Responses to the reviewers:

Reviewer #00227375:

This is an interesting manuscript about the effects of abdominal paracentesis drainage (APD) on severe acute pancreatitis (SAP)-associated cardiac injury. APD treatment improved cardiac morphological changes, inhibited cardiac dysfunction, decrease cardiac enzymes, and reduced cardiomyocyte apoptosis. In addition, APD significantly decreased serum level of high mobility group box (HMGB) 1. The authors have demonstrated that APD treatment could exert protective effects on SAP-associated cardiac injury through suppression HMGB1-mediated oxidative stress. This manuscript is nicely structured and well written. I have no question about this manuscript.

Response: We were pleased to read this Reviewer's conclusion that "This is an interesting manuscript about the effects of abdominal paracentesis drainage (APD) on severe acute pancreatitis (SAP)-associated cardiac injury", and "This manuscript is nicely structured and well written". Thank you very much for your recognition of our work.

Reviewer #03647305

Excellent work, congrats.

Response: We were delighted to read this Reviewer's positive comment that "Excellent work, congrats", which gives us encouragement and motivation.

Reviewer # 0***2446694***

The authors investigated the preventing effect of abdominal paracentesis drainage (APD) on myocardial injury in severe experimental pancreatitis. They showed that APD can improve cardiac dysfunction including the histopathological changes, increase in cardiac enzyme, hemodynamics, ultrasonographic findings, oxidative stress, apoptosis, pancreatic injury, and inflammation. They concluded that APD treatment can exert cardioprotective effects on severe acute pancreatitis (SAP)-associated cardiac injury. The results of present study seem to be superior, however, there are two major problems.

Response: The Reviewer commented that "The results of present study seem to be superior, however, there are two major problems" and he/she proposed some valuable and constructive issues, which we have addressed in the revised manuscript as outlined below.

Questions:

1. In the present study, there are many statistical significances between SAP group and SAP+APD group (n = 6 in each arm). As for me, I cannot accept these results without any questions. The authors should provide some supplemental information regarding their results.

Answer: We thank the Reviewer for the critique. In our experiment, considering death rate in SAP group and the number of testing indexes we included fifteen rats in each group to guarantee enough samples. Six samples for each index was sufficient statistically. We used SPSS version 18.0 to analyze the data, normally distributed data were compared using a one-way analysis of variance followed by the SNK test for multiple comparisons, and nonparametrically distributed variables were compared by the Mann-Whitney test with Bonferroni corrections. Values of $P < 0.05$ was recognized as statistically significant. The statistical and grouping method were used in our previous experiments (Jing Zhou, et al. Free Radical Biology and Medicine. 2016; Ruo-Hong Liu, et al. World J Gastroenterol. 2018).

2. To my knowledge, I have not experienced any patients with SAP-associated cardiac dysfunction. In addition, APD is not the standard therapy for acute pancreatitis. Thus, the authors should mention the clinical implications in the "Discussion" section.

Answer: We thank the Reviewer for this valuable comment. For the first question, severe acute pancreatitis (SAP) can easily cause pancreatic necrosis and non-pancreas organ injuries, among the multiple organ system dysfunctions in SAP, cardiovascular manifestations are frequent (Yegneswaran B, et al. Journal of

critical care. 2011). Cardiovascular failure occurs in a variable proportion of patients with SAP, may be affected alone or with other organ systems. One prospective study showed that elevated CK-MB level (more than two times normal) was seen in 18 (27.7%) patients and was associated with increased necrosis, higher CTSI, the severity of AP, cardiovascular failure, prolonged hospital stay, LVDD, and mortality (Raghavendra Prasada, et al. Indian J Gastroenterol. 2018). Experimental studies also confirmed that myocardial ultrastructure exhibits disturbances, including intercellular edema, cardiomyocyte hypoxia, apoptosis and hypertrophy (Saulea A, et la. Rom J Physiol. 1997; Shanbhag, S. T, et al. Surgery. 2018; Li, L, et al. Life Sci. 2018). These research results all manifest that SAP can cause cardiac changes. For the second question, at present, the minimally invasive step-up approach has been gradually replaced various kinds of traditional treatments and dominated the main trends in AP therapy. However, this approach may not be optimal and needs further improvement. Especially for early fluid collection in the abdominal or pelvic cavity, there is no explicit conclusion that whether intervention is needed. Address this gap, we pioneered the APD approach to deal with early fluid collection. In our previous clinical study, early APD was found to effectively relieve or control the severity of SAP without increase in infection rate, improve tolerance of enteral nutrition, and reduce intra-abdominal pressure. It was an important development and supplement for the minimally invasive step-up approach with important clinical implications (Liu W, et al. Crit Care Med. 2015; L.Liu, et al. J.Clin. Gastroenterol. 2015; Tao Wang, et al. Pancreas. 2016). As suggested, we have mentioned the clinical implications of APD in the "Discussion" section in the current manuscript. The corresponding revisions are highlighted red in the revised manuscript (Page, Line).

Reviewer # 02951945

This study is very interesting since they report novel findings about cardiac injury. Previous studies demonstrated the effect of APD on systemic inflammatory response and other organ injuries. • All outcomes and parameters were explored extensively to

cover cardiac-related issues. • Methods of experiments were thoroughly described.

Response: We are pleased to read this Reviewer's conclusion that "This study is very interesting since they report novel findings about cardiac injury." The Reviewer also commented that "All outcomes and parameters were explored extensively to cover cardiac-related issues. Methods of experiments were thoroughly described." The Reviewer also made many constructive comments, which we have addressed in the revised manuscript as outlined below.

