

Jan 09, 2018

Dr. Li-Jun Cui

Science Editor, *World Journal of Gastrointestinal Oncology*

Dear Cui,

I am pleased to submit our revised manuscript (37307) entitled “**A case of pancreatic metastasis from colon cancer in which cell block using the Trefle® endoscopic scraper enables differential diagnosis from pancreatic cancer**” for reconsideration for publication in *World Journal of Gastrointestinal Oncology*.

The reviewers' comments were helpful and gave us a better perspective on our work. In accordance with your recommendation, we thoroughly addressed all the comments of the reviewers and modified the manuscript accordingly.

Responses to the comments of Reviewer 1

There have been probably few reports dealing with the combination of a scraper Trefle and cellblock method for histocytological diagnosis of malignant biliary strictures. Therefore, this is an interesting report. However, there are several points, which should be revised.

Major points:

- Pancreatic metastasis from rectal carcinoma is very rare. It seems to be difficult to make a definitive diagnosis in this case. There is a possibility of primary bile duct/pancreas carcinoma rather than pancreatic metastasis from the colon.

1) The biliary stricture by ERC (Figure 1C) looks like a stricture caused by a primary bile duct carcinoma rather than one by a pancreatic carcinoma (primary or metastatic).

We agree this point. At first, we also considered that the biliary stricture caused by a primary bile duct carcinoma during performing ERC as reviewer pointed out. However, Contrast-enhanced CT revealed a 1.5 cm mass associated with upstream main pancreatic duct dilatation. These findings is different from those of typical primary bile duct carcinoma. Furthermore, final histological diagnosis using immunohistochemical staining was pancreatic metastasis from colon cancer. We speculate that metastatic tumor invade into the bile duct. ERC finding look like the occupying lesion inside the bile duct.

2) Noda Y, et al. [Dig Endosc 25: 444–452, 2013] reported that the sensitivity of cell block method for bile duct cancer (80%) was much better than that for pancreatic head cancer (20%). Therefore it is probably difficult to obtain cancer tissue/cells from the metastatic pancreatic tumor unless tumor exposure to the bile duct exists even if using a scraper Trefle.

We completely agree the reviewer's opinion that it is difficult to obtain tumor tissue/cells unless tumor exposure inside the bile duct. In this case, as described above, metastatic tumor may destroy the biliary epithelium and invade into the bile duct, hence we considered that we could obtain tumor tissue/cells using endoscopic device. In our retrospective study (Journal of Digestive Diseases 2016; 17; 44–51), the sensitivity of conventional brush cytology is 30% in pancreatic cancer. However, a scraper Trefle® has higher chance of obtainment for tumor tissue/cells with consideration of device shape than conventional brush cytology even if tumor small invasion.

3) As Chu et al. [7] reported 62% of cases of pancreatic carcinoma, 43% of cases of cholangiocarcinoma and 5% of cases of colorectal adenocarcinoma were CK7+/CK20+ by immunostaining. On the other hand, Werling RW [Am J Surg Pathol. 2003; 27: 303-10] reported that immunohistochemistry showed expression of CDX-2 in 32% of pancreatic adenocarcinomas, 25% of cholangiocarcinomas (bile duct carcinoma/gallbladder carcinoma) and 99% of colonic adenocarcinomas. In the present case, the results of immunostaining for cell block specimens (CK7+/CK20+/CDX-2+) seem to be compatible to primary pancreatobiliary carcinoma rather than metastatic carcinoma from the colon.

Thank you for nice suggestions. We also considered that immunohistochemical findings of the present case are not typical pattern of colon cancer especially in CK7 immunostaining as reviewer pointed out. However, immunohistochemical stainings of both cell block specimen and previous resected specimen were focally positive, and mostly negative for CK 7 as reviewer kindly indicated as those of the typical colon cancer from previous studies. We described „focal positive' because a small portion of both cell block specimen and previous resected specimen showed positive. Therefore, these findings of cell block specimen that were completely consistent with those of previous resected specimen including CK 7 focal positive indicated metastatic carcinoma from the colon origin rather than pancreatobiliary carcinoma.

4) The HE image of the cell block specimen shows well differentiated adenocarcinoma

while that of the rectal resection specimen shows moderately differentiated adenocarcinoma. Usually metastatic carcinomas show less differentiated features than their primary tumor.

