Dear Editors and Reviewers:

Thank you for your letter and the reviewers' comments on our manuscript entitled " Prognostic value of preoperative enhanced computed tomography as a quantitative imaging biomarker in pancreatic cancer " (Manuscript NO: 70312). Those comments are very helpful for revising and improving our paper, as well as the important guiding significance to the other research. In response to the critiques from reviewers, we have included detailed point-by-point responses. The comments from reviewer are labeled as blue and the responses to reviewers are marked in black.

Round 1

Reviewer #1 (Comments to the Author):

The authors proposed a really interesting paper regarding quantitative analysis images in pancreatic cancer. All the main sections of the article (title, abstract, key words, background, methods, and results) seem to be adequate and the topic is very interesting. Just one minor issue to be reviewed: considering what in the scientific literature is currently available regarding radiomics and delta radiomics in pancreatic cancer, in the discussion can be useful to add some consideration regarding the delineation of the region of interest. The authors specified in fact that they considered only the largest axial image of the tumor and only some random area of the healthy pancreas, but in scientific literature whole volume quantitative analysis is currently available. I think that the author should consider to discuss this topic.

Response:

Thank you very much for your suggestions on our article. Because tumour heterogeneity is affected by tumour blood supply, the ratio of tumour cells to stromal cells and tumour necrosis will lead to different CT values in different parts of the tumour. Although whole-volume quantitative analysis of tumour CTs is currently available, it has not been analysed in our study for the following reasons: 1. At the largest and most

visible level of the tumour in CT images, the change of the tumour relative to surrounding tissue is relatively obvious, and it is easy to identify the boundary of the tumour. Moreover, it is simple to obtain the average CT value of ROT; 2. Compared with the largest level of the tumour, the CT value of the whole volume may be more easily affected by the obvious blood vessels and dilated pancreatic duct in the tumour.

The above analysis has been added to the penultimate paragraph of the discussion section.

Special thanks to you for your good comments.

Reviewer #2 (Comments to the Author):

[Methods] 1. This study was performed with a retrospective design. In real clinical practice, the timing that supposed to be taken for PV phase or PP phase scan may have some disparity when compared with the ideal timing. How do you convince that the CT protocol was performed by the exact way you explained in the method section?

Response:

Thank you very much for your suggestions on our article. In the past, when our department cooperated with the department of radiology, an agreement had been reached through communication. When patients with pancreatic tumour were undergoing enhanced CT examination, the department of radiology will follow the procedure of “CT image acquisition” in the methods section. The contrast agent was injected with an automatic syringe linked with CT instrument. The bolus-tracking technique (explained in "CT image acquisition" in the methods section) can greatly reduce individual differences between patients. In the process of collecting and reviewing the CT imaging results of the patient's imaging data, we tried our best to make the quality of each patient's tumour image meet our requirements. However, this study is retrospective, and it is indeed difficult to avoid disparity in the data of a small

number of patients between the timing that supposed to be taken for PV phase or PP phase scan and the ideal timing. In the future, we will further adopt prospective studies and implement a stricter supervision mechanism to make the CT protocol performed by the exact for every patient.

Part of the above reply has been appropriately added to the last sentence of the first paragraph of CT image acquisition in the methods section.

2. Furthermore, Is the statistical significance of TRER still maintained after the 17 patients that showed inverse enhancement patten of PV phase < PP phase as shown in Table 3 were excluded?

Response:

Previous study has analyzed the ratio of CT value change amplitude between tumour and normal pancreatic tissue from the PP phase to the PV phase, but we found that the CT value in the PP phase of PTOT in 17 cases was greater than that in the PV phase. Therefore, for some patients with significant enhancement of CT, the ratio became negative because the CT value in the PP phase was greater than that in the PV phase (This part is mentioned in the second half of paragraph 5 of the discussion section). It significantly interferes with the predictive prediction efficiency of the results. In order to avoid this phenomenon, we analyzed the ratio of CT value increase from the nonenhancement phase to the PV phase between ROT and PTOT, rather than the changes from the PP phase to the PV phase. In this way, we make TRER suitable for all patients. In addition, if 17 patients are excluded, the sample size will be further reduced, and the statistical results will be more obviously biased. But we also tried to do statistical analysis after 17 patients are excluded, TRER is statistically significant in the univariate analysis ($p = 0.003$) and the multivariate analysis ($p = 0.014$).

