

Delta HU is a potential marker to predict chemotherapy response for unresectable pancreatic ductal adenocarcinoma

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ABSTRACT

Background: FOLFIRINOX and gemcitabine plus albumin-bound paclitaxel (AG) regimens are recommended as first-line therapy for both locally advanced and metastatic pancreatic cancer. However, there were no specific markers to conduct personalized regimen choice. The research is to assess delta Hounsfield unit (delta HU), which is the difference in CT attenuation value (in HU) between enhanced and nonenhanced phase of region of interest, as a marker for predicting chemotherapy response of unresectable pancreatic cancer.

Methods: A total of 179 unresectable pancreatic cancer patients were enrolled in the study. Kaplan-Meier analysis and COX regression analysis were performed for progression-free survival (PFS) and overall survival. The differences of clinical characteristics were analyzed by χ^2 test. Microvessel density (MVD) was calculated by immunohistochemistry staining of CD34.

Results: Delta HU was an independent risk factor for unresectable pancreatic cancer ($P = 0.017$, HR 0.672, 95%CI 0.485–0.930). Patients with higher delta HU were associated with better PFS ($P = 0.004$). For modified FOLFIRINOX (mFOLFIRINOX) group, delta HU was an independent risk factor ($P = 0.045$, HR 0.571), but not for AG group ($P = 0.473$, HR 0.855). Delta HU was correlated with stroma MVD ($P = 0.000$, $R = 0.483$), not with parenchyma MVD ($P = 0.074$, $R = 0.199$).

Conclusions: Delta HU was a marker predicting chemotherapy response for unresectable pancreatic cancer. Higher delta HU was associated with better survival for patients receiving mFOLFIRINOX rather than AG. The delta HU was positively correlated with stroma MVD, explaining the relationship between delta HU and prognosis.

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Abbreviations: AG, gemcitabine plus albumin bound paclitaxel; CECT, contrast-enhanced computed tomography; CI, confidence interval; CT, computed tomography; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group performance status; HU, Hounsfield unit; Δ HU, delta HU, delta Hounsfield unit; HR, hazard ratio; mFOLFIRINOX, modified FOLFIRINOX; MVD, microvessel density; NCCN, National Comprehensive Cancer Network; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; RECIST 1.1, Response Evaluation Criteria in Solid Tumors 1.1; ROI, region of interest; TER, tumor enhanced rate.

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Introduction

Pancreatic cancer is a fatal malignancy with a 5-year survival rate of 10% [1]. Radical surgery is the potential curative treatment, whereas 80%–85% patients cannot undergo surgical resection, in which detected with locally advanced pancreatic cancer or metastatic cancer initially [2]. To prolong survival and improve quality of life, FOLFIRINOX (oxaliplatin, leucovorin, irinotecan and fluorouracil) and gemcitabine plus albumin-bound paclitaxel (AG) are recommended as first-line therapy for both locally advanced and metastatic pancreatic cancer by National Comprehensive Cancer Network (NCCN) guidelines [3]. However, there were no accomplished clinical trials to compare two regimens and no specific

markers to conduct personalized regimen choice. It is urgent to seek a marker predicting chemotherapy response and selecting proper regimen.

Contrast-enhanced computer tomography (CECT) is the most common detection of initial diagnosis and applied to evaluate chemotherapy response. CT value has been used to evaluate chemo response and prognosis for advanced pancreatic ductal adenocarcinoma (PDAC). Shin Il Kim et al. demonstrated that the delta Hounsfield unit (HU) was defined that arterial phase HU of region of interest (ROI) subtracted HU in pre-contrast phase, with cutoff of 31.5 and 18 respectively applied to predict chemotherapy responders in pancreatic tumor (sensitivity 62.8%, specificity 91.3%) and liver metastasis (sensitivity 76.0%, specificity 85.7%) [4]. Zhi-qiang Meng et al. confirmed that locally advanced pancreatic cancer patients with tumor enhanced rate (TER) less than 80.456% had 3.587-fold risk of poorer overall time (OS) than who with TER more than 80.456% [5]. However, in unresectable patients with regular chemotherapy, it was unclear whether prognosis was related to delta Hounsfield unit (delta HU, Δ HU), which is the difference in CT attenuation value (in HU) between enhanced and nonenhanced phase of ROI.

In regard to the correlation between CT value and histology, Xiao-Hua Chen et al. reported that the HU enhancement was negatively related to pathological grade ($P < 0.05$, $R = -0.784$) and tumor microvessel density (MVD, $P < 0.05$, $R = -0.790$) in pancreatic cancer [6]. On the contrary, Yuki Hattori et al. reported that aorta ratio was correlated with MVD positively ($P = 0.033$, $R = 0.649$) [7]. Also, Zhongqiu Wang et al. reported that HU enhancement was correlated with pathological grades ($P = 0.001$, $R = 0.7857$) and MVD ($P < 0.05$, $R = 0.3613$) [8]. The conflicting correlation of delta HU and MVD should be proved by histopathological characteristics explaining the relationship between delta HU and prognosis in different regimens.

