139-0.569
Tumor-aorta enhancement ratio (late)	0.487 ± 0.129	0.483	0.295-0.816

The absolute value of pre-contrast CT correlated significantly with none of the histopathological findings, or the level of VEGF, MVD or fibrosis. The absolute value in the arterial phase correlated significantly with the level of VEGF and MVD ($P = 0.047$, $P = 0.001$) (Figure 1A and B, Figures 2 and 3). The absolute value in the arterial, pancreatic and late phases correlated significantly and negatively with the extent of fibrosis ($P = 0.006$, $P = 0.018$, $P = 0.035$) (Figure 1C-E and Figure 4). None of the relatively enhanced values in any phase correlated significantly with the level of VEGF. The relatively enhanced value in the arterial phase correlated significantly with MVD ($P = 0.003$) (Figure 5A). All of the relatively enhanced values in the arterial, pancreatic and late phase correlated significantly and negatively with the extent of fibrosis ($P = 0.003$, $P = 0.020$, $P = 0.039$) (Figure 5B-D).

Correlation between tumor-aorta enhancement ratio and histopathological findings

The averages of the tumor-aorta ratio are shown in Table 3. None of tumor-aorta enhancement ratios in any phase correlated significantly with the level of VEGF. Tumor-aorta enhancement ratio (arterial) was correlated significantly with MVD ($P = 0.022$) (Figure 6A), and significantly and negatively with the extent of fibrosis ($P = 0.004$) (Figure 6B).
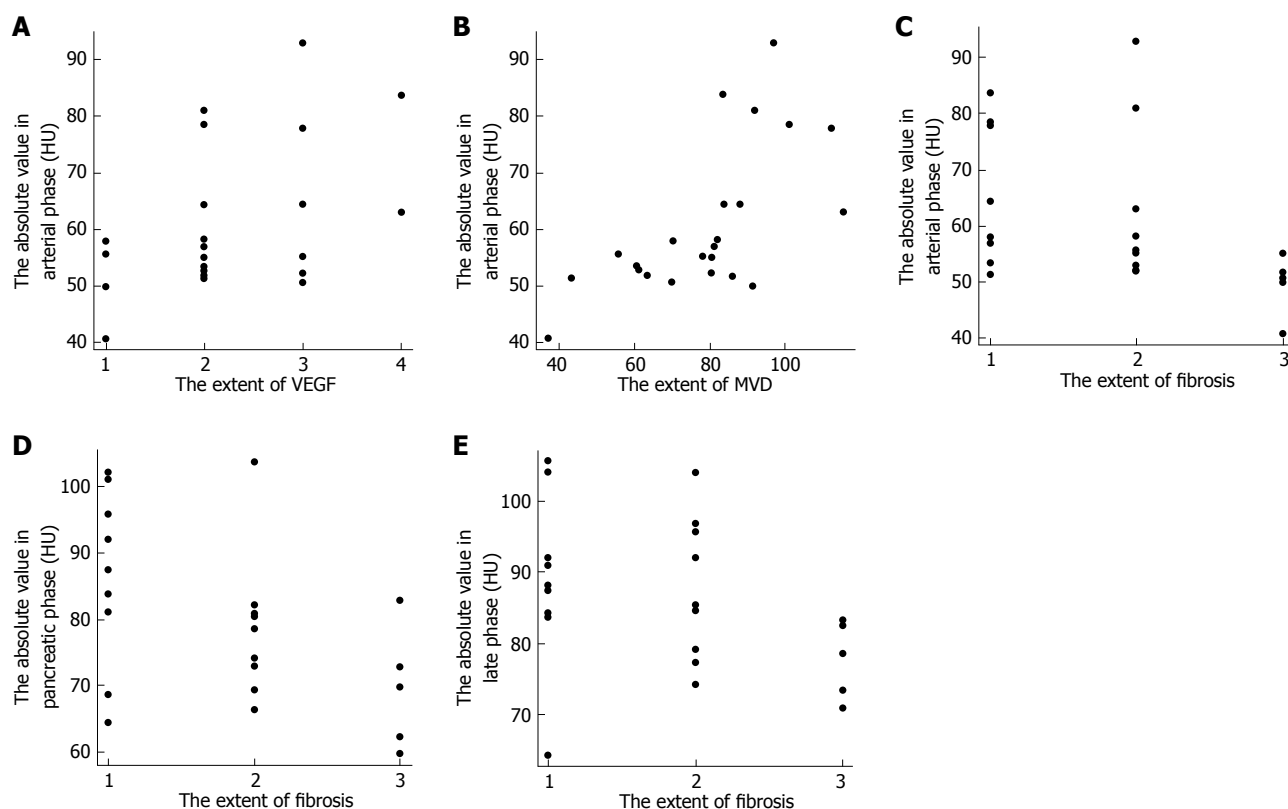
Figure 1 Scatter plots showing correlation between absolute values and histopathological findings. A: The absolute value in the arterial phase correlated significantly with the level of VEGF (r = 0.418, P = 0.047); B: The absolute value in the arterial phase correlated significantly with MVD (r = 0.649, P = 0.001); C: The absolute value in the arterial phase correlated significantly and negatively with the extent of fibrosis (r = -0.556, P = 0.006); D: The absolute value in the pancreatic phase correlated significantly and negatively with the extent of fibrosis (r = -0.488, P = 0.018); E: The absolute value in the late phase correlated significantly and negatively with the extent of fibrosis (r = -0.442, P = 0.035).
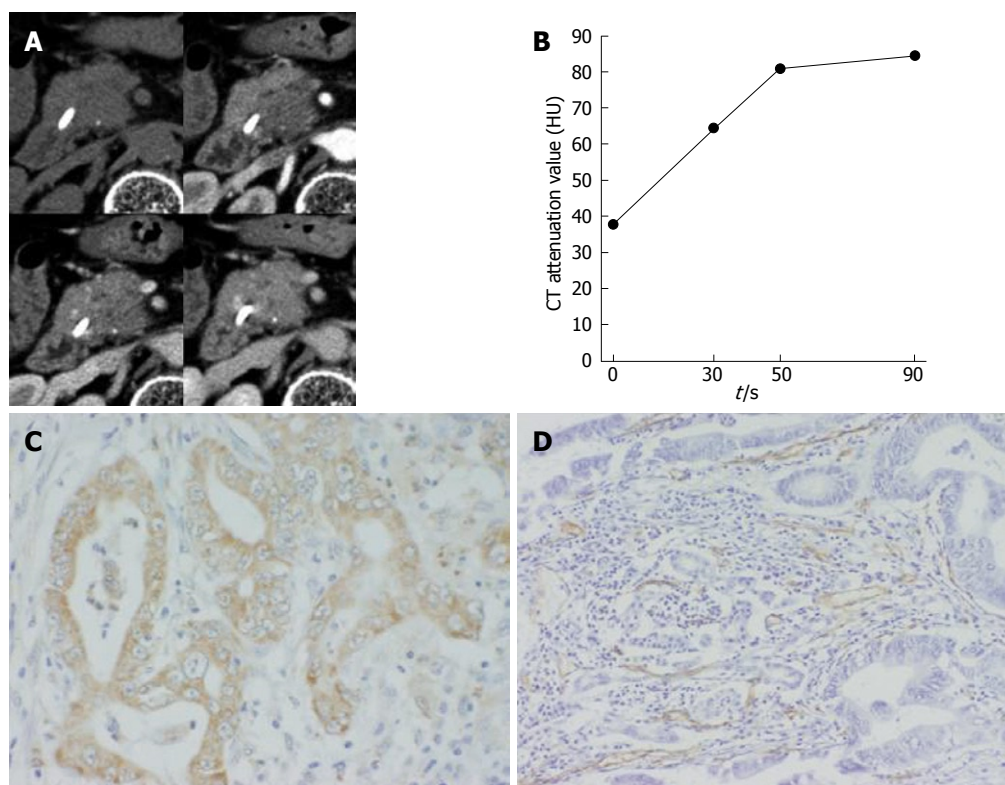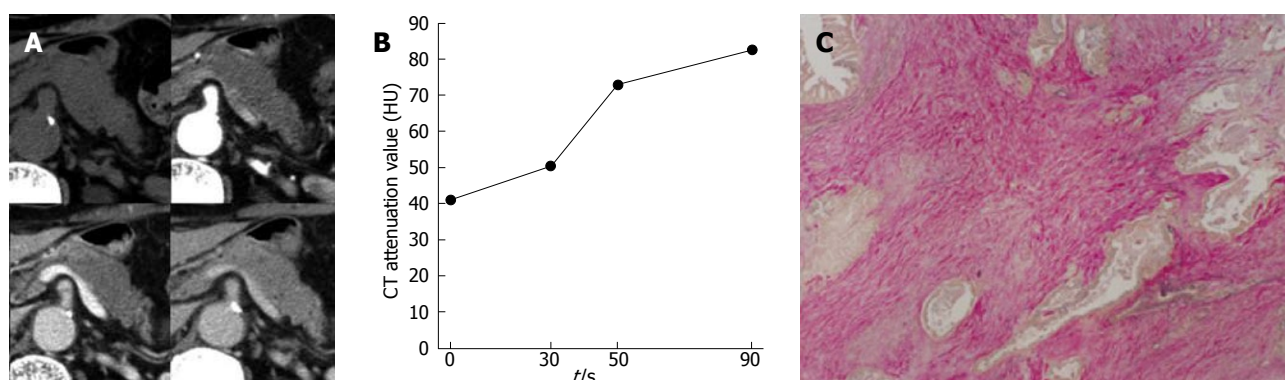