3. The authors described that the ROT (the region of the tumor) was delineated at the largest and most visible level. However, there could be variable enhancement

pattern even within the same cancer mass. How did you deal with the heterogeneity CT texture within the same tumor mass of the same imaging slide? Furthermore, in such cases, how did you decide the measure point of the ROT?

Response:

The region of the overall tumour (ROT) was delineated along the tumour edge at the largest and most visible level (This point has been appropriately modified in the second sentence of the second paragraph of CT image acquisition in the methods section). Because tumour heterogeneity is affected by tumour blood supply, the ratio of tumour cells to stromal cells and tumour necrosis will lead to different CT values in different parts of the tumour. We obtained the corresponding average CT value after delineating ROT, which was used as a reflection of the overall situation of the tumour. After we delineated ROT, the average CT value of ROT was obtained through the CT instrument software, instead of measuring the CT value of a certain measure point inside the tumour.

4. Please describe the method to derived the cut-off value of the ROC in details in the method section.

Response:

We are very sorry for our negligence. When the ROC curve is obtained, the "Coordinates of the Curve" and the corresponding "Sensitivity" and "1 - Specificity" can be obtained, and their corresponding Youden indices can be calculated. The TRER corresponding to the maximum value of all Youden indices is the cut-off value of the ROC curve.

And we have accepted your suggestion and described it in detail in the statistical analysis of the method section.

[Results] 1. Based on the Table 1, this study included 29 patients with AJCC stage III which means that the patients had T4 and unresectable; however, all of the enrolled

67 patients had undergone surgical resection. Please explain this contradictory findings.

Response:

The 67 patients were considered resectable during preoperative analysis. Not all of the 29 patients with AJCC stage III had T4, of which 13 were T4 and the rest were T1-3N2. 13 patients with postoperative T4 stage were identified by intraoperative conditions or postoperative pathology. 6 patients with pancreatic body or tail cancer invading the celiac axis were surgically removed by distal pancreatectomy with en bloc celiac axis resection (DP-CAR) in these 13 patients; the remaining 2 patients with pancreatic body or tail cancer and 5 patients with pancreatic head or uncinate process carcinoma were classified as the positive part of surgical margin.

2. I am very curious about the tumor stage (AJCC) was not significant in multivariate analysis in Table 6.

Response:

The tumour stage (AJCC) was statistically significant in the univariate analysis, but not in the multivariate analysis. We consider that this situation is due to the relatively small sample size. We have supplemented the analysis of this result in the third and fourth sentences of the second paragraph of the discussion section. In the future, we will continue to accumulate the sample size for analysis.

3.Please show additional results of univariate and multivariate analysis after the tumor stage categorized into resectable and LAPD. Please add the additional results in the Table 4, 5, and 6.

Response:

Regarding the reclassification of resectable and LAPD for all patients, we reply as follows. We think the classification of the resectability of pancreatic cancer is mainly to distinguish whether the tumour can be completely removed or R0 removed, and to a

certain extent, it also reflects the AJCC stage of the tumour. For example, the majority of patients with borderline resectable and LAPD are T4 or III stage. Therefore, we think that when the surgical margin and AJCC stage have been analyzed in the multivariate analysis, the results of tumour resectability classification in the multivariate analysis will be greatly disturbed, so the additional results of tumour resectability classification were not added in the table 4, 5, and 6.