The purpose of study was to assess the prognosis prediction value of Δ HU in unresectable pancreatic cancer personalization therapy, exploring the potential underlying mechanisms of the correlation between delta HU and prognosis by histopathology.

Methods and materials

Patients

The retrospective study was approved by the institutional ethics committee of Tianjin Medical University Cancer Institute and Hospital. Written informed consents were obtained from all participants. 179 unresectable PDAC patients between June 2016 and January 2019 were enrolled, who underwent AG or modified FOLFIRINOX (mFOLFIRINOX) chemotherapy at Tianjin Medical University Cancer Institute and Hospital. Following up has continued 18–40 months.

The inclusion criteria included: locally advanced and metastatic stage pancreatic cancer assessed according to NCCN guideline; PDAC pathologically diagnosed by tumor biopsy; treated initially with AG or mFOLFIRINOX; underwent at least three cycles chemotherapy; estimated CECT every three cycles or three months after chemotherapy finished.

Exclusion criteria included: underwent radical resection; diagnosed of other histologic types of pancreatic neoplasms or not with pathological diagnosis; with double primary tumors or other tumors; received palliative surgery or additional radiotherapy or other therapy before; treated with other second-line regimens; treated less than three cycles; cannot examine by CECT due to any reasons; without complete follow-up information or clinical information.

81 patients who were performed pancreatic cancer radical

resection and received adjuvant chemotherapy were enrolled in another group to explore the correlation between MVD and Δ HU. All patients were pathologically diagnosed of PDAC and without neoadjuvant therapy. Following up had continued 18 months–48 months.

Chemotherapeutic regimens

All patients received normative chemotherapy recommended by NCCN guideline. For AG regimen, a 30-min intravenous infusion of albumin bound paclitaxel was given at dose of 125 mg per square meter, following an infusion of gemcitabine at dose of 1000 mg per square meter of body-surface area, on days 1, 8 every 3 weeks. Patients with Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 and 1 were adopted mFOLFIRINOX every 2 weeks, which was consisted of oxaliplatin 65 mg, leucovorin 400 mg, irinotecan 150 mg and fluorouracil 2400 mg per square meter. Oxaliplatin delivered as a 2-h intravenous infusion, following leucovorin a 2-h intravenous infusion, after 30 min, following a 90-min intravenous infusion of irinotecan, immediately following by fluorouracil continuously intravenous infusion over a period of 46 h.

Contrast-enhanced CT measurement and delta HU definition

CECT was performed with a 64-slice CT scanner (GE Discovery CT, General Electric, WI, USA) during breath-hold. Using a high-pressure syringe, 90–100 ml (1.0–2.0 ml/kg) of nonionic contrast material (standard iodine concentration, 300 mg/mL, Ultravist 300; Bayer Health Care, Wayne, NJ) was injected intravenously at 2 ml/s. Nonenhanced CT with 1.5 mm section thickness was recorded before enhanced scan. Enhanced phase was recorded at 60sec with a thickness of 1.5 mm.

Three 1 cm $\times$ 1 cm ROIs were chosen in the maximum tumor area to measure the CT value by two experienced radiologists. Cystic areas, necrotic areas and margin near areas were avoided. Both CT value in nonenhanced and enhanced phase of ROIs were measured. Delta HU is the difference in CT attenuation value (in HU) between enhanced and nonenhanced phase of ROI. Two sample graphs were presented in Fig. 1a,b. Average over delta HU of three ROIs was recorded for a patient. In unresectable PDAC group, we set Δ HU median 27 as cutoff value, in addition, we set median 33 as cutoff value in resected group.

Immunohistochemistry and MVD assessment

MVD was calculated by immunohistochemical staining of CD34. Tumor specimens were obtained at the time of surgery. After deparaffination, pathological sections were heated with high pressure for 2min to retrieve antigens in EDTA buffer. The endogenous peroxidase activity was blocked with 3% H_2O_2 for 30 min at room temperature without light. Following PBS washing, primary antibodies were incubated with anti-CD34 antibodies (ab81289, 1:2500; Abcam, Cambridge, MA, USA) overnight at 4 °C. The antibody binding was detected using peroxidase-conjugated secondary antibody at 37 °C for 30 min. A DAB Substrate Kit (ZLI-9019, ZSGB-BIO) was used for the chromogenic reaction.

