PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effectiveness of Peritoneal Lavage Fluid Circulating Tumor Cells and Circulating Tumor DNA in the Prediction of Metachronous Peritoneal Metastasis of Gastric Cancer (pT4NxM0/pT1-3N+M0) after Radical Resection: Protocol of a Prospective Single-center Clinical Study
AUTHORS	Bai, Long; Guan, Yujing; Zhang, Yeqian; Gu, Jiayi; Ni, Bo; Zhang, Hao-yu; Aimaiti, Muerzhate; Wang, Shuchang; Yue, Ben; Xia, Xiang; Zhang, Zizhen

VERSION 1 - REVIEW

REVIEWER NAME	Rawicz-Pruszyński, Karol
REVIEWER AFFILIATION	Medical University of Lublin
REVIEWER CONFLICT OF INTEREST	none
DATE REVIEW RETURNED	06-Feb-2024

GENERAL COMMENTS	Thank you for the opportunity to review this interesting study protocol. - My main concern is about exclusion of patients after neoadjuvant treatment in locally advanced setting and inclusion of early GC patients - please address these two clinically important issues. - In the study flowchart there's a description of intraoperative cytological assessment, whereas it's not mentioned in the full-text. I assume pCyt+ patients would be also excluded? - Additionally, language of the manuscript should be revised by a native English speaker

REVIEWER NAME	Sammartino, Paolo
REVIEWER AFFILIATION	Umberto I Policlinico di Roma, Pietro Valdoni
REVIEWER CONFLICT OF INTEREST	No disclosure
DATE REVIEW RETURNED	02-Apr-2024

GENERAL COMMENTS	A limit of this paper is that of not making a selection regarding some prognostic parameters in these patients. For example different histologies are considered together (intestinal vs poorly cohesive) and no mention is made about cases with or without her2/neu

	overexpression or patients dMMR, or suitable for immune checkpoint blockade therapy.

REVIEWER NAME	Brandl, Andreas
REVIEWER AFFILIATION	Heidelberg University
REVIEWER CONFLICT OF INTEREST	none
DATE REVIEW RETURNED	13-Apr-2024

GENERAL COMMENTS	It was a pleasure reading your manuscript named “A Protocol for a Prospective Single-center Clinical Study Based on Peritoneal Lavage Fluid Circulating Tumor Cells and Circulating Tumor DNA in the Prediction of Metachronous Peritoneal Metastasis of Gastric Cancer (pT4NxM0/pT1-3N+M0) after Radical Resection.”, which evaluates the predictive effect of peritoneal lavage fluid CTC and ctDNA on metachronous peritoneal metastasis after gastric cancer. The study addresses an important topic as peritoneal metastasis are a common problem in patients with gastric cancer, and preventive strategies are currently under evaluation in e.g. France and Germany (GASTRICHIP, PREVENT trial) There are a few points, who need to be addressed in order to improve the quality of the manuscript: Major points: 1. Your study selected a mainly radiologic endpoint. Could you please comment on the weakness of this endpoint as radiologic findings can be sometimes misleading? I understand that not all patients might have access for pathological proof of recurrence or metachronous metastasis, but your endpoint has disadvantages. 2. The discussion part should include a section about future outline of your findings. It will be enriching if you could elaborate on the clinical consequences of your findings. What will change for the patient. Are there any therapeutic options to prevent metachronous peritoneal metastasis for identified high risk patients, etc… 3. CTC and ctDNA in patient blood has shown interesting results regarding the recurrence and overall survival of patients with gastric cancer. Have you thought about taking pre- and post-operative blood samples of these patients for comparison? 4. One of the inclusion criteria was pT4. It might increase reproducibility as well as validity if you would choose cT4 as inclusion criteria. There are some therapeutic options you might want to use in the future for patients with high risk, such as IP chemotherapy during the procedure and not after pathological exam, which sometimes arrives 5-7 days postoperative, depending on the country.

