Author’s response to reviews

Title: Multiplex PCR of bronchoalveolar lavage fluid in children enhances the rate of pathogen detection

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Version: 2 Date: 16 May 2019

Author’s response to reviews:

Dear editor and reviewers:

Thank you very much for the very helpful review of our manuscript. According to your review and advice, we have changed important aspects of the manuscript as follows.

Additionally, we made use of a professional editing service to improve the language.

Editor:

“The methodological and contamination concerns raised are the key issues that require addressing. Please see all reviewers comments and respond to them in full with detailed explanation. Please note that the contamination issue is a significant one and with the lack of controls is difficult to assess.”

Answer: Certainly I responded to all issues in full and with detailed explanation.
As stated by reviewer 2/3 (peer reviewer) himself it is a challenge (and unjustifiable) to acquire BAL samples from healthy children as controls. As recommended I expanded in the discussion on the significant potential for false-positives using SF for respiratory pathogen detection instead.

In the following you find my answers to the reviewers’ comments and questions in detail.

Reviewer 1:

“It is interesting study to demonstrate the enhanced pathogen detection rate of multiplex PCR detection system in the bronchoalveolar lavage fluid in children under suspicion of respiratory tract infection.

Even though authors are demonstrating the usefulness of SeptiFast in the current study it is difficult to reach an indicated conclusion because this kit is not originally designed to detect major organisms causing respiratory infectious diseases. While this aspect is mentioned as a limitation of the study it is not reasonable to apply this kit to respiratory infectious diseases. The idea to use multiplex nuclear PCR methods for clinical samples to obtain rapid diagnosis is right and interesting, but to select this kit for respiratory infectious diseases is appropriate. Therefore it is difficult to find proper significance in the current study.

Answer:

Until the second quarter of 2018, no multiplex PCR kit for respiratory pathogens was commercially available. Therefore, the explicit goal of our study was to evaluate the applicability and diagnostic benefit of SeptiFast® for respiratory secretions.

“As children are thought to be main focus in this manuscript, I recommend to exclude any data obtained from patients of age more than 15 years old.”

Answer:

In Germany, children are defined as patients until the age of 18 years. These patients are required to be treated in children’s hospitals. The study was conceptualized for the patient population treated in our children’s hospital. In exceptional cases, this can also include patients with chronic diseases who are older than 18 years old. Usually, these patients’ constitutions correspond more to those of children than to those of adults. In concrete terms, our patient cohort includes 2 patients older than 18 years old (22 y and 25 y). We assume that these patients do not differ from the younger patients in terms of infection or the identification of pathogens.
Reviewer 2:

“In the manuscript entitled "Multiplex PCR of bronchoalveolar lavage fluid in children enhances the rate of pathogen detection" (PULM-D-18-00124R1) the authors describe a case of PCR of bronchoalveolar lavage fluid in children. This case is interested and educational but it needs a basic revision!

This issue should be considered in any revised manuscript.

In addition, there are some grammatical and spelling errors that should be correct by a person expert.

Answer: We now made use of a professional editing service to improve the language of the entire manuscript.

The following comments should be considered.

Abstract:

“The diagnostic gold standard refers only to histopathology documents not culture!”

Answer: The statement was modified in the text.

“SF is abbreviation of ??”

Answer: As stated in the abstract (line 31) and in the main document (line 73), SF is the abbreviation for “SeptiFast®”.

"S. pneumoniae (27/28; 96%) and S. aureus (11/16; 69%)" the numbers what means?”

Answer: The numbers indicate the detection rate by the SeptiFast® method alone for the indicated bacterial strain. The numbers have now been removed from the text to improve readability.

"Severe bacterial and fungal infections in critically ill patients require immediate anti-infective treatment to reduce mortality [1,2]” needs another references. You should add them:


Answer: We have listed two references that show the reduction of mortality by immediate anti-infective treatment of severe infections.

Without a doubt, all three suggested references provide very interesting data, but they do not refer to mortality reduction by immediate antibiotic treatment of infections. Therefore, we cannot add these references in this context.

"Bronchoalveolar lavage (BAL) is frequently performed in patients with severe pneumonia for microbiological workup." needs a reference. You can add: Mycological Microscopic and Culture Examination of 400 Bronchoalveolar Lavage (BAL) Samples. Iranian J Publ Health. 2012; 41(7): 70-76.

Answer: The subject of the suggested reference study was the “incidence of fungal elements in at-risk patients by direct microscopy and culture of BAL samples”. As far as we understand, neither topic nor results refer to the frequency of BAL performance for microbiological workup. Therefore, we cannot add the reference in this context.

“Line 58: "gold standard" should be revised.”

Answer: The wording has been revised as stated above.