Parenchyma micro vessels were defined as vessels within malignant ducts less than 100 μ m, while the stroma micro vessels were defined as vessels away from malignant ducts more than 100 μ m. Total MVD was the average of parenchyma and stroma MVD. MVD were calculated according to Weidner correction MVD measure method. Three most vascularized regions, named hot spots, were selected at a low magnification ($\times 100$). MVD was counted in each region at high magnification ($\times 400$) by staining

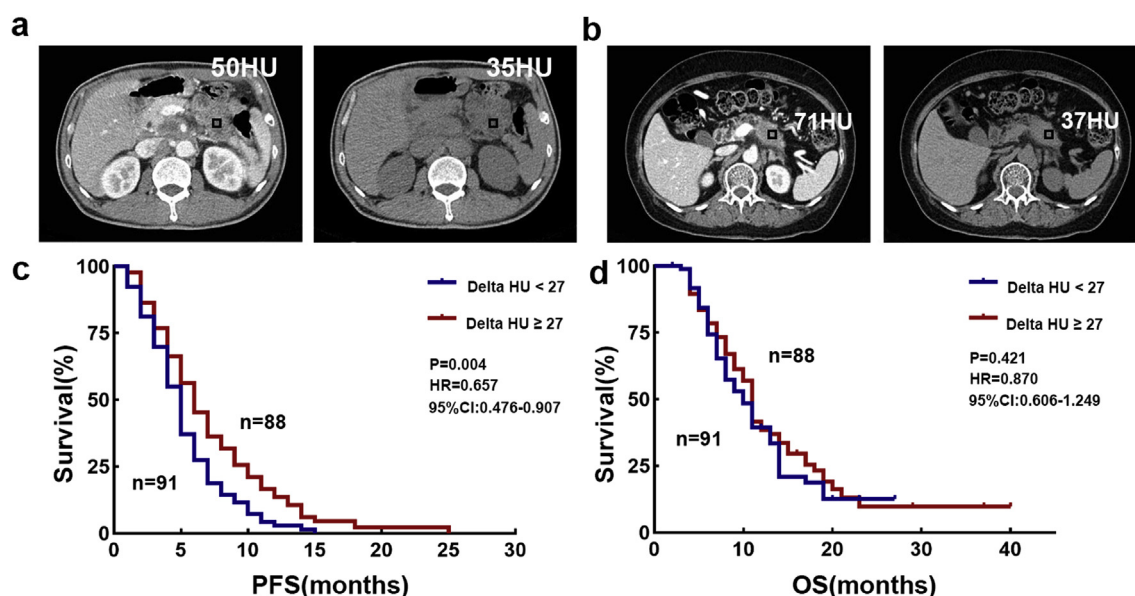


Fig. 1. Delta HU was significantly associated with PFS in unresectable patients. Two samples were presented explaining measurement of delta HU, a 64-year old female advanced PDAC patient with lower Δ HU (a) and a 56-year-old male advanced PDAC patient with higher Δ HU (b), PFS(c) and OS (d) survival curves of Δ HU < 27 and $\geq$ 27 in 179 patients. delta HU, Δ HU, delta Hounsfield unit; PFS, progression-free survival; PDAC, pancreatic ductal adenocarcinoma.

micro vessels of CD34. The average counts of three views were recorded. Any single endothelial cell or cell mass stained with antibodies, whether or not forming a lumen, was considered a countable micro vessel. Vessels with smooth muscle wall or diameter larger than 8 red blood cells were excluded. All the measurement assessed by two experienced pathologists. We set median as cutoff for each MVD, 26 for stroma MVD (range 11–57), 31 for parenchyma MVD (range 14–63) and 29 for total MVD (range 14–51).

Other factors measurement

Baseline target lesions of all patients were recorded according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) less than 2 weeks before the beginning of chemotherapy by CECT. Gender, age and other characteristic were collected from medical records. ECOG PS was assessed objectively by experienced oncologists before therapy. Baseline CA19-9 was detected by ELISA with the cutoff of 37U/ml. Tumor size, lymph nodes, tumor location and metastasis were assessed by baseline CECT. Clinical TNM stage confirmed according to AJCC TNM staging of pancreatic cancer (8th ed., 2017). Differentiation was confirmed by fine needle aspiration tissue or surgical specimen by pathologists. Therapy response was identified by RECIST 1.1 for regular follow-up.

Statistical analysis

All statistical analyses were performed using SPSS Statistics Program (version 22.0; IBM, Armonk, NY, USA). The clinical characteristics demonstrated with proportion for classified variable, analyzed using χ^2 test between groups. The cutoff of Δ HU was determined by the means of 27 for unresectable group and 33 for resected group. Survival curves were generated by Kaplan-Meier analysis and comparison was analyzed by Log-Rank tests or Breslow tests. Cox regression models for univariate and multivariable analyses were used to identify prognostic factors and estimate hazard ratio (HR) based on progression-free survival (PFS). We considered a two-side P value less than 0.05 to be significant. The P

value of factor in univariate analysis less than 0.2 was considered in multivariate COX analysis. Correlation between MVD and Δ HU were calculated using Spearman correlation analysis. Paired *t*-tests were used for pairwise comparisons in stroma, parenchyma, total MVD.

Results

Delta HU is significantly correlated to prognosis in unresectable PDAC

The baseline characteristics of 179 patients were compared according to the Δ HU, only the factor of age showed significant difference ($P = 0.021$, Table 1). Baseline characteristic of gender, T stage, N stage, M stage and TNM stage, tumor location, differentiation, ECOG PS, chemotherapy regimens, CA19-9 showed no difference in two groups ($P > 0.05$).