VERSION 1 – AUTHOR RESPONSE

Respond to the Reviewer’s Comments:

Reviewer: 1 Dr. Karol Rawicz-Pruszyński, Medical University of Lublin

1. My main concern is about exclusion of patients after neoadjuvant treatment in locally advanced setting and inclusion of early GC patients - please address these two clinically important issues.

Response:

We greatly appreciate your review of our manuscript and the issues you have raised.

This study includes patients with untreated gastric cancer (pT4N0M0/pT1-3N+M0). As you noted, patients with locally advanced disease who have undergone neoadjuvant therapy were indeed part of our exclusion criteria (Table 1 - Exclusion Criteria - Point 2). This study represents our center's first exploration into detecting CTC and ctDNA in peritoneal lavage fluid. And the main objective is assessing their sensitivity and specificity in predicting metachronous peritoneal metastasis after radical resection. Including patients who have undergone neoadjuvant treatment may unpredictably affect the levels of CTC and ctDNA detected, potentially introducing more confounding factors to our study and impacting the analysis of our results.

Regarding your question about including early GC patients, we do have a significant interest in this group. However, considering the current uncertainty of this detection technology's efficacy in predicting peritoneal metastasis, as well as budgetary and manpower constraints. We have prioritized enrolling high-risk patients for peritoneal metastasis, including pT4a and pT4b, as well as those with lymph node metastasis. Upon completing this study, it is highly likely that we will conduct further research detecting CTC and ctDNA in peritoneal lavage fluid to evaluate their predictive performance for early-stage and post-neoadjuvant therapy gastric cancer patients. Even more, further studies may conduct on peritoneal metastasis gastric cancer patients during their therapy to monitor the efficacy of different regimens.

Thank you once again for your valuable feedback.

2. In the study flowchart there's a description of intraoperative cytological assessment, whereas it's not mentioned in the full-text. I assume pCyt+ patients would be also excluded?

Response:

Thank you for pointing out this issue. Indeed, if the cytology results of the peritoneal lavage fluid are positive, the patient does not meet our study inclusion criteria. Point 1 of the exclusion criteria in Table 1 specifies that CY1 patients are excluded. We greatly appreciate your valuable feedback.

3. Additionally, language of the manuscript should be revised by a native English speaker
Response:

We sincerely apologize for the poor writing quality in our initial manuscript .We greatly appreciate your patient review of our work. In this revised version, we have meticulously edited the manuscript and enlisted the assistance of colleagues proficient in written English to enhance its readability. We hope these efforts will make the manuscript more accessible to native English speakers. If you identify any issues, please do not hesitate to inform us. Once again, thank you for your valuable feedback and understanding.

Reviewer: 2 Dr. Paolo Sammartino, Umberto I Policlinico di Roma

1.A limit of this paper is that of not making a selection regarding some prognostic parameters in these patients. For example different histologies are considered together (intestinal vs poorly cohesive) and no mention is made about cases with or without her2/neu overexpression or patients dMMR, or suitable for immune checkpoint blockade therapy.
Response:

Thank you very much for highlighting such an important research design issue. This is crucial for both patient selection and data analysis. Our study includes GC patients classified as pT4NxM0/pT1-3N+M0, who are at high risk for peritoneal metastasis. However, we have included the pathology of adenocarcinoma without distinguishing specific types. This is because we are equally concerned about peritoneal metastasis in both intestinal and diffuse types of gastric cancer. Additionally, given that current research does not suggest that HER2 or MMR status impacts peritoneal metastasis, we have not restricted HER2 and MMR status as criteria for inclusion. However, as you rightly pointed out, these are important stratification factors. Therefore, upon completion of data collection, all data will be stratified and analyzed based on the patients' pathology type, HER2 status, MMR status, and whether they received targeted or immunotherapy. This point is mentioned in the Statistical Analysis section. Thank you for your patient review and valuable feedback on this manuscript.