“Lines 58-59: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Line 60: "invasive" should be semi invasive.”
Answer: The wording has been changed as recommended.

“Lines 65-66: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 70-72: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.


Answer: The recommended reference deals with detection of galactomannan for the diagnosis of Aspergillosis and not with PCR-based methods: “Here, we utilized the Platelia Aspergillus GM enzyme immunoassay (Bio-Rad) to evaluate the GM index in BAL fluid samples from patients with proven, probable or putative IA due to Aspergillus flavus versus Aspergillus fumigatus”.

Therefore, we have mentioned this method in our manuscript and added the reference as recommended.

“Lines 82-84: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 87-89: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.
“Lines 91-92: "defined as immunosuppressed (59%), 36 from patients (51%) under". The percent has became 110% !!”

Answer: Here, we list two independent subgroups. One subgroup consists of the patients under immunosuppression. The other subgroup consists of patients under antibiotic treatment. Thus, the percentages are not conflicting.

“Line 96: "previously" what means now??”

Answer: In this context, “previously” means “before suffering from pneumonia”. The wording was changed to “patients without underlying disease”.

“Table 1 and 2 has not been mentioned in the text!!”

Answer: Table 1 and Table 2 are now mentioned and explained in the text.

“In Table 1, 29/70 (41%) has mentiond only in haematologic patients!!”

Answer: The missing numbers were added in Table 1.

“In Table 1, "Haematooncologic" what means!!”

Answer: “Haematooncologic” means “patients with underlying haematological or oncological disease”. This is now formulated more distinctly in Table 1.

“In Table 1, "Gender" should be "Gender (f/m) and 12f/17m should be 12/17 and".”

Answer: Table 1 has been changed as recommended.

“In Table 1, "Immunosussion [patients]" should be "Immunosupresive patients (%)".”

Answer: Table 1 has been changed as recommended.
“In Table 1, "Anti-infective pre-treatment [No. of patients]" should be "Patients with anti-infective pre-treatment (%)".”

Answer: Table 1 has been changed as recommended.

“Lines 106-110: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 111-112: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 113-114: "Enterococcus faecium" sould be E. faecium, and "Streptococcus pneumonia" be S. pneumonia.”

Answer: The spelling has been changed as recommended.

“Lines 115-118: The sentence is not clear and the English needs revision.”

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 115: "and BAL" should be deleted.”

Answer: “and BAL” has been deleted as recommended.

“Lines 118: "BAL" should be Bronchoscopy.”

Answer: I agree to change the name for the whole procedure into “Bronchoscopy” (see above). However, in this passage (line 118), BAL is explicitly meant. During bronchoscopy, the whole bronchial system is usually examined, whereas for lavage, the worst affected area is specifically selected.
“Lines 120-122: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 123: What about direct examination!! Done? PCR details??”
Answer: Direct examination was also routinely performed. This information and PCR details have been added to the text.

“Lines 129-131: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 132: The details of DNA extraction!! What protocol??”
Answer: The details concerning DNA extraction has been added to the text. Literature was added.

“Lines 135-140: The genus and species should be written C. glabrata … and the same for all organisms.”
Answer: The spelling has been changed as recommended.

“Lines 143-145: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Line 151: "Frequency of pathogen detection" should be omitted.”
Answer: The indicated subtitle has been omitted as recommended.
“Lines 152-154: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Table 2 and 3 should be combined together.”
Answer: Knowing that “contamination” is difficult to approach and to prevent misunderstanding in this context, I find it clearer to keep both tables separate.

“Lines 159-160: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 161-162: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 163-167: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Line 172: The sentence is not clear and the English needs revision.”
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

“Lines 172-178: If the data exist in the tables, they shouldn't be in the text again.”
Answer: Data that are listed in the table have been removed from the text as recommended.
“Table 4, "both correspondingly" is unclear!!”
Answer: The wording has been changed to “consistently”.

“Lines 186-193: The sentence is not clear and the English needs revision.”
Answer: This sentence has been completely omitted.

“Line 193: "He was known to be colonized with Aspergillus" to be omitted.”
Answer: The sentence has been omitted as recommended.

“Be careful for all organisms, line 194, be P. variotii”
Answer: The spelling has been changed as recommended.

“Lines 198-199 should be omitted.”
Answer: The indicated subtitle has been omitted as recommended.

“Line 200: The numbers at the beginning of the sentence must be written in alphabetical order.”
Answer: The spelling has been changed as recommended.

“Tables of 5 and 6 should be as graphs.”
Answer: It is difficult for me to understand why these tables should be presented as graphs and find it even less clear.

Nevertheless, Tables 5 and 6 have been replaced by figures:

Fig. 1: Spectrum of detected pathogens in patients under immunosuppression
Fig. 2: Spectrum of detected pathogens in immunocompetent patients
"Lines 219-221: The sentence is not clear and the English needs revision."
Answer: This sentence has been completely omitted.