On univariate COX analysis among factors (Table 2), gender (HR 1.347, 95% CI 0.971–1.870, $P = 0.075$), clinical T stage (HR 1.354, 95% CI 0.969–1.893, $P = 0.076$), ECOG PS (HR 1.520, 95% CI 1.097–2.106, $P = 0.012$) and Δ HU (HR 0.645, 95% CI 0.466–0.892, $P = 0.008$) were associated with prognosis. ECOG PS (HR 1.458, 95% CI 1.051–2.023, $P = 0.024$) and Δ HU (HR 0.672, 95% CI 0.485–0.930, $P = 0.017$) were independent risk factors for PFS of unresectable PDAC identified by multivariate COX analysis. Gender and clinical T stage did not affect the prognosis. Patients with Δ HU $\geq$ 27 ($n = 88$) had 0.672-fold risk of shorter PFS compared with those lower than 27 ($n = 91$).

The median PFS of patients with Δ HU < 27 was 5 months, showing poorer survival than 6 months of Δ HU $\geq$ 27 ($P = 0.004$, HR 0.657, 95% CI 0.476–0.907, Fig. 1c). Although, the significant difference didn't find in OS of two groups ($P = 0.421$, HR 0.870, 95% CI 0.606–1.249, Fig. 1d). Delta HU was significantly associated with PFS in unresectable PDAC.

The relationship between delta HU and prognosis in different regimens

Given to the difference in the chemo regimens, subgroup

Table 1
Characteristics of unresectable patients at baseline.

Characteristic	Delta HU < 27		Delta HU ≥ 27		P Value
	(N = 91)		(N = 88)		
	n (%)		n (%)		
Gender					0.063
female	32	(35.2)	43	(48.9)	
male	59	(64.8)	45	(51.1)	
Age, yr					0.021*
< 60	54	(59.3)	37	(42.0)	
≥ 60	37	(40.7)	51	(58.0)	
Clinical T stage					0.496
T1	1	(1.1)	2	(2.3)	
T2	31	(34.1)	38	(43.2)	
T3	29	(31.9)	26	(29.5)	
T4	30	(33.0)	22	(25.0)	
Clinical N stage					0.920
N0	48	(52.7)	44	(50.0)	
N1	32	(35.2)	32	(36.4)	
N2	11	(12.1)	12	(13.6)	
Clinical M stage					0.898
M0	22	(24.2)	22	(25.0)	
M1	69	(75.8)	66	(75.0)	
TNM stage					0.898
III	22	(24.2)	22	(25.0)	
IV	69	(75.8)	66	(75.0)	
Tumor location					0.519
head/neck	36	(39.6)	39	(44.3)	
body/tail	55	(60.4)	49	(55.7)	
Differentiation					0.407
moderately	30	(33.0)	24	(27.3)	
poorly	61	(67.0)	64	(72.7)	
ECOG PS					0.584
0	43	(47.3)	38	(43.2)	
1	48	(52.7)	50	(56.8)	
Chemotherapy regimen					0.777
AG	55	(60.4)	55	(62.5)	
mFOLFIRINOX	36	(39.6)	33	(37.5)	
CA19–9, U/ml					0.924
≤ 37	16	(17.6)	15	(17.0)	
> 37	75	(82.4)	73	(83.0)	

delta HU, delta Hounsfield unit; ECOG PS, Eastern Cooperative Oncology Group performance status; AG, gemcitabine plus albumin bound paclitaxel; mFOLFIRINOX, modified FOLFIRINOX.

analyses were performed. The group of AG (n = 110, [Supplementary Table 1](#)) and mFOLFIRINOX (n = 69, [Supplementary Table 2](#)) were separately compared with clinical characteristics by $\Delta\text{HU} < 27$ and ≥ 27 , no statistically significant differences were detected in AG group, whereas gender (P = 0.041), age (P = 0.044) showed significant difference in mFOLFIRINOX group.

In AG group, after performing univariate Cox regression analysis ([Table 3](#)), data on the variables of clinical T stage (P = 0.132, HR 1.466, 95%CI 0.892–2.410), clinical M stage (P = 0.113, HR 1.499, 95%CI 0.909–2.473), TNM stage (P = 0.113, HR 1.499, 95%CI 0.909–2.473), tumor location (P = 0.114, HR 0.701, 95%CI 0.452–1.089) were entered into multivariate Cox regression analysis of PFS for AG patients. Although it was not related to PFS (P = 0.241, HR 0.780, 95%CI 0.514–1.182), delta HU as the major subject was included in multivariate COX analysis. The result of multivariate COX analysis showed that clinical T stage (P = 0.006, HR 2.150, 95%CI 1.250–3.699), clinical M stage (P = 0.004, HR 2.318, 95%CI 1.316–4.082), TNM stage (P = 0.004, HR 2.318, 95%CI 1.316–4.082), tumor location (P = 0.012, HR 0.551, 95%CI 0.347–0.875) were independent risk factors for AG patients. Unexpectedly, no significant differences were demonstrated in delta HU (P = 0.473, HR 0.855, 95%CI 0.558–1.311).

