Author's response to reviews

Title: The added value of using mutational profiling in addition to cytology in diagnosing aggressive pancreaticobiliary disease: review of clinical cases at a single center

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Author's response to reviews: see over
Dear Dr Phillips, Dr Matthaei and Dr Gupta,

On behalf of my co-authors, I would like to thank you for your review of our manuscript *The added value of using mutational profiling in addition to cytology in diagnosing aggressive pancreaticobiliary disease: review of clinical cases at a single center*, and the invitation to submit a revised manuscript.

We have considered the reviewers’ comments and have addressed each one in point-by-point responses below, which identify the changes made in the revised manuscript.

We believe that the revised manuscript is improved with the changes made and additional information included, and we hope that you will now find it acceptable for publication in *BMC Gastroenterology*.

We will be pleased to address any additional questions that may arise, and look forward to your decision.

Sincerely yours,

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RESPONSES TO REVIEWERS’ COMMENTS

Reviewer 1

In their Article “The added value of using mutational profiling in addition to cytology in diagnosing pancreaticobiliary malignancy: review of clinical cases at a single center” Malhotra et al analyzed the value of mutational analyses (MP=mutational profiling) of DNA from microdissected cytology slides and supernatant specimens from biliary brushings in order to predict histology/malignancy. The authors used an established panel of DNA alterations in their analyses and found that these assays are capable of providing additional information regarding the presence of cancer. The authors concluded that MP is particularly useful in patients that were hard to diagnose by cytology alone.

In the past years large scale DNA profiling (such as next generation sequencing) has revealed many cancer specific genetic alterations. Malhotra et al demonstrate an important and elegant translational application of this genetic knowledge and a very promising approach. Cytology with its known limited sensitivity and specificity can obviously be supported by genetic testing. Even if the authors show only a small series the translational approach is intriguing. The manuscript is well written and altogether I think very suitable for publication in BMC Gastroenterology.

Major points

1. The paper is missing some important studies in the field. For example, when investigating the use of pancreaticobiliary cancer specific DNA alterations comprehensive DNA profiling publications such as Jiao et al. Nat Gen 2013 (intrahepatic CCC) and Jones et al. Science 2008 (PDAC) should be cited.

Response: We agree with the reviewer and have, thus, included the Jiao et al. Nat Gen 2013 reference (line 23) and Jones et al. Science 2008 reference (line 23 & line 230) within the background and discussion sections given their relevance to the study.

Minor points

1. Line 41: It should be “than” instead of “that”

Response: The typo has been corrected (line 43).

2. Line 65: The long sentence “Patient outcomes…” should be rewritten. Outcomes should not be categorized as malignant etc. (rather histologies). Also: Please refer to the classification of tumors in the methods. Which lesions are being investigated (e.g. pancreatic ductal adenocarcinoma, ampullary adenocarcinomas, cholangiocarcinome) and according to which reference (WHO blue book)?

Response: This paragraph (lines 70-76) in the methods section has been rewritten to more clearly define the patient outcome categories used in this study. The disease outcomes have also now been changed from three categories (“Benign”, “Stable”, “Malignant”) to two categories (“Non-aggressive, “Aggressive”) in an attempt to meet the reviewer’s expectations. This paragraph now also includes further detail on the types of lesions being investigated. Since the majority of the patients (20/30) did not have outcomes related to surgical pathology results, we
could not use histological language in designating their disease outcomes. However, the final pathologic diagnoses in terms of lesion type for the 10/30 patients who had surgical pathology results were added in lines 162-164 within the results section per the reviewer's request.

Reviewer 2

In this study, Drs Malhotra and her colleagues present data supporting the increased diagnostic value of mutational profiling in the study of pancreatobiliary malignancies by cytology. They analyzed about two dozen cytologic specimens and used later dissection and supernatant samples for mutational studies.