Questions:

1. Does the abstract summarize and reflect the work described in the manuscript? • Please mention the full word of SACI prior to the acronym

Answer: The abstract summarizes and reflects the work described in the manuscript. The full word of SACI prior to the acronym has been mentioned.

2. Results should not contain the discussion or hypothesis as the sentence "These data suggest that APD treatment can exert ……, which may be a novel mechanism behind the effectiveness of APD on SAP patients.

Answer: The sentence has been deleted as required.

3. The results regarding PAAF should be added

Answer: The results regarding PAAF have been added as required. The corresponding revisions are highlighted red in the section of abstract in the revised manuscript.

4. Key words. Do the key words reflect the focus of the manuscript? • Keywords were not mentioned in the manuscript.

Answer: Sorry for the omission. We have added the key words in the manuscript.

5. The experiment of mild acute pancreatitis (MAP): Why do you use MAP

model in this experiment? Is the objective of this study focused on severe acute pancreatitis?

Answer: We thank the Reviewer for raising such a valuable issue. The objective of this study focused on severe acute pancreatitis definitely. Intra-peritoneal injections of caerulein have been shown to reliably cause mild pancreatitis, and in this model, the pathological changes mainly include edema and inflammation of the pancreas and increases in peritoneal permeability (Noel, P, et al. Gut. 2016). In the second part of our animal experiment, we wanted to determine if PAAF could aggravate mild pancreatitis and affect the expression of NADPH oxidase, so we chose mild acute pancreatitis for this model system. This experiment approach has been used in our previous study (Zhou J, et al. Free Radic Biol Med. 2016).

6. The experiment of mild acute pancreatitis (MAP): Is 'three groups of six" correct? It is unclear what it means. The detail of 6 groups were mentioned in the sentence after this so is it 3 or 6 groups? It was mentioned that there were 36 rats for this experiment.

Answer: We are very sorry the for incorrect description which lead to your misunderstandings. In the second experiment, the total rats were 36, and the rats were randomly divided into 6 groups, six rats in each group. The corresponding revisions are highlighted red in the section of animals and experimental design in the revised manuscript.

7. Please add the significance of each Nox: Nox-2 and -4.

Answer: We have added the the significance of each Nox as required.

8. Please add details of histological scores (referred to Figure 6B)

Answer: Thank you very much for your professional suggestion. The histological scoring of the pancreas was evaluated based on a 0-4 scoring method as described previously (Schmidt J, et al. Ann Surg. 1992. 215:44-56). There are six different variables determining severity of injury, including edema, acinar necrosis,

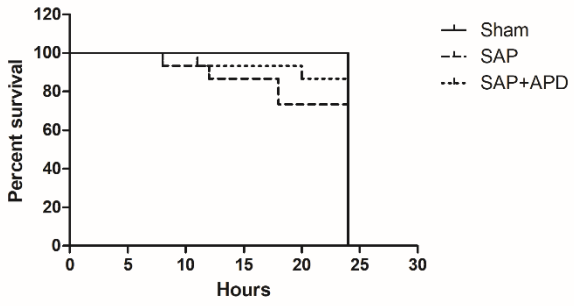
hemorrhage and fat necrosis, inflammation and perivascular infiltrate. This method has been widely used and reported in several literatures (Lei Li, et al. Life Sci. 2018. 202:167-174). The corresponding revisions are highlighted red in the section of histological assessment in the revised manuscript.

9. Can you discuss why LVEDD is not different between SAP and SAP+APD groups

Answer: We thank the Reviewer for raising such an important issue. In the present study, we found the decline of cardiac contractility was prior to the possible change of myocardial compliance, that means the inhibition of myocardial contractility may occur first in this model system. Besides, twenty-four hours after SAP induction is still at the early stage, the decrease of myocardial compliance maybe not enough to cause obvious change in end-diastolic dimension of left ventricular. Thus, we did not observe the change in left ventricular. If we extended the experiment time, changes in ventricular diastolic function may be observed.

10. Do you have the data on death of rats in groups of with and without APD?

Answer: Thank you for this important question and apologize for missing the death data. There were 4 rats died in SAP group within the first 24 hours after SAP induction, the survival rate was 73.3%. While there were 2 rats died in SAP+APD group, the survival rate was 86.7%. The specific situation is shown below.



11. Please add references to the sentences containing "our previous studies".

What are those studies

Answer: We have added the references.

12. Please add strengths and limitations of this study. • What is the future direction?

Answer: Thank you very much for the professional suggestion. In the present study, we revealed the potential mechanism of APD for remote organ function maintenance, and that provided a more abundant theoretical basis for the clinical treatment of APD. In addition, our study has several limitations. Firstly, we gave a certain amount of sterile saline by subcutaneous injection in the back to compensate for anticipated fluid loss instead of monitoring the effective blood volume, therefore we could not neglect the possible hypovolemia which can cause bias to this experiment. Secondly, HMGB1 exert function need its specific membrane receptors, we still do not know HMGB1 how to modulate NADPH oxidase under SAP condition. In the next experiments, we should detect the expression profile of HMGB1 receptor protein in the heart, and utilize a special receptor protein knockout model to clarify the precise mechanisms.

13. Several sentences in Discussion referring to 'our previous studies' need references.

Answer: We have added the references as required.