"Line 250: "(SF)" be SF."
Answer: The spelling has been changed as recommended.

"Lines 252-255: The sentence is not clear and the English needs revision."
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

"Lines 256-258 should be omitted."
Answer: The sentence has been omitted as recommended.

"Lines 272-274: The sentence is not clear and the English needs revision."
Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

"Be careful for all organisms, line 281, be A. fumigatus."
Answer: The spelling has been changed as recommended.

Answer: The reference has been added as recommended.
"Line 285: "Relevance of data and assessment of results" should be omitted."

Answer: The indicated subtitle has been omitted as recommended.

"Lines 285-289: The sentence is not clear and the English needs revision."

Answer: The sentence has been reworded, and the entire manuscript has been revised by a professional language editing service.

"Line 285: "Method" should be omitted."

Answer: Most likely, you mean line 307? Here, the indicated subtitle has been omitted as recommended.


Answer: The passage has been omitted as recommended. The reference has been added.

Reviewer 2 (Reviewer 3): PEER REVIEWER ASSESSMENTS:

"GENERAL COMMENTS: The study assesses the applicability of a molecular diagnostic tool for use in pneumonia diagnosis with bronchoalveolar lavage samples. This test, SeptiFast (SF) is well validated for use with blood samples in sepsis, but has not previously been tested with BAL. The study uses a retrospective design, which somewhat limits the conclusions the authors can make from the findings. However, they acknowledge this limitation in the discussion. The study is well designed and well powered. Data are clearly presented in a series of tables. The topic is of obvious clinical significance and the findings will be of interest to the field. The authors describe the limitations of the study well, but not enough consideration is given to the problem of contamination during BAL sampling."
REQUESTED REVISIONS:

“The authors make interesting observations and discuss them carefully. Their findings suggest that SF may be a useful diagnostic tool in pneumonia but it is impossible to determine this for certain at present as it is unclear what proportion of the increased pathogen detection in SF vs culture is due to contamination with upper airway flora acquired during BAL sampling. It is notable that the biggest increases in species detection in SF vs culture are amongst the Gram positive upper airway colonisers S. pneumoniae (31% vs 2.9%) and S. aureus. Both are prevalent in the upper airways of children and the high sensitivity of molecular methods means even minor contamination could lead to a positive SF result. The high potential for false positive results in SF is indicated by the frequency of detection of non-respiratory pathogens in SF vs culture (40% vs 7% - these percentages should be added to the section on contamination at line 159). To determine how much of an issue upper airways contamination is would require testing BAL samples from children without pneumonia or lower respiratory tract infection (i.e. a group with a high prevalence of upper airway bacterial carriage but where a negative test result would be expected). I accept that acquiring such samples may be a challenge, as BAL is an invasive procedure. If not possible, the authors should expand in the discussion on the significant potential for false-positives using SF for respiratory pathogen detection.”

Answer: Percentages for the frequency of detection of non-respiratory pathogens in SF vs. culture has been added as recommended. The discussion section has been expanded regarding the issue of contamination.

“More detail is needed on sample collection. Where were samples collected and what were the case definitions of suspected pulmonary infection?”

Answer: As explained in the “Methods” section, BAL was performed in the lobe where the major pathology was displayed radiographically or in the right middle lobe.

“Suspected pulmonary infection” was defined as persistent or threatening respiratory symptoms such as coughing or shortness of breath without another cause and/or matching radiological findings. This information was added to the text.

“I found the title of Table 3 confusing. The data show pathogen detection after removing contaminated samples, but the title suggests the opposite.”

Answer: The title of Table 3 has been changed to clarify its meaning.
“What is the rationale for comparing detection rates after removal of the Paecilomyces-infected sample? It would seem to be a spurious comparison as the absence of the species from the SF panel does not indicate it isn't a relevant causative agent of infection. Removing it leads to artificially inflated detection rates for SF.”

Answer: You are correct. Knowing that Paecilomyces can cause infections and even being aware that the patient with P. variotii identified in the culture had possible invasive fungal infection, we should take into account that a possible disadvantage of SF is that SF cannot detect this organism. Concerning this detail, we deleted the statistical calculation after removal of Paecilomyces, re-evaluated it and corrected the results in the text.

ADDITIONAL REQUESTS/SUGGESTIONS:

“Gram positive and Gram negative rather than grampositive and gramnegative at line 173.”

Answer: The spelling of Gram positive and Gram negative has been corrected throughout the manuscript.

We believe that with the help of the reviewer’s comments, we have substantially improved the quality of this manuscript.

Sincerely,

Eva Tschiedel