However, we have carefully considered your suggestions for our article. First of all, all patients were considered to be resectable in the preoperative analysis, but a small number of patients were confirmed to be borderline resectable and LAPD during the operation or by postoperative pathology. Therefore, we think that all patients cannot be simply divided into resectable and LAPD. In addition, the number of LAPD cases is very small, so we combined borderline resectable and LAPD patients into one group. In our response to your comments, we tried to put resectability into Table 4, 5, and 6 for analysis. The results are shown below.

Resectability is not statistically significant in the univariate and multivariate analyses. We consider the reasons for this result as the following: 1. Surgical margin and AJCC stage interfered with it to a certain extent. 2. The sample size is relatively small.

As Cox regression (forward LR model) was used in the multivariate analysis, the results in Table 6 remained unchanged.

Table 4 Baseline characteristics of the low-and high-enhancement groups

Variable	TRER ≤ 0.7 Number of patients	TRER > 0.7 Number of patients	P value
Age (year)			0.397
≤ 60	22 (50.0%)	9 (39.1%)	
> 60	22 (50.0%)	14 (60.9%)	
Sex			0.170
Male	25 (56.8%)	17 (73.9%)	
Female	19 (43.2%)	6 (26.1%)	
Tumour location			0.117
Head/Uncinate	26 (59.1%)	18 (78.3%)	
Body/Tail	18 (40.9%)	5 (21.7%)	

CA19-9			0.230
< 37 ng/ml	11 (25.0%)	9 (39.1%)	
≥ 37 ng/ml	33 (75.0%)	14 (60.9%)	
AJCC stage (2017)			0.015
I	4 (9.1%)	7 (30.4%)	
II	16 (36.4%)	11 (47.8%)	
III	24 (54.5%)	5 (21.7%)	
Tumour differentiation			0.634
Highly/Moderately	33 (75.0%)	16 (69.6%)	
Poorly/Undifferentiated	11 (25.0%)	7 (30.4%)	
Vascular invasion			0.071
No	30 (68.2%)	21 (91.3%)	
Yes	14 (31.8%)	2 (8.7%)	
Surgical margin			0.501
Negative	36 (81.8%)	21 (91.3%)	
Positive	8 (18.2%)	2 (8.7%)	
Adjuvant chemotherapy			0.087
No	25 (56.8%)	8 (34.8%)	
Yes	19 (43.2%)	15 (65.2%)	
Resectability			0.014
R	26 (59.1%)	21 (91.3%)	
BR/LAPD	18 (40.9%)	2 (8.7%)	

R: Resectable; BR: Borderline resectable.

Table 5 Univariate analysis using Cox regression for postoperative OS in all patients

Variable	Number of patients	Number of events	Median OS (months) (95% CI)	P value
Age (year)				0.699
≤ 60	31 (46.3%)	27	14.6 (9.1-20.0)	
> 60	36 (53.7%)	30	10.2 (5.7-14.7)	
Sex				0.651
Male	42 (62.7%)	34	10.2 (4.3-16.1)	
Female	25 (37.3%)	23	13.6 (9.2-18.1)	
Tumour location				0.836
Head/Uncinate	44 (65.7%)	36	12.0 (7.7-16.3)	
Body/Tail	23 (34.3%)	21	12.8 (4.1-21.6)	
AJCC stage (2017)				0.005
I	11 (16.4%)	6	27.3 (14.9-39.6)	
II	27 (40.3%)	23	12.0 (10.2-13.8)	
III	29 (43.3%)	28	8.9 (5.0-12.7)	
Resectability				0.129