We appreciate their valuable insights and suggestions, and agree that metastatic carcinomas usually show less differentiated features than their primary tumor. Actually, most lesion of cell block specimen showed moderately differentiated adenocarcinoma as described in the main text. However, we provided the image of the lesion indicated „CK 7 focal positive’ in Figure 2, resulting in confusing as the reviewer pointed out.

Minor points:

- The report by Noda Y, et al. " Prospective randomized controlled study comparing cellblock method and conventional smear method for bile cytology. Dig Endosc 25: 444–452, 2013" should be cited.

Thank you for your nice suggestion. We have cited this article in Introduction section.

- Discussion: You should mention the reason that you selected the Trefle device rather than biopsy forceps. Looking at the location of the biliary stricture (Figure 1C) it is probably not so difficult to perform biopsy (under fluoroscopic or endoscopic guidance). Sometimes specimens obtained from the biliary stricture using biopsy forceps show important findings such as “invasive cancer clusters under nonneoplastic biliary epithelium”that suggests invasive carcinoma from the outside of the bile duct. Can you show such findings in the specimen obtained by the Trefle device?

We completely agree that we should perform forceps biopsy to differentiate pancreatic cancer from metastatic cancer because we can get larger specimen than Trefle device. We can observe important findings such as “invasive cancer clusters under non-neoplastic biliary epithelium” by larger samples. In the present case, we prioritized shorter procedure time because the condition of patient was not so good and the main purpose of ERCP is to insert SEMS for the treatment of obstructive jaundice. Therefore, we selected Trefle device before SEMS insertion in the present case because we do not need to consume procedure time compared to biopsy forceps. We fortunately diagnosed the present case the metastasis from colon cancer using Trefle with immunostaining. However we could not observe the finding above mentioned by the Trefle device. We should have performed forceps biopsy if the patient’s condition was good and the main purpose of ERC was to differentiate primary pancreatic cancer from metastatic cancer.

- It is recommended that you cite the reports “Yasuda I, et al. Diagnostic value of

transpapillary biopsy using double lumen introducer for determination of mucosal extent in extrahepatic bile duct cancer. Dig Endosc 15: 200–206, 2003”, “Noda Y, et al. Intraductal ultrasonography before biliary drainage and transpapillary biopsy in assessment of the longitudinal extent of bile duct cancer. Dig Endosc 20: 73–78, 2008” and “Werling RW, et al. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. Am J Surg Pathol. 2003; 27: 303-10.

According to the reviewer's recommendation, we cited these article in Introduction or Discussion section.

Responses to the comments of Reviewer 2

The Authors describe a case of pancreatic metastasis from colon cancer in which the cell block technique, together with immunohistochemistry, enabled differential diagnosis from pancreatic cancer. The combination of the cell block technique and the Trefle® device shows promise for diagnosis of biliary strictures as it is as easy as conventional brush cytology. Generally speaking, in my opinion the paper is clear, well and concisely written, with well done procedures. In order to solve the diagnostic bias regarding primary or metastatic pancreatic masses, the application of cell-block related to EUS-FNAC appears to be the best practice. Furthermore, at least in a small subset of neoplasms in which cytological diagnosis may be hard to achieve, due to the presence of extensive tumor necrosis, associated inflammation, limited sampling and, mainly, contamination by intestinal epithelial cells, the cell-block technique should be emphasized and more largely applied, furnishing the opportunity to do immunohistochemical procedures, as elsewhere reported (Ieni et al, Cell-block procedure in endoscopic ultrasound guided fine needle aspiration of gastrointestinal solid neoplastic lesions World J Gastrointest Endosc 2015; Ieni et al, Diagnostic relevance of cell block procedure in secondary tumors of the pancreas,). Therefore, I suggest that Authors may introduce and discuss some considerations about these latter previous references. In addition English grammar and style should be improved.

Thank you very much for your nice comments. We cited 2 articles by Ieni et al. which reviewer recommended and described in Discussion section. The English has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: <http://www.textcheck.com/certificate/download/NnoH93>

We thank the reviewers for their helpful and insightful comments. As a result of addressing these comments, our study has significantly improved. We hope that our manuscript is now acceptable for publication in *World Journal of Gastrointestinal Oncology*.

Yours sincerely,

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