While the result was diverse in mFOLFIRINOX group ([Table 4](#)). Gender (P = 0.073, HR 1.693, 95%CI 0.952–3.012), differentiation

(P = 0.185, HR 0.702, 95%CI 0.416–1.185), ECOG PS (P = 0.002, HR 2.400, 95%CI 1.379–4.179) and ΔHU (P = 0.009, HR 0.490, 95%CI 0.287–0.836) were associated with PFS in univariate COX analysis. Furthermore, ECOG PS (P = 0.012, HR 2.065, 95%CI 1.169–3.646) and ΔHU (P = 0.045, HR 0.571, 95%CI 0.330–0.988) were independent prognostic factors for mFOLFIRINOX group.

With Kaplan-Meier survival analysis, different results showed in two regimens. In AG group, no significant statistical difference was observed among patients with $\Delta\text{HU} < 27$ or ≥ 27 in PFS (5mo vs. 6mo, P = 0.190, HR 0.786, 95%CI 0.521–1.186, [Fig. 2a](#)) and OS (11mo vs. 11mo, P = 0.347, HR 1.246, 95%CI 0.766–2.026, [Fig. 2b](#)). In mFOLFIRINOX group, patients with $\Delta\text{HU} < 27$ had a shorter median PFS (4mo vs. 6mo, P = 0.004, HR 0.524, 95%CI 0.313–0.878, [Fig. 2c](#)) and OS (9mo vs. 11mo, P = 0.012, HR 0.538, 95%CI 0.310–0.934, [Fig. 2d](#)). The delta HU was associated with survival in mFOLFIRINOX group, but not in AG group.

Relationship between delta HU and MVD

To investigate underlying reasons for predicting prognosis by delta HU, we focused on histopathological characteristic of PDAC, based on previous results, we verified the relationship between ΔHU and MVD in resectable PDAC group. MVD was calculated by immunohistochemical staining of CD34 in both parenchyma and stroma areas of 81 patients ([Fig. 3a](#)). The stroma MVD was significantly less than parenchyma MVD in PDAC (P < 0.05, [Fig. 3b](#)). Compared the clinical characteristics of these 81 patients according to ΔHU ([Supplementary Table 3](#)), pathology T stage was associated with ΔHU (P = 0.041), meaning that the patients with $\Delta\text{HU} \geq 33$ had smaller masses. Moreover, the stroma MVD (P = 0.000) and total MVD (P = 0.014) were relevant to delta HU. The Spearman correlation between ΔHU and stroma MVD (P = 0.000, R = 0.483, [Table 5](#)) or total MVD (P = 0.001, R = 0.370) was significant, while no significance was indicated in the correlation between ΔHU and parenchyma MVD (P = 0.074, R = 0.199).

In resected PDAC patients, the prognostic significance of ΔHU was identified by Kaplan-Meier analysis in disease-free survival (DFS, 7mo vs. 12mo, P = 0.032, HR 0.609, 95%CI 0.370–1.002, [Fig. 3c](#)), not in OS (35mo vs. 29mo, P = 0.994, HR 1.003, 95%CI 0.487–2.065, [Supplementary Fig 1a](#)). Significant statistical difference of DFS also was observed in stroma MVD (9mo vs. 11mo, P = 0.049, HR 0.736, 95%CI 0.454–1.194, [Fig. 3d](#)) and but not in parenchyma (10mo vs. 11mo, P = 0.760, HR 0.930, 95%CI 0.573–1.510, [Supplementary Fig 1c](#)) and total MVD (10mo vs. 11mo, P = 0.349, HR 0.801, 95%CI 0.494–1.299, [Supplementary Fig 1e](#)). No statistical difference was detected in OS ([Supplementary Fig. 1b, d, f](#)). Delta HU and stroma MVD is associated with prognosis in resected PDAC.

Discussion

The decision of chemotherapy regimen for unresectable PDAC patients was based on performance status recommended by guideline [9]. For individualized therapy, searching for a marker of predicting treatment response would be necessary. Delta HU, which is the difference in CT attenuation value (in HU) between enhanced and nonenhanced phase of ROI, is objectively available in CECT. We validated the relationship between delta HU and prognosis in this study, focused on the group of unresectable PDAC that completely relying on the chemotherapy. Delta HU, as an independent prognosis factor for unresectable PDAC, could be applied to personalized chemotherapy regimen selection. In two regimens subgroups, delta HU was detected merely significant difference in survival for mFOLFIRINOX rather than AG. Therefore, for locally advanced or metastasis PDAC, patients with higher delta HU were

Table 2
Univariate and multivariate analyses of the PFS of unresectable patients.

