Reviewer's report

Title: Validation of intraluminal and intraperitoneal microdialysis in ischemic small intestine - Clinical feasibility of equilibration dialysis.

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Reviewer: Norbert Cibicek

Reviewer's report:

• Major Compulsory Revisions

1, I disagree with the use of some words. For example „ex vivo“ may be defined as „out of the living“. The organ must be completely removed from the body and exist in an artificial medium during the testing procedure. The authors declare that they used an ex vivo model, but in fact what they did was a determination of metabolites in microdialysates obtained from ischaemic gut BEFORE it was removed out of the body (pg. 8). This means we are dealing in fact with sampling performed in the living system, or „in vivo“. If I just do not understand the description of the procedure given by the authors (at different places in the manuscript) and the resecate was placed in the water bath, where the dialysate was collected, there is no place for „intraperitoneal“ approach – since this must take place in the peritoneal cavity and not in a sterile cup.

2, Concerning the intraperitoneal position of a catheter, it was defined by Sommer and Larsen differently (see Fig. 1 in the reference No 9. in the manuscript, pg. 17). This position requires free placement of the probe in the peritoneal cavity, not its direct and tight association with the serosa of the gut with limited access to the peritoneal fluid (what is described in the present manuscript). Therefore, the latter should be termed „extramural“ position instead.

3, Some statements are false – e.g. the third one in the Background section of the manuscript. Intestinal luminal application of microdialysis has already been validated by other authors, at least in the large gut in terms of detection of ischaemic events.

4, What is the „gut hypothesis“ (pg. 4 in the middle)? Such word connections need to be clearly defined if they are to be used.

5, Glycerol can by no means be considered a „specific marker of the damage of the gut epithelia“, as stated in the manuscript. There is a number of different sources of gut glycerol including dietary triacylglycerols, glycerol enema or infused lipids. Moreover, various events may contribute to gut glycerol besides ischaemia. Some of these factors may be found in the latter parts of the manuscript (pg. 12).

6, If the authors used CMA 600 analyzer, they must have been able to measure urea as well. Microdialysate urea (added to the perfusion fluid) has recently been
validated for blood perfusion estimation in a number of studies, none of which have been cited by the authors. This marker would bring important additional information and enable a more correct interpretation of the results of metabolic markers. Other validated markers of blood perfusion (such as ethanol) have not been mentioned in the background.

7. Very importantly, the collection of fractions is wrongly described. For example, why did the authors collect 10-min fractions and then presented fractions collected in 30-min intervals? My own experience with gut ischaemia and CMA 600 analyzer tell me that there is a huge problem with the analysis of frozen samples. After thawing, bubbles are formed in the samples and if there is only 10 microlitres, it is highly probable that the analyzer takes insufficient sample volume (small dead volume) resulting in very low concentration of the analyte(s). If the sequence of analyte is always the same, the last in the row suffers the most. It is likely that, by default, the last analyte was pyruvate (I experienced this in my own experiments). If this was the case, the authors may have a plausible explanation for their rather unexpected and inconsistent results. For instance, high LP ratio may not be only a result of high lactate, but also very low pyruvate (the colorimetric principle of the measurement allows the release of very low results even if there is almost no sample present in the reaction mixture). Because it would be very difficult (almost impossible) to repeat the analyses from only 10 microlitres, the authors would have no possibility to check whether the sample dilution problem occurred. There are more data indicating high probability of the existence of this problem – very high variability of the results even during baseline conditions, discrepancy between LP and LG ratios and unexpectedly high baseline values. None of these have been satisfactorily addressed by the authors in the discussion. By the way, was this problem the reason why the authors finally decided to put the samples together and analyze them en bloc (in 30-min intervals instead of 10-min intervals)? Or did they publish the means of three serial values?

8. As to the number of patients and their description, different parts of the manuscript offer different figures (11, then 21 and finally 20) that are confusing for a reader. The data (age of the group) must correspond to the group’s homogeneity, e.g. if there are men and women (two subgroups), two figures should be provided.