Major points

1. The question posed is well defined and merit investigations. However, in my opinion, the sample size, locations and size of lesions, whether solid or cystic, number of cases studied by dissection and supernatant analysis, final pathologic diagnoses and reporting terminology leave much to be desired; there are either poorly or not documented in the present manuscript.

Response: Our goal was to examine the mutational analysis of pancreatobiliary masses that we have seen in our clinical practice upon cross-sectional imaging. We wanted to correlate mutational analysis results with patient outcomes so that we could determine the added value of mutational analysis to that of standard clinical assessments, in particular cytology, at our institution. The study was designed to closely resemble the real-life process of diagnosing malignancy in pancreatobiliary masses. Since in practice we do not know the surgical pathological classification when diagnosing malignancy and making subsequent surgery or surveillance decisions, patients who had both surgical and other outcome determinants of aggressive or non-aggressive disease (other than surgical pathology) were included in the study.

All outcome determinates as well as lesion descriptions have been further clarified in the methods section (lines 70-76) and in the results section, for those who had surgical pathology (lines 162-164), in an attempt to satisfy the reviewer comments. Disease outcomes have also now been changed from three categories (“Benign”, “Stable”, “Malignant”) to two categories (“Non-aggressive, “Aggressive”) in an attempt to meet the reviewer’s concerns regarding terminology.

The number of cases studied by microdissection and by supernatant analysis can be found in lines 185-188.

2. What were the cases that had positive mutational profile and were negative by cytology; their location, size, nature of morphologic changes and final diagnoses should be spelled out. Similarly, with is indeterminate diagnosis, it appears similar to unsatisfactory, and should be clarified. These concerns lead me to question the validity of results.
Response: The number of cases that had positive mutational profiling and were negative by cytology can be found in lines 198-200 and in Table 3.

All outcome determinates as well as lesion descriptions have been further clarified in the methods section (lines 70-76) and in the results section, for those who had surgical pathology (lines 162-164), in an attempt to satisfy the reviewer comments. Disease outcomes have also now been changed from three categories (“Benign”, “Stable”, “Malignant”) to two categories (“Non-aggressive”, “Aggressive”) in an attempt to meet the reviewer’s concerns.

The definition of “indeterminate” cytology diagnosis and other cytological diagnostic categories have been clarified in the methods section lines 84-93, per the reviewer’s comment. These categories were defined based on the pathology language found/used in the cytology reports, as indicated in this section.

Minor points
1. Ten months is not one year (L57,58).
Response: This inconsistency has been corrected (line 60).

2. How was aggressive disease determined? (L58,59).
The definition of “Aggressive” disease is now outlined in the methods section (lines 70-73).

3. What is independent cytology (L64).
Response: The typo has been corrected. It should read ‘indeterminate’ (line 66).

4. Benign or stable, please define the criteria used (L66).
Response: Disease outcomes have now been changed from three categories (“Benign”, “Stable”, “Malignant”) to two categories (“Non-aggressive”, “Aggressive”). The definition of patient outcome categories has been more clearly defined in the methods section (lines 70-76) and corresponding language has been changed throughout the manuscript.

5. Why was surgery performed on the benign cases? (L66)
Response: None of the 10/30 patients who had surgery had a “benign” outcome (now designated as “non-aggressive” outcome). We hope that this misinterpretation has been clarified by the revised and more detailed outcome definitions and terminology in lines 73-76 of the methods.

6. Which case had false positive cytology? (L226).
Response: This was a typo and has been corrected. It now reads ‘false negative’ cytology (line 244).

7. How would you handle the Mutational data (242-244) in the patient management?

Response: Lines 246-250 have been revised to more clearly demonstrate the role that mutational profiling can play in patient management. It now reads: “…mutational profiling has the potential to improve diagnostic sensitivity for aggressive disease without compromise to specificity when used as a supplement to first line cytology testing. Such characteristics are of particular utility in cases of low or non-diagnostic cellularity and uncertain cytology diagnoses (i.e. negative, atypical, indeterminate).”