Figure 2 Moderately differentiated tubular adenocarcinoma in a 73-year-old woman. A: Transverse dynamic CT images; B: Time-attenuation curve. Dynamic CT scans showing marked enhancement in the arterial phase; C: Photomicrograph showing immunoreactivity to VEGF, which is depicted as brown cytoplasm. The score was 4 (high expression) (Anti-VEGF stain; original magnification, × 400); D: Photomicrograph showing abundant microvessels and depicting vessel walls that appeared brown (Anti-CD34 stain; original magnification, × 200).
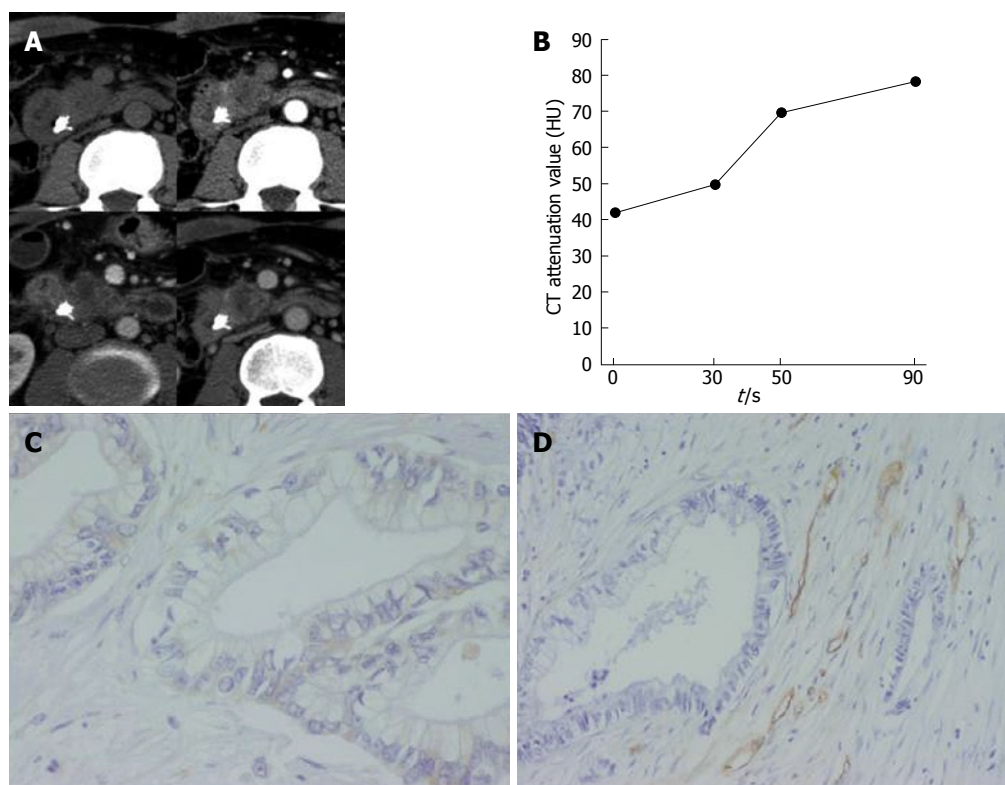
Figure 3 Well-differentiated tubular adenocarcinoma in a 44-year-old man. A: Transverse dynamic CT images; B: Time-attenuation curve. Dynamic CT scans showing low enhancement in the arterial phase; C: Photomicrograph showing immunoreactivity to VEGF, which is depicted as brown cytoplasm. The score was 1 (extremely weak) (Anti-VEGF stain; original magnification, × 400); D: Photomicrograph showing few microvessels and depicting vessel walls, which appear brown (Anti-CD34 stain; original magnification, × 200).