R	47(70.1%)	37	12.8 (8.1-17.6)	
BR/LAPD	20(29.9%)	20	10.8 (4.0-17.6)	
CA19-9				0.033
< 37 ng/ml	20 (29.9%)	14	15.8 (10.4-21.2)	
≥ 37 ng/ml	47 (70.1%)	43	11.3 (7.2-15.3)	
Tumour differentiation				0.255
Highly/Moderately	49 (73.1%)	42	14.7 (11.9-17.6)	
Poorly/Undifferentiated	18 (26.9%)	15	8.2 (5.7-10.7)	
Surgical margin				0.141
Negative	57 (85.1%)	47	12.3 (7.5-17.1)	
Positive	10 (14.9%)	10	12.0 (2.9-21.1)	
Vascular invasion				0.435
No	51 (76.1%)	41	12.3 (7.7-16.9)	
Yes	16 (23.9%)	16	12.0 (4.8-19.2)	
Adjuvant chemotherapy				0.000
No	33 (49.3%)	32	8.2 (6.6-9.7)	
Yes	34 (50.7%)	25	17.7 (14.4-20.9)	
TRER				0.001
≤ 0.7	44 (65.7%)	42	10.0 (5.9-14.1)	
> 0.7	23 (34.3%)	15	22.0 (12.4-31.6)	

CI: confidence interval.

Table 6 Multivariate analysis using Cox regression for postoperative OS in all patients

Variable	Hazard ratio	95% CI	P value
CA19-9 (ng/ml)	2.279	1.174-4.422	0.015
Tumour differentiation	3.057	1.585-5.898	0.001
Surgical margin	2.860	1.315-6.222	0.008
Adjuvant chemotherapy	0.200	0.106-0.380	0.000
TRER	0.432	0.229-0.812	0.009

4.There are duplicate results in the Table 5 and Table 7. Furthermore, the statistical value was different even though the variable had the identical values. What is the correct statistical values?

Response:

We think that the results of Table 5 and Table 7 in the article are not duplicates. In Table 5, Cox regression (enter model) was performed for the univariate analyses. In Table 7, the bivariate correlation method was used to analyze the correlation between TRER and clinicopathological characteristics. The statistical method of Table 7 is supplemented in the penultimate sentence of the last paragraph of the method section. In addition, Table 4 and Table 7 in our article are partially similar in structure, but the meaning and statistical methods of them are different. In Table 4, chi-squared test or Fisher's exact test is used for comparison between groups. The above statistical methods are described in "statistical analysis" in the methods section.

Discussion] It is plausible to explain the prognostic value of TRER for the unresectable patients with pancreatic cancer, because chemotherapeutic drugs could be well delivered in patients with relative high TRER which means that the vascularity are relatively sufficient when compared with low TRER. However, all of the 67 patients enrolled in this study had undergone surgical resection. Is it the real effect of the tumor nature that shows high TRER, or just a surrogates for another important factors such as nodal status, tumor size, and stage. Please discuss the meaning of TRER in resected patients.

Response:

In 67 patients with surgically resected pancreatic cancer, TRER was found to be an independent prognostic factor in our study. Although TRER is associated with AJCC stage, T stage, and N stage, it is not a substitute for lymph node status, tumour size, or stage. Pancreatic cancer is a kind of cold tumour with abundant stroma, and the stroma contributes to tumour growth and progression and plays an important role in the chemoresistance. This pathological feature of pancreatic cancer is similar to the pathological differentiation of tumours. It represents the characteristics of the pathology and growth of pancreatic cancer itself and will not disappear because the tumour is removed. The low-enhancement mode of CT in pancreatic cancer is partly due to the

high stromal ratio of pancreatic cancer. Based on this, TRER is used as a quantitative reflection of the low-enhancement mode of CT in pancreatic cancer and the richness of pancreatic cancer stroma, which is used to predict postoperative OS. Moreover, because the postoperative prognosis of patients with low TRER is poor, such patients can consider whether to receive neoadjuvant chemotherapy.

The above analysis has been added to the third-to-last paragraph of the discussion section.

Once again, thank you very much for your constructive comments and suggestions which would help us both in English and in depth to improve the quality of the paper.

Round 1

Reviewer (Comments to the Author):

In general, correlation analysis such as Pearson and Spearman's correlation analysis requires at least one side variable of numerical data. But, in Table 7, there are many statistics performed only with categorical data. Thus, I think the analyzing method seems to be inappropriate, especially for the part where the analysis was conducted only with categorical variables. Furthermore, If the authors want to perform correlation analysis, please present the results with graph and correlation co-efficient.

