Author's response to reviews

Title: Evaluation of lipase levels in patients with nephropathia epidemica - no evidence for acute pancreatitis

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Author's response to reviews: see over
Dear Prof. Dobler,

Please find enclosed the revised manuscript entitled “Evaluation of lipase levels in patients with nephropathia epidemica - no evidence for acute pancreatitis”.

The resubmission includes a detailed, point-by-point response to each of the reviewers’ comments.

The authors are very grateful for the reviewers’ comments, which improved the manuscript significantly.

We hope you will find our paper now worthy for publication in “BMC Infectious Diseases”.

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Sincerely yours

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RESPONSE TO REVIEWER

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„Evaluation of lipase levels in patients with nephropathia epidemica - no evidence for acute pancreatitis“

Editorial requests to the Authors:

1. Please name all of the authors who contributed to the interpretation of the data and the writing of the paper

As stated in the contributions section: Latus J, Braun N and Dominik Alscher M conceived the study; Latus J, Braun N, Kitterer D, Segerer S and Artunc F analyzed the data; all authors contributed to the interpretation of the data and the writing of the paper; Latus J, Braun N, Segerer S, Kitterer D, Dominik Alscher M and Artunc F were involved in the drafting of the manuscript; and all authors contributed to the final report.

2. Copyediting

Prior to submission of the manuscript “Charlesworth publishing Services Ltd” performed a complete language editing.
Reviewer 1

Dear Referee,

thank you for the time invested in our manuscript. We feel that your comments helped us to significantly improve the manuscript. We have addressed your comments in the revised manuscript as follows.

1. Abstract: Background is not a background but seems an objective. P value is not written with a capital p

The abstract was modified according to your suggestions.

“Background: The most common causative agent for hemorrhagic fever with renal syndrome in Germany is Puumala virus (PUUV) and a high percentage of patients with PUUV infection have gastrointestinal (GI) symptoms. The aim of the present study was to determine the prevalence of increased lipase levels and acute pancreatitis during nephropathia epidemica (NE) in 166 patients from Germany.” (Page 2, first paragraph)

2. Title: In my opinion the title is misleading and incorrect. Actually it may even cause confusion among people who only screen title and abstract. A title must represent the data in the article. I would suggest something in line with Evaluation of lipase levels in acute hantavirus patients in Germany, no evidence for acute pancreatitis. Also hantavirus is without a capital as is nephropathia epidemica.

Thanks a lot for this well taken comment. Following your suggestions, the title of the manuscript was changed to “Evaluation of lipase levels in patients with
nephropathia epidemica - no evidence for acute pancreatitis”. The authors modified the manuscript accordingly. (Page 1)

3. Background: The beginning paragraph of the background is not of proper scientific quality. It actually states things that are not true and reads not easily.

Frist two sentences do not read well and are actually not correct. HCPS is indeed caused by new world hantaviruses but these do not only circulate in the USA as a matter of fact most infections in recent years are due to Andes virus infection in South America. Furthermore only pathogenic hantaviruses cause disease which currently are 23 out of the 43 known hantaviruses. I would suggest to rewrite to:

Pathogenic hantaviruses are able to cause disease in humans and after inhalation of virus containing aerosols to known syndromes may arise: haemorrhagic fever with renal syndrome (HFRS), with the milder subtype nephropathia epidemica (NE) and the hantavirus cardiopulmonary syndrome (HCPS). HFRS is endemic in large parts of Eastern and Northern Europe and Asia, while small clustered outbreaks of HCPS have been reported in North and South America” I think the current references can be used to back up this introduction. I am quite sure that a reference from 1989 doesn’t hold through for what is known today about hantavirus epidemiology.

The Background section was modified according to your suggestions including removal of the “old” citation (thank you for the kind help with the text, Page 3).

Thanks a lot for this well taken comment. The case fatality rates were changed according to your suggestions, please see the revised text.

“HFRS is endemic in large parts of Eastern and Northern Europe and Asia, whereas clustered outbreaks of HCPS (with case fatality rates up to 60%)”, page 3

5. “Along with norovirus, rotavirus, influenza and 77 hepatitis C, hantavirus infections are associated with the five most common viral diseases [5]”

This is wrong in so many ways. What do the authors want to say? I really hope this statement if about Germany but even that I doubt. Now it reads like it is about the world. How about HIV, dengue and hep a and B????

Thanks a lot for this well taken comment. The statement was a direct citation from the data presented in the cited manuscript (about the situation in Germany). Due to your comment the sentence was removed from the manuscript.

This sentence was added to the manuscript “In Germany, the incidence of HFRS increased from 0.09 cases/100.000 persons in 2001 to 2.47 cases/100.000 persons in 2010”

6. Methods: There should be also a case definition for the diagnosis of acute Puumala hantavirus infection. What is considered as an acute
hantavirus case by the Robert Koch institute? Since hantavirus serology done by ELISA (especially Progen or Focus) and/or IFA (especially Euroimmun mosaic) results in a remarkably high number of false positive test result I doubt if all patients included in this study are actually hantavirus infected patients.