Figure 4 Moderately differentiated tubular adenocarcinoma in a 79-year-old man. A: Transverse dynamic CT images; B: Time-attenuation curve. Dynamic CT scans showing gradual enhancement; C: Photomicrograph showing abundant fibrosis and collagen fibers, which appear pink. The score was 3 (EVG stain; original magnification, × 40).
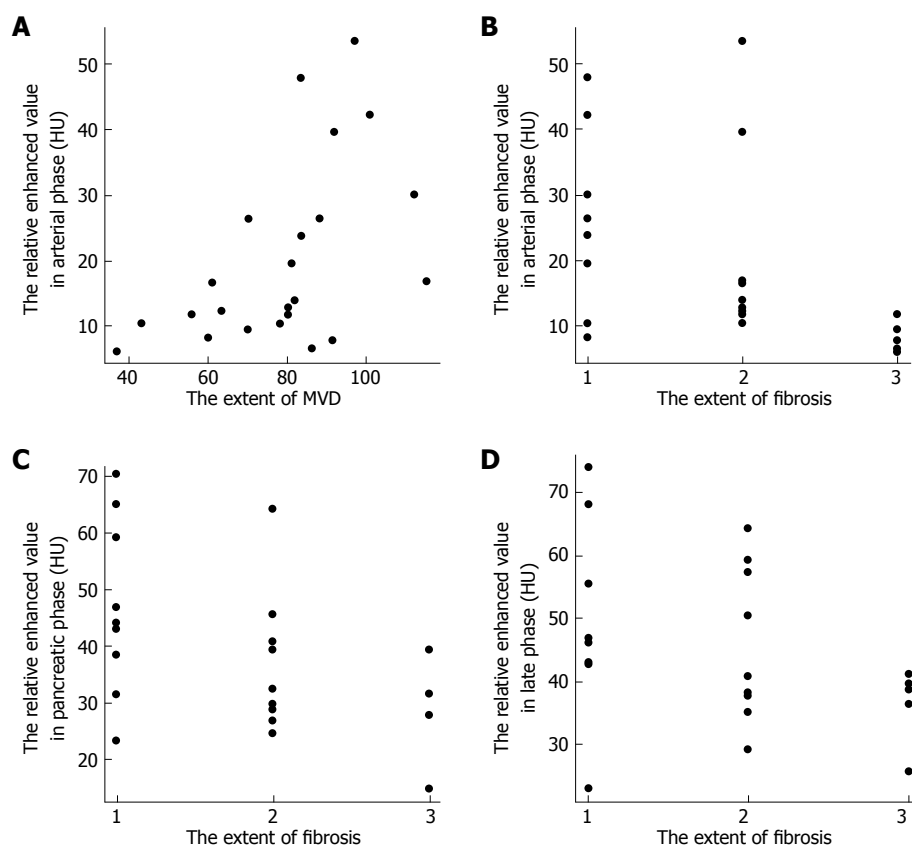
Correlation among histopathological findings

The level of VEGF was correlated significantly with MVD ($P = 0.037$). The extent of fibrosis was not correlated significantly with the level of VEGF and MVD.

DISCUSSION

The correlation between conventional dynamic MDCT findings and angiogenesis in lung[15] and renal cell[16] cancer has been reported previously. These studies have revealed that the attenuation value of the peak enhancement of the tumor and the enhancement ratio (peak enhancement value divided by time) are correlated positively with the extent of angiogenesis. However, it is not realistic to apply these results to pancreatic cancer, which usually has abundant fibrosis and tends to show gradual enhancement with the peak enhancement in the equilibrium phase[28,29]. To overcome this important problem in the common type of pancreatic cancer, we analyzed the correlation with enhancement of each phase and angiogenesis and fibrosis. In general, contrast agents have two-compartment pharmacokinetics with intravascular and extravascular-extracellular (interstitium) components. The enhancement of the tumor depends on the concentration of the injected agent, blood flow, blood volume, permeability, and extravascular-