Reviewer: 3 Dr. Andreas Brandl, Heidelberg University
1. Your study selected a mainly radiologic endpoint. Could you please comment on the weakness of this endpoint as radiologic findings can be sometimes misleading? I understand that not all patients might have access for pathological proof of recurrence or metachronous metastasis, but your endpoint has disadvantages.

Response:

Thank you very much for your correction. It was an oversight on our part to provide an incomplete definition of peritoneal metastasis.

The potential for misdiagnosis due to imaging tests that you mentioned does indeed exist. Some patients exhibit no significant abnormalities on imaging, yet experience weight loss, ascites, and consistently elevated tumor markers.

In terms of criteria for determining peritoneal metastasis, the commonly used clinical imaging test s such as CT, MRI, and positron emission tomography/computed tomography (PET/CT), including [18 F]-FDG and [68Ga]-FAPI-PET/CT. Notably,

[68Ga]-FAPI-PET/CT has higher sensitivity compared to [18F]-FDG PET/CT in detecting peritoneal metastasis in gastric cancer, which can reduce the incidence of misleading results[1]. Therefore, for pe ritoneal metastatic lesions that are inconclusive on CT, MRI, or [18F]-FDG PET/CT, patients are often recommended to undergo [68Ga]-FAPI-PET/CT.

When imaging studies do not reveal significant abnormalities but there is a high clinical suspicion of peritoneal metastasis, invasive procedures may be considered, such as peritoneal puncture or laparoscopic exploration. For patients with ascites, cytological examination of the ascites from a peritoneal puncture may provide a definitive pathological diagnosis. However, as noted in the background section, the detection rate of cytology examination is only 6.25-54.4%. Therefore, for patients with cardiopulmonary function that can tolerate anesthesia, laparoscopic exploration may be a more accurate method. Laparoscopy allows for a direct assessment of the presence of peritoneal metastasis and enables pathological diagnosis through peritoneal biopsy.

Additionally, as you mentioned, not all patients can have access for pathological proof of recurrence or metachronous metastasis. Some patients may be unable to tolerate invasive laparoscopic procedures, may not have ascites, or may have negative results from ascites examinations. In such cases, clinicians can make a clinical diagnosis of peritoneal metastasis based on the patient's symptoms and laboratory findings.

Therefore, we have revised the “**definition of peritoneal metastasis**” in the manuscript to include the following criteria:

(1) In at least one of the CT, MRI, or PET-CT (including [18F]-FDG and [68Ga]-FAPI-PET/CT) imaging tests peritoneal metastasis is identified, and the results of multiple imaging tests are consistent. For patients who can afford it or for whom other imaging tests are inconclusive, [68Ga]-FAPI-PET/CT is preferred.
(2) Peritoneal metastasis is diagnosed when a cytological examination of the ascites obtained from a peritoneal puncture confirms the presence of cancer cells.
(3) For patients who can tolerate general anesthesia, laparoscopic exploration is performed to confirm the presence of peritoneal lesions, and pathological examination of the biopsy confirms peritoneal metastasis.
(4) For patients who cannot tolerate invasive procedures and have negative ascitic fluid examination and/or inconclusive imaging tests, clinicians can diagnose peritoneal metastasis based on the patient's signs, symptoms, and laboratory findings.
(5) The earliest date of peritoneal metastasis detection by the above examinations is considered the "date of peritoneal metastasis."

Thank you once again for your patience in reviewing this manuscript and for raising such important issues.

[1]Zhao L, Pang Y, Luo Z, et al. Role of [68Ga]Ga-DOTA-FAPI-04 PET/CT in the evaluation of peritoneal carcinomatosis and comparison with [18F]-FDG PET/CT[J]. Eur J Nucl Med Mol Imaging. 2021;48(6):1944-1955.

2. The discussion part should include a section about future outline of your findings. It will be enriching if you could elaborate on the clinical consequences of your findings. What will change for the patient. Are there any therapeutic options to prevent metachronous peritoneal metastasis for identified high risk patients, etc…

Response:

Thank you very much for raising this important issue. We deeply apologize for the insufficient consideration given to this part. We have made the following additions to the discussion section.

