Author Response 1

Dear Editor,

We thank the both reviewers for their careful study of our manuscript and for their useful and thoughtful remarks. We are confident that filling of our paper (red writing) in accordance with their recommendation will improve its value.

Reviewer 1

Comments to the Author

The authors describe the metabolism mechanism of neutrophil predominant pleural effusions using coefficient of energy balance(lactate and glucose). This was compared to Light's criteria with a new criteria for exudate (glucose, lactate, LDH, tProt). Neutrophilic effusions had a lower KEB as well as a higher LDH and neutrophil %. However the authors do not demonstrate that KEB is any different than the conventional exudate criteria or simply a cell count/differential. This especially matters since a purulent complication of surgery has a surprisingly normal KEB compared to chest infections. Post surgery with purulent complication would warrant the use of antibiotics so it is best if there is a stronger discriminator. It would be useful to chart an ROC curve with KEB, LDH and glucose so see which has the best AUC.

It is also good to have more physiologic information about pleural effusions and I believe the study would be strengthened with more robust statistical analysis (treat this as analysis of a new diagnostic tool).

Our response to Reviewer 1:

The cytological-energy analysis of pleural effusions compared with Light's criteria presents different views on detection of the pathological processes in the pleural cavity. Light's criteria distinguish between transudative and exudative effusions using the current values of few particular parameters. The cytological-energy analysis observes the local metabolic presentation of immunity system as the reaction to changes in the pleural cavity. Therefore it is not easy to compare both views only by using of particular parameters. Despite this fact we have constructed requested ROC curves with KEB, LDH and glucose, calculated their AUC and added them in the manuscript (see "Material and Methods", "Results" and "Discussion").

We also added some basic information about the pleural effusion origin (see "Introduction").

Reviewer 2

Comments to the Author

TO THE AUTHORS

The aim of the study was to determine whether separation of the aetiology of pleural effusions containing neutrophils was possible using cytological-energy analysis, and thereby early identification of purulent complications following surgery.

Our response to Reviewer 2:

MAJOR COMMENTS

- How are the sensitivity, specificity and diagnostic efficiency of Lights criteria calculated if the diagnosis is made using those same criteria?

Every final diagnosis was made using the clinical condition of patients, the microbial investigation, an assessment of the response to antibiotic therapy and the RDG imaging. The cytological-energy investigation of the pleural effusions was only one part of complex diagnostics procedures.

- There is no specific description as to how patients were recruited to the study – were these 635 samples randomly or consecutively selected?

This is the retrospective study and our patients were selected randomly. This information is added into "Material and Methods".

- No timeframe is specified in relation to the collection of pleural fluid specimens, in particular when post operatively the pleural fluid samples were collected and whether the patient had been diagnosed with a purulent effusion at this point, or even on this sample and how this diagnosis was made.

Every specimen was collected during the first nine days after operation. This information is added into "Material and Methods". The purpose was to specify an immunity response after chest operation. Our findings were on the scale from slight reaction to very intensive purulent inflammation.

- The results section states that 'Significantly lower frequency of neutrophils was found in the pleural effusions of patients without purulent inflammation after chest surgery'. Where does this appear in the statistical analysis.

Frequencies of neutrophils in the pleural effusions of patients after chest surgery with purulent complication, purulent pneumonia and chest empyema which were analyzed by ANOVA Kruskal-Wallis test are not significantly different. They simultaneously represent the group "C" in Table 1. Frequencies of neutrophils in pleural effusions of patients after chest surgery without purulent complication were compared with patients of "group C" using the same statistical test. We found statistical lower frequencies of neutrophils in these patients ($p < 0.05$) and we present them as group "B" in Table 1. –

In the results section the authors state that 'We found the high diagnostic efficiency of high glucose concentrations and low lactate and LDH catalytic activity concentrations in patients with transudative pleural effusions.' This is not a novel finding.

Yes, I agree with your opinion. Our goal was to highlight even better results of the KEB contrary to very good results of glucose, lactate and LDH.

- In the discussion it is stated that there was a confirmed correlation between KEB values and concentrations of LDH and AST in pleural effusions – what is the benefit then of doing the KEB over and above these investigations?

Correlation between KEB values and LDH and AST catalytic activities in the laboratory pattern of pleural effusions shows the direct relationship between intensity of the local inflammatory response and seriousness of tissue injury in the pleural cavity. Simultaneous assessment of all these parameters allows us to disclose the local damage effect of intensive inflammation. It also allows us to monitor the development of local inflammation as a reaction to the primary tissue injury. Both these views considerably improve information about pathological changes in the pleural cavity.

- Would combining KEB, LDH and AST results improve the diagnostic efficiency?

See above.

- What is the cost and time entailed in performing the KEB? What is the reproducibility?

The measurement of glucose and lactate for calculation of KEB is inexpensive (under 1 USD) and takes only a few minutes. In general the reproducibility is very high as certified precise enzymatic methods are used (see in "Material and Methods").

- It would be preferable for a validation cohort of patient samples be analyzed to assess the ability of the KEB results to group the patients accurately according to results.

We accept your recommendation very seriously. But our selected group of pleural effusions with predominance of neutrophils is not suitable for a cohort validation. Our purpose is to make its assessment by using of more specimens without regard to their cell composition and to present it in the independent study. Our current research is based on our studies which results have been published recently (see References 22-25).

- There is no discussion as to the clinical relevance of the findings.

We have added discussion to the manuscript (see "Discussion").

MINOR COMMENTS

- Page 3, line 28 – 'phagocyte' should likely be 'phagocytose'

- Page 3, line 30 – 'we can find them' should likely read 'they can be found'

- Page 10, line 19 – 'excellent' should likely be changed

We have corrected all text in accordance with recommendation.

Thank you very much.

Yours Sincerely,

Petr Kelbich et al.