Variable	Univariate			Multivariate		
	HR	95% CI	P Value	HR	95% CI	P Value
Gender						
female vs. male	1.347	0.971–1.870	0.075	1.273	0.913–1.774	0.154
Age, yr						
< 60 vs. ≥ 60	0.922	0.669–1.271	0.619			
Clinical T stage						
T1/T2 vs. T3/T4	1.354	0.969–1.893	0.076	1.147	0.810–1.627	0.440
Clinical N stage						
N0 vs. N1/N2	1.180	0.856–1.625	0.312			
Clinical M stage						
M0 vs. M1	1.154	0.794–1.677	0.453			
TNM stage						
III vs. IV	1.154	0.794–1.677	0.453			
Tumor location						
head/neck vs. body/tail	0.910	0.657–1.261	0.572			
Differentiation						
moderately vs. poorly	0.876	0.619–1.240	0.455			
ECOG PS						
0 vs. 1	1.520	1.097–2.106	0.012	1.458	1.051–2.023	0.024*
Chemotherapy regimen						
AG vs. mFOLFIRINOX	1.035	0.743–1.441	0.839			
CA19–9, U/ml						
≤ 37 vs. > 37	1.147	0.760–1.729	0.514			
Delta HU						
< 27 vs. ≥ 27	0.645	0.466–0.892	0.008	0.672	0.485–0.930	0.017*

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; AG, gemcitabine plus albumin bound paclitaxel; mFOLFIRINOX, modified FOLFIRINOX; delta HU, delta Hounsfield unit.

Table 3
Univariate and multivariate analyses of the PFS of patients with AG.

Variable	Univariate			Multivariate		
	HR	95% CI	P Value	HR	95% CI	P Value
Gender						
female vs. male	1.198	0.791–1.814	0.395			
Age, yr						
< 60 vs. ≥ 60	1.113	0.736–1.681	0.612			
Clinical T stage						
T1/T2 vs. T3/T4	1.466	0.892–2.410	0.132	2.150	1.250–3.699	0.006*
Clinical N stage						
N0 vs. N1/N2	1.134	0.733–1.754	0.573			
Clinical M stage						
M0 vs. M1	1.499	0.909–2.473	0.113	2.318	1.316–4.082	0.004*
TNM stage						
III vs. IV	1.499	0.909–2.473	0.113	2.318	1.316–4.082	0.004*
Tumor location						
head/neck vs. body/tail	0.701	0.452–1.089	0.114	0.551	0.347–0.875	0.012*
Differentiation						
moderately vs. poorly	1.206	0.732–1.986	0.463			
ECOG PS						
0 vs. 1	1.315	0.834–2.074	0.239			
CA19–9, U/ml						
≤ 37 vs. > 37	1.070	0.621–1.843	0.808			
Delta HU						
< 27 vs. ≥ 27	0.780	0.514–1.182	0.241	0.855	0.558–1.311	0.473

PFS, progression-free survival; AG, gemcitabine plus albumin bound paclitaxel; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; delta HU, delta Hounsfield unit.

supposed be recommended by mFOLFIRINOX as chemo regimen, providing a novel method to select treatment regimen. In this study, there was no difference in both PFS ($P = 0.074$) and OS ($P = 0.467$) in AG and mFOLFIRINOX.

Independent risk factors in AG group included T, M, TNM stage and tumor location, except ECOG PS. Patients with tumor in pancreatic body or tail had better survival. TNM stage was not associated with prognosis in mFOLFIRINOX group, but ECOG PS was significant as independent risk factor. Patients were selected into two groups by ECOG PS before, so the significance of ECOG PS was

different. In all unresectable patients, the difference of ECOG PS in prognosis was significant, either. The results confirmed the reliability of the data that were consistent with identifying regimen by ECOG PS in clinical practice [10]. Whereas, delta HU was superior to ECOG PS in personalization treated choice because of objective record.

Previous researches had indicated the positive or negative correlation between MVD and delta HU, incompatibly [11]. This study verified the positive relationship between delta HU and MVD, emphasizing MVD in stroma rather than parenchyma related to

Table 4
Univariate and Multivariate Analyses of the PFS of Patients with mFOLFIRINOX.

Variable	Univariate			Multivariate		
	HR	95% CI	P Value	HR	95% CI	P Value
Gender						
female vs. male	1.693	0.952–3.012	0.073	1.292	0.694–2.406	0.419
Age, yr						
< 60 vs. ≥ 60	0.740	0.432–1.267	0.272			
Clinical T stage						
T1/T2 vs. T3/T4	1.392	0.824–2.353	0.217			
Clinical N stage						
N0 vs. N1/N2	1.206	0.706–2.061	0.492			
Clinical M stage						
M0 vs. M1	0.897	0.497–1.621	0.720			
TNM stage						
III vs. IV	0.897	0.497–1.621	0.720			
Tumor location						
head/neck vs. body/tail	1.129	0.678–1.880	0.641			
Differentiation						
moderately vs. poorly	0.702	0.416–1.185	0.185	0.665	0.376–1.174	0.159
ECOG PS						
0 vs. 1	2.400	1.379–4.179	0.002	2.065	1.169–3.646	0.012*
CA19–9, U/ml						
≤ 37 vs. > 37	1.157	0.618–2.168	0.648			
Delta HU						
< 27 vs. ≥ 27	0.490	0.287–0.836	0.009	0.571	0.330–0.988	0.045*

PFS, progression-free survival; mFOLFIRINOX, modified FOLFIRINOX; HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; delta HU, delta Hounsfield unit.