9. The results of sensitivity and specificity cannot be compared when there is no identical reference. As a reference, 95 % specificity is usually selected. So the authors should present the values for sensitivity at 95 % specificity, otherwise “apples are compared with pears”.

10. Levels of glycerol cannot be put as 9 (median) and 34 – 37 (interquartile range).

11. The statistical analysis is insuffciently described. If the ROC analysis is performed, there must be a reference (healthy) group / data. These were obviously the baseline values (however questionable due to very high variability) – this group must be specified, even though no “sham-operated group” or
“control catheter location” was tested.

12, I can see no reason why the authors selected 68 as the threshold for intraluminal LP ratio, if the value 109 has better characteristics in terms of sensitivity and specificity. Is it because it is closer to 20, which was defined in a number of other studies and confirmed by our group (Cibicek N et al. Physiol Meas 2010)?

13, The discussion in general is very weak – the authors were unable to acceptably discuss the metabolic parameters, especially the discrepancies between the findings of other groups and their data and discrepancies between different locations (e.g. why the thresholds differ so much between the intraluminal and “intraperitoneal” markers and why there is so high variability?)

Regarding the equilibration dialysis, the LP and LG ratios (including glycerol) cannot be equal to 0. It means lactate (and glycerol) must have been equal to 0, which was not documented in the previous findings (even in the same manuscript).

14, NJ feeding tube with equilibration dialysis could be considered a feasible method for clinical purposes provided the results were adequately discussed. The authors, however, failed to do this.

15, The placement of the probes is not fully delineated. How was the intraluminal probe implanted? Was there a puncture and/or suture?

16, If there was so great effect of ischaemia on other markers, why glycerol was unaffected (in the i.p. catheter)?

17, The discussion of LG ratio includes results of glucose obtained by other groups. However, no information on the perfusion rates (that determine the recoveries of probes) is provided for the reader, so the given values are of no use.

The reference No. 22 cannot be used at the beginning of the page 14. The cited paper did not study the transient increase in glucose concentration - it has only been speculated by Deeba et al. in an attempt to account for their unexpected results. The authors cite Deeba et al. here without knowing what they really meant by additional glucose delivery etc.

• Minor Essential Revisions

18, The article contains numerous English grammatical errors. They are most often of minor importance (US English vs. British English spelling, spelling in general, wrong use of articles, missing articles, wrong use of prepositions, frequently repeated words and/or their incorrect use – e.g. to depict), sometimes, however, the reader gets confused by long and incomprehensible sentences (especially the last sentence on the pg. 6). Other sentences and explanations (such as peritoneal cavity) are redundant or repeated more times in the paragraphs (such as the absence of a clinical report on the page 4 and 5 or the major results on the pg. 11). Word connections such as „serosal metabolic
signalling“ on the page 4 are used in a wrong manner – the word „signalling“ means something completely different. The same sentence must have a reference – how do the authors know that gut motility influences the (recovery of) metabolic markers? English has to be reviewed by a professional. The authors must decide if they are to use a „Whipple procedure“ or "Whipple´s procedure". Specificity must be changed for specificity in the figures and tables.

19. There is a number of errors in the References as well. I have identified at least five (e.g. intraperitioneal, Apperent, reable ...).

20. The key words were not selected properly. ROC analysis should not be included as a key word, there was no glucose to pyruvate ratio (as stated in the key words) but lactate to glucose ratio studied by the group.

21. Table 1 contains numbers of patients with different measurements. Why there were only 12 (13) patients with lactate (pH) measurements and 20 patients with the measurement of saturation? Is it due to technical reasons (inability to collect the sample) or was the protocol changed in time?

22. Finally, due to the number of grammatical mistakes, I do not believe that all (seven!) authors read the manuscript. In such case at least some of them must have identified the mistakes.

• Discretionary Revisions

None.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.