The main objective of this study was to explore the predictive effect of peritoneal lavage fluid CTC and ctDNA on metachronous peritoneal metastasis after gastric cancer surgery. We anticipate that this study can create an effective tool for targeting peritoneal metastasis in gastric cancer patients. Additionally, considering the current treatment modalities, such as Hyperthermic Intraperitoneal Chemotherapy (HIPEC), Intraperitoneal Chemotherapy (IP), and Neoadjuvant Intraperitoneal and Systemic Chemotherapy (NIPS), which can improve the prognosis of patients with peritoneal metastasis, our center may conduct further research based on this study. This future research will focus on peritoneal chemotherapy guided by peritoneal lavage fluid CTC and ctDNA, as well as patient follow-up studies.

We believe that the maturation of this tool can contribute to the prophylactic use of HIPEC/IP for high-risk gastric cancer patients with peritoneal metastasis, the monitoring of therapeutic efficacy and outcomes in patients with peritoneal metastasis, and serve as a more sensitive diagnostic method for peritoneal metastasis during postoperative follow-up of gastric cancer patients.

Once again, thank you for raising this important issue.

3. CTC and ctDNA in patient blood has shown interesting results regarding the recurrence and overall survival of patients with gastric cancer. Have you thought about taking pre- and post-operative blood samples of these patients for comparison?

Response:

Thank you for your question.

Current research indicates a strong correlation between circulating tumor cells (CTC) and circulating tumor DNA (ctDNA) in the blood of gastric cancer patients and their prognosis. During the design phase of this study, we also considered that blood CTC and ctDNA could serve as supplementary prognostic indicators, potentially being more closely associated with hematogenous and lymphatic metastasis. However, due to budgetary constraints, manpower limitations, and the primary objectives of the study, our current focus is on the feasibility and predictive efficacy of using peritoneal lavage fluid as a liquid biopsy sample for predicting peritoneal metastasis. If this study yields preliminary positive results, our center may undertake a prospective study involving both peritoneal lavage fluid and blood, encompassing a broader range of gastric cancer stages.

Thank you once again for your question.

4. One of the inclusion criteria was pT4. It might increase reproducibility as well as validity if you would choose cT4 as inclusion criteria. There are some therapeutic options you might want to use in the future for patients with high risk, such as IP chemotherapy during the procedure and not after pathological exam, which sometimes arrives 5-7 days postoperative, depending on the country.

Response:

Thank you for your suggestion.

We have considered this issue from the following perspectives. Firstly, the inclusion of the pT4 population in the study design is based on the fact that patients with pathological stages T4a/T4b are at high risk for peritoneal metastasis. Selecting this subset of patients is likely the most indicative for demonstrating the predictive value of CTC and ctDNA in peritoneal lavage fluid for peritoneal

metastasis, which is the primary concern of this study. Additionally, in China, intravenous chemotherapy and intraperitoneal perfusion therapy are typically conducted 3-4 weeks post-surgery, depending on the individual recovery of the patient. Currently, hyperthermic intraperitoneal chemotherapy performed concurrently with surgery is not widely practiced at our center. Therefore, during the study design, we considered that the pathological results would be formally reported within one week post-surgery, which coincides with the patients' postoperative recovery and does not affect their treatment decisions. Including cT4 patients would also entail additional financial and manpower resources. Following preliminary positive results from this study, our center may continue to conduct research involving peritoneal lavage fluid in a broader range of gastric cancer stages.

Thank you for raising this important issue.

VERSION 2 - REVIEW

REVIEWER NAME	Rawicz-Pruszyński, Karol
REVIEWER AFFILIATION	Medical University of Lublin
REVIEWER CONFLICT OF INTEREST	none
DATE REVIEW RETURNED	28-Jul-2024

GENERAL COMMENTS	Authors have addressed numerous questions from several Reviewers, strengthening the quality of the manuscript.