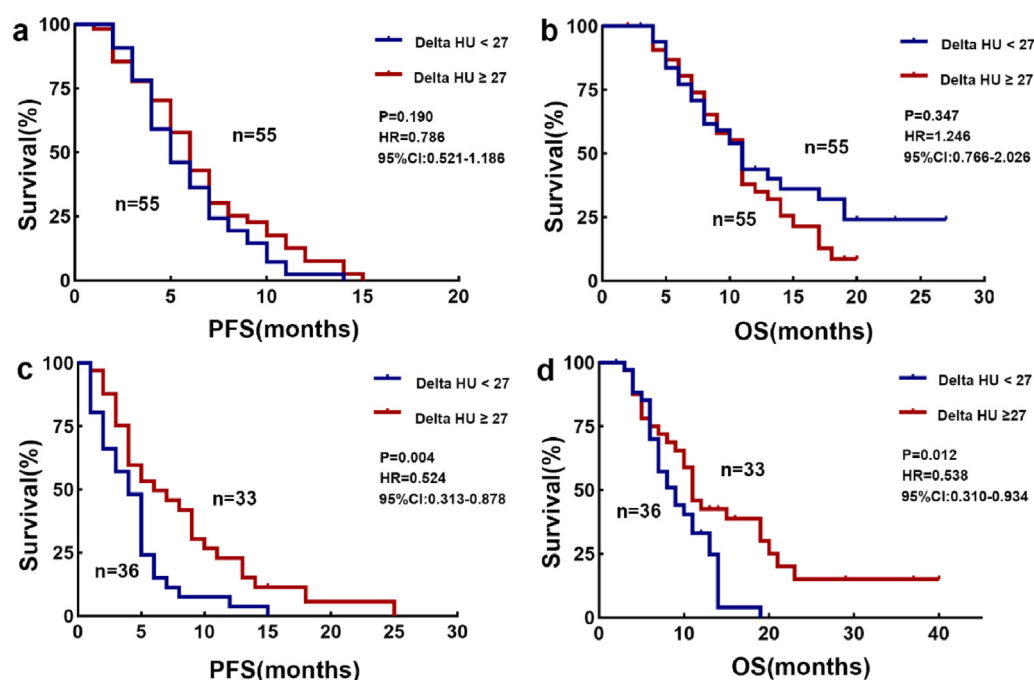


Fig. 2. Kaplan-Meier analysis of survival in AG group and mFOLFIRINOX group. PFS(a) and OS(b) survival curves of Δ HU < 27 and $\geq$ 27 in AG group. PFS(c) and OS(d) survival curves of Δ HU < 27 and $\geq$ 27 in mFOLFIRINOX group. delta HU, delta Hounsfield unit; AG, gemcitabine plus albumin bound paclitaxel; mFOLFIRINOX, modified FOLFIRINOX; HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; OS, overall survival.

delta HU. Speculating from this, micro vessels probably played different roles in stroma and parenchyma, stroma compressed vasculature in the tumor resulting in impaired drug delivery, more micro vessels possibly delivered more drug to tumor [12]. We inferred that the chemo response was influenced by stroma micro vessels strongly in mFOLFIRINOX, on the contrary, weakly in AG leading to no significance in analysis of AG regimens.

We announced that higher MVD was associated with poorer

prognosis for resectable PDAC before [13]. Kenji Takagi et al. demonstrated that tumor peripheral MVD assessed by the lumen method was associated with liver metastasis and a poorer survival [14]. MVD was calculated jointly in both stroma and parenchyma in most research, nevertheless, large area of stroma surrounded by pancreatic cancer cells was one of the histology characteristics of PDAC, micro vessels in stroma were much more than in parenchyma [15,16]. Takahiro Nishida et al. indicated that vessels in