Response:

After querying the literature and consulting several statistical experts again, we quite agreed with your suggestion that Pearson and Spearman's correlation analysis should not be used between two unordered categorical variables. Meanwhile, our statisticians believed that the ordinal categorical variable was similar to the continuous numerical variable, so Spearman rank correlation could be used for correlation analysis when there was an ordinal categorical variable between the two variables.

In the TRER and related clinicopathological features we analyzed, except that "AJCC stage (2017)" and "N stage" are ordinal categorical variables, the rest are unordered categorical variables.

Statisticians gave us the following suggestions: In the case of correlation analysis, when the clinicopathological features were grouped as unordered categorical variables, the chi-squared test was used for analysis and the Cramer’s V correlation coefficient was calculated; When the clinicopathological features were grouped as ordinal categorical variables, Spearman rank correlation was used for analysis and the Spearman's correlation coefficient was calculated.

The above statistical methods and results have been added and modified in the penultimate sentence of the statistical analysis section, the last paragraph of the results section, and the last sentence of the research methods section respectively, and the table 7 has been modified.

The revised table 7 and remarks are as follows.

Table 7 Correlation between TRER and clinicopathological characteristics

Variable	TRER ≤ 0.7 Number of patients	TRER > 0.7 Number of patients	P value	Coefficient of correlation
Tumour location			0.117	0.192
Head/Uncinate	26 (59.1%)	18 (78.3%)		
Body/Tail	18 (40.9%)	5 (21.7%)		
CA19-9			0.230	0.147
< 37 ng/ml	11 (25.0%)	9 (39.1%)		
≥ 37 ng/ml	33 (75.0%)	14 (60.9%)		
AJCC stage (2017) *			0.003	-0.353
I	4 (9.1%)	7 (30.4%)		
II	16 (36.4%)	11 (47.8%)		
III	24 (54.5%)	5 (21.7%)		
T stage			0.005	0.343
T1/2	13 (29.5%)	15 (65.2%)		
T3/4	31 (70.5%)	8 (34.8%)		
N stage*			0.046	-0.245
N0	12 (27.3%)	11 (47.8%)		
N1	15 (34.1%)	8 (34.8%)		
N2	17 (38.6%)	4 (17.4%)		
Lymph node metastasis			0.138	0.181
Negative	13 (29.5%)	11 (47.8%)		
Positive	31 (70.5%)	12 (52.2%)		
Vascular invasion			0.071	0.258
No	30 (68.2%)	21 (91.3%)		
Yes	14 (31.8%)	2 (8.7%)		
Tumour differentiation			0.634	0.058
Highly/Moderately	33 (75.0%)	16 (69.6%)		
Poorly/Undifferentiated	11 (25.0%)	7 (30.4%)		

*: Spearman rank correlation was used, and Spearman correlation coefficient was calculated. The other variables were tested by chi-square test, and Cramer's V correlation coefficient was calculated.

When we performed the correlation analysis again, it was found that there was no significant correlation between vascular invasion and TRER (P = 0.071), but this change did not affect the main conclusion of our paper.

This result has been revised or explained in the last sentence of the results section of the abstract, the penultimate sentence of the core tip section, the last paragraph of the results section of the text, the last sentence of the third paragraph of the discussion section, and the last sentence of the research results section.

Once again, thank you very much for your constructive comments and suggestions which would help us in depth to improve the quality of the paper.

I'm very sorry that this modification took so long to reply. As it coincides with the Chinese Spring Festival, the statisticians we need to consult took a long vacation; Later, we spent a lot of time with statisticians searching and studying the relevant data and literature; Moreover, in order to make the uploaded figures meet the requirements of the journal, we also asked a number of colleagues, so it also took a long time.

Best regards,

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