Figure 5 Scatter plots showing correlation between the relative enhanced values and histopathological findings. A: The relatively enhanced value in the arterial phase correlated significantly with the extent of MVD (r = 0.593, P = 0.003); B: The relatively enhanced values in the arterial phase correlated significantly and negatively with the extent of fibrosis (r = -0.590, P = 0.003); C: The relatively enhanced values in the pancreatic phase correlated significantly and negatively with the extent of fibrosis (r = -0.483, P = 0.020); D: The relatively enhanced values in the late phase correlated significantly and negatively with the extent of fibrosis (r = -0.433, P = 0.039).
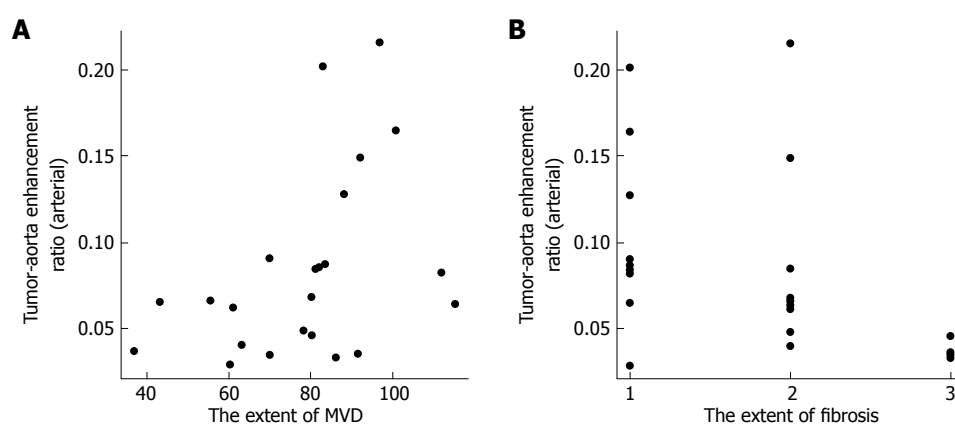
Figure 6 Scatter plots showing correlation between tumor-aorta enhancement ratio and histopathological findings. A: Tumor-aorta enhancement ratio (arterial) was correlated positively with MVD (r = 0.477, P = 0.022); B: Tumor-aorta enhancement ratio (arterial) was correlated negatively with the extent of fibrosis (r = -0.575, P = 0.004).

extracellular components. The contrast agents in the arterial dominant phase are predominantly the intravascular component. In the pancreatic phase (near portal dominant phase), they pass into the extravascular-extracellular components. The enhancement in this phase is considered to be a mixture of intravascular and extravascular-extracellular components. Tissues with adequate blood supply generally show the highest enhancement in this phase. The contrast agents in late phase (equilibrium phase) are both intravascular and extravascular-extracellular components, and the enhancement depends mainly on the extravascular-extracellular components. In addition to absolute and relative enhanced values, we employed the tumor-aorta enhancement ratio, which was calculated by dividing

the attenuation value (HU) of pancreatic cancer by that of the abdominal aorta in each phase of contrast-enhanced CT, as a parameter of the grade of tumor enhancement. This parameter was decided in order to exclude the influence of the intravascular concentration of an injected contrast agent that is dependent on cardiac output and circular blood volume. We think that this parameter reflects tumor enhancement more exactly than the absolute and relative attenuation values. Tumor-aorta enhancement ratio (arterial) is considered to reflect mainly the amount of arterial blood flow and volume of intratumoral blood spaces. Tumor-aorta enhancement ratio (pancreatic) may depend on vascular permeability in addition to intratumoral blood flow and/or blood volume. Tumor-aorta enhancement ratio (late) may

reflect mostly the extravascular-extracellular component.

In the present study, several findings of dynamic CT showed significant correlations with the histopathological findings. MVD in the tumor correlated significantly with the absolute value in the arterial and pancreatic phases, relative enhanced value in the arterial phase and the tumor-aorta enhancement ratio (arterial). This may have resulted from the increased vascular space and/or increased blood flow in tumors with increased MVD. The absolute value in the arterial phase also correlated significantly with the level of VEGF, probably for the same reason as in the case of MVD. On the other hand, the absolute value and relatively enhanced value in the arterial and pancreatic phases and tumor-aorta enhancement ratio (arterial) correlated significantly and negatively with the extent of fibrosis. This may have resulted from the smaller intratumoral blood spaces and blood flow in the tumors, with abundant fibrosis resulting in an absolutely lower volume of contrast inflow into this kind of tumor. Both the absolute value and relatively enhanced value in the late phases correlated significantly and negatively with the extent of fibrosis. It is known that the tumors with abundant internal fibrosis show prominent delayed enhancement in the late-phase of dynamic CT because of an increased extravascular-extracellular component. Therefore, the results obtained in our tumor were not consistent with previous speculations. This may also have been caused by the smaller amount of blood inflow into the tumors with more abundant fibrosis. We need to study this issue further with a more delayed phase on dynamic CT.