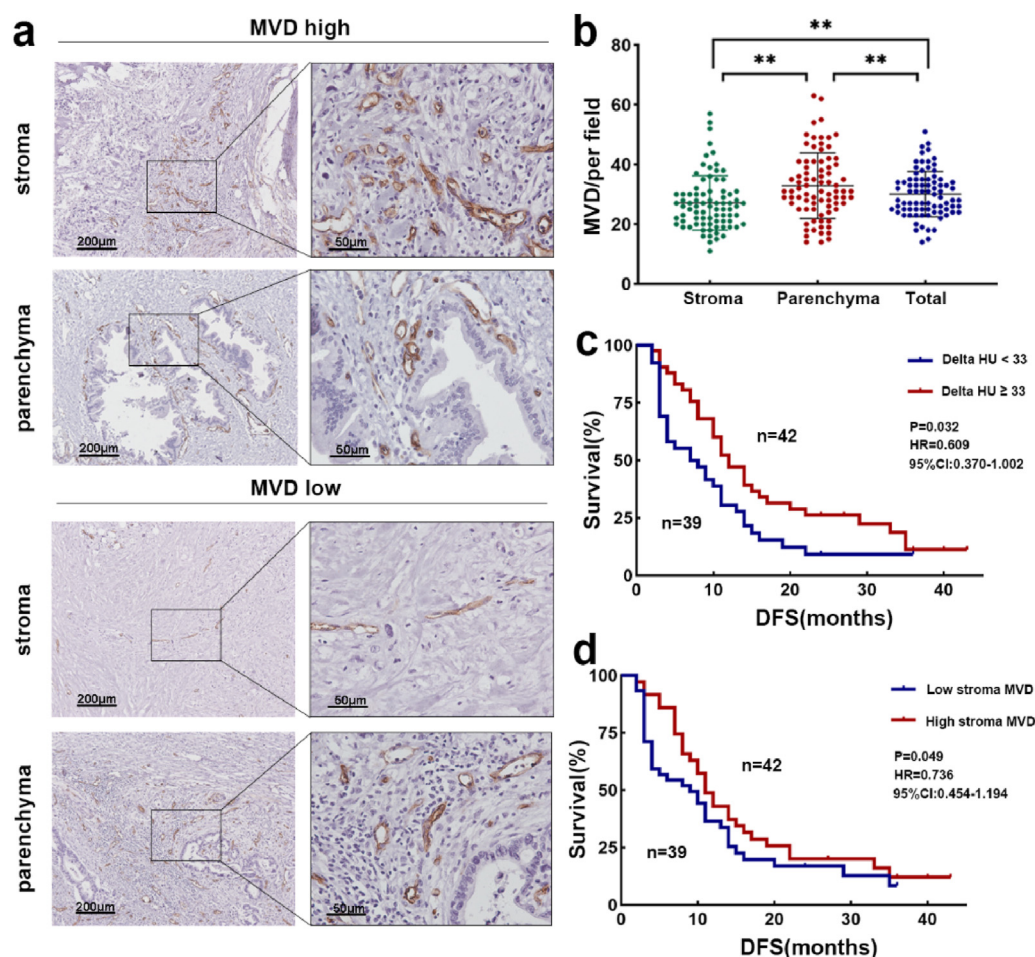


Fig. 3. Delta HU was positively associated with MVD. a, CD34 immunohistochemistry staining of PDAC specimens in stroma and parenchyma. b, Significant difference of MVD in stroma, parenchyma and total. c, DFS survival curves of Δ HU < 33 and $\geq$ 33. d, DFS survival curves of low and high stroma MVD. MVD, microvessel density; delta HU, delta Hounsfield unit; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

Table 5

Correlation of delta HU with MVD by Spearman analysis.

		Stroma MVD	Parenchyma MVD	Total MVD
Delta HU	r	0.483**	0.199	0.370**
	P	0.000	0.074	0.001

delta HU, delta Hounsfield unit; MVD, microvessel density.

different areas separately calculated showed differently significance, low stromal area and high stromal MVD predicted poor prognosis in pancreatic cancer [17]. However, in this study, patients with higher stroma MVD had better survival, potentially due to different calculation methods or small sample size. Different results in regimens indicated the clinical significance of stroma MVD [18]. More micro vessels in stroma appear to be better prognosis for unresectable PDAC. It may explain that combined anti-angiogenic therapy and chemotherapy failed in clinical trials for advanced PDAC [19,20]. From now on, the reason was unclear, it was worthy to deeply explore the different function of micro vessels in PDAC.

However, there were some study limitations. Firstly, this retrospective study was performed in only one center, and the sample of groups was small, the results may not apply to other centers. Large scale multicenter prospective clinical trials are needed to confirm these conclusions. Secondly, the mechanism of the difference between regimens is still not clear. The basic research of different

chemo drugs and micro vessels are needed. Thirdly, the correlation between MVD and delta HU was indirectly verified not in biopsy tissue of unresectable group. And the sample of resected group should be enlarged for further study.

In summary, we demonstrated the prognostic value of delta HU for both locally advanced PDAC and advanced PDAC. Delta HU was as a potential marker of predicting chemotherapy response. Patients with higher delta HU will benefit from mFOLFIRINOX treatment. The delta HU was positively correlated with stroma MVD, probably explaining the reason of lower Δ HU associated with worse prognosis.

Author contributions

All authors listed have contributed substantially to the design, data collection and analysis, and editing of the manuscript. Yizhang Zhou and Song Gao contributed equally to this work.

Conception and design: YZ ZHOU, S GAO; statistical analysis: YZ ZHOU, H REN, XF GUO; data acquisition: YZ ZHOU, BD ZHOU, S YUAN, YF WANG, J REN, ZQ CHEN; drafting of the manuscript: YZ ZHOU; critical revision of the manuscript: JH HAO, S GAO; study supervision: JH HAO, S GAO.

Declaration of competing interest

The authors have declared that no competing interest exists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pan.2021.02.016>.

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