Thanks a lot for this well taken comment and sorry for not being clear in this regard. All included patients met the national case definition (see ref. in manuscript). Remarkably, all patients (456 patients) of our study population were followed up to years after the infection and all patients had Hantavirus-specific IgG antibodies years after the infection [1]. The authors modified the manuscript accordingly. (Page 4)


7. On what ground was the Lipase level cut off decided? Preferably backed up with a reference.

Sorry for not being in this regard. Diagnosis of acute pancreatitis: „serum lipase activity (or amylase activity) at least three times greater than the upper limit of normal” [1-3]. Normal range in our laboratory: lipase (< 60 U/L).


8. at the treating physician’s discretion’ is a huge selection bias. This is not a deal breaker since still the data could be of interest for physicians in infectious diseases but this should be clearly stated in the discussion.

The Discussion section was modified according to your suggestions (Line 273, 274)

9. Definition were there amylase levels from any patient?

Thanks a lot for this well taken comment, Unfortunately no amylase levels were available within our study population.

10. Results: The result section does not read well. First of all there is duplication of the results (Abdominal ultrasound was performed in 88%
of the 153 patients with elevated serum lipase but without signs of acute pancreatitis. In two patients, CT scans of the abdomen were performed, revealing no signs of pancreatitis.) is written twice (first and third paragraph)

The manuscript has been revised according to your suggestion (see lines 209-231).

11. I thought that in the PCT paper from the same group and the same cohort also PCT levels were available in 166 out of the 456 patients (correct me if I’m wrong). Where this the same patients? Is it still the physicians choice to test either amylase or PCT or are we looking at a in depth studied cohort of 166 patients in a total of 456 reported cases?

Sorry for not being clear in this regard. A total of 7,476 patients with serologically and clinically confirmed NE were reported to the Robert Koch Institute in Berlin (Robert Koch Institute, Survstat, www3.rki.de/SurvStat) from 2001 to 2012. In cooperation with four selected local health authorities in southern Germany (Stuttgart, Boeblingen/Sindelfingen, Esslingen, Reutlingen), we identified 1,570 serologically confirmed patients with NE infected between 2001 and 2012. These patients were contacted by mail and asked to attend the outpatient clinic at the Robert-Bosch hospital, Stuttgart between September 2012 and April 2013 for follow-up examination. Overall, 456 patients (29%) were included in the study. In the PCT paper, PCT levels at the time of diagnosis were available for 43 out of 456 patients, and in 24 of these patients (56%) PCT levels were elevated (“PCT positive”). In the current study, lipase levels were measured in 166 out of 456 patients during acute course of the disease. Therefore, 141 lipase-neg. patients were compared with
25 lipase-pos. patients. The authors modified the manuscript accordingly (page 4 and 5).

12. Table 1: Is there anything known about level of lipase in serum and correlation to age or to creatinine levels?

Regarding impaired kidney function and lipase levels, there is still a discussion whether pancreatic enzymes were elevated within threefold normal in patients with impaired kidney function. There are no data about correlation with age and lipase levels. The authors modified the manuscript accordingly (page 7 and 8).

13. Table 2: Does not read well consider turning it to a landscape page and try to be more comprehensive

We absolutely agree with your comment regarding table 2 as the information of the 25 patients is already given in table 1. The style of the Table was changed. The authors modified the manuscript accordingly (page 6 and 14).
Reviewer 2

Dear Referee,

thank you very much for the evaluation of our manuscript. We respond to your comments as follows.

1. In their introduction the authors should mention that reports on sensitivity of lipase testing for the diagnosis of pancreatitis vary from approx 60-100%.

Thanks a lot for this well taken comment. The authors modified the manuscript according to your suggestions, please see the revised text, page 4.

2. Also, renal impairment is known as a contributor to elevated lipase levels due to impaired clearance, the authors should mention this in the introduction and discussion; however as the 7 patients with >3xULN lipase did not show markedly impaired clearance as compared to the other lipase-pos patients, impaired clearance may not be the reason for elevated lipase.

Regarding impaired kidney function and lipase levels, there is still a discussion whether pancreatic enzymes were elevated within threefold normal in patients with impaired kidney function. The authors modified the manuscript accordingly, page 8.

3. it would be quite helpful if alternative biomarkers such as amylase or elastase in stool have been determined in some of the patients.

We agree absolutely with you that this is a very interesting issue. Unfortunately, due to the retrospective character of the study, we are unable to provide data about amylase or elastase in stool. This should be performed in
our prospective study with patients with acute NE. We have included this in the discussion section (page 8): Furthermore, we performed a retrospective non-blinded study of medical case reports, with all known associated limitations, “e.g. it would be quite helpful to have alternative biomarkers such as amylase or elastase measured in stool of the patients.”

4. Although amylase is probably less sensitive than lipase it would still be interesting whether some of the patients were "double positive". A solely elevated lipase could rise the clinician's confidence that no pancreatitis is taking place.

Thanks a lot for this well taken comment. Unfortunately, no amylase levels were available within our study population. This was added to the limitation section, the authors modified the manuscript accordingly, page 8.