Histologically, the extent of VEGF expression was correlated significantly with MVD. However, the extent of fibrosis was not correlated significantly with the level of VEGF or MVD. These results support the similarity of the findings of dynamic CT between tumors with increased MVD and expression of VEGF. However, these findings may be modified by the extent of intratumoral fibrosis, which has no direct correlation with VEGF expression and MVD.

There are several limitations in the present study. First, the protocol of dynamic CT was not entirely appropriate. The late phase was earlier than the widely accepted equilibrium phase of dynamic CT of the pancreas. We used a fixed amount of contrast material. The grade of enhancement might be influenced by several factors such as patient weight, cardiac output, and CT tube wear[30,31]. Since we analyzed the validity of conventional dynamic CT retrospectively and preliminarily, we did not adopt these factors in the present study. Second, the attenuation value (HU) reflects not only the vascular characteristics of immature vessels formed by tumor angiogenesis, but also those of pre-existing mature vessels. Third, there are several other factors that affect the enhancement pattern of pancreatic cancers, such as the shape of intratumoral blood spaces and vasoactive elements. Fourth, the attenuation value (HU) in each phase on conventional dynamic MDCT may reflect various levels of blood flow, blood volume, vascular permeability and extravascular-

extracellular components. In particular, new capillaries formed by tumor angiogenesis are immature and have greater permeability than normal capillaries[32]. Further investigation, including by perfusion CT, is needed in this regard. In spite of these limitations, we think that our results provide some useful indication for the estimation of angiogenesis and intratumoral fibrosis in pancreatic cancer. After clinical application of anti-angiogenesis agents for pancreatic cancer, evaluation of the results obtained this study should be performed.

In conclusion, there was a significant correlation between the enhancement in conventional dynamic CT and angiogenesis and fibrosis in pancreatic adenocarcinoma. The tumors with greater MVD and expression of VEGF tended to show high enhancement in the arterial dominant phase. On the other hand, the tumors with a larger amount of fibrosis showed a negative correlation with the grade of enhancement during the arterial phase. Dynamic CT features that are caused by angiogenesis may be modified by the extent of intratumoral fibrosis.

COMMENTS

Background
Prognosis of pancreatic cancer is poor. Recently, it has been clarified that the grade of tumor angiogenesis is a useful prognostic marker in human cancer, including pancreatic cancer. Therefore, evaluation of the grade of angiogenesis by imaging may be important as a prognostic marker and is necessary for deciding the indications and evaluating the effect of anti-angiogenic treatment.

Research frontiers
To establish the grade of tumor angiogenesis by non-invasive imaging may be important clinically. However, there are only a few such reports on pancreatic adenocarcinoma. In the present study, the authors analyzed the correlation between enhancement on dynamic multidetector computed tomography (MDCT) and histopathological findings, including the grade of tumor angiogenesis, and the extent of fibrosis in surgically resected pancreatic adenocarcinoma.

Innovations and breakthroughs
This study predicted the grade of angiogenesis by conventional dynamic MDCT that is performed most often for the diagnosis of pancreatic cancer. The tumors with strong angiogenesis tended to show high enhancement in the arterial dominant phase. On the other hand, tumors with a larger amount of fibrosis showed a negative correlation with the grade of enhancement during the arterial phase.

Applications
Anti-angiogenesis agents are still not approved for the treatment of pancreatic cancer at present. However, as a preliminary investigation for future application, prediction of the grade of angiogenesis by conventional dynamic MDCT would be useful clinically.

Terminology
Angiogenesis is the process of new blood vessel formation. This consists of endothelial sprouts of preexisting vessels and is stimulated by angiogenic factors such as vascular endothelial growth factors. Recently, angiogenesis has been recognized as an important factor of tumor growth and metastasis.

Peer review
The author retrospectively evaluate the correlation between enhancement patterns on dynamic computed tomography and angiogenesis and fibrosis in pancreatic adenocarcinoma.

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