Reviewer's report

Title: Aspergillus-PCR in bronchoalveolar lavage for detection of invasive pulmonary aspergillosis in immunocompromised patients

Version: 1 Date: 15 April 2012

Reviewer: Mark Reinwald

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Major Compulsory Revisions

I. Abstract:
In your analysis you counted the “possible” cases, which make up the majority of your “suspected aspergillosis population” as having IFD (invasive fungal disease). However, I find it questionable to include the possible cases as having IFD. The clinical reality reflects the fact that most cases where aspergillosis is suspected you fail to obtain a microbiological criterion (based on EORTC like GM, culture, BDG) and therefore these patients may have IFD based on radiological and clinical signs however the true cause cannot be identified. This is reflected by the relative number of cases (proven&probable vs. possible). I would at least comment on that because in most available diagnostic studies that adhere to the EORTC/MSG criteria the “positive “ IA population are the patients who would be classified as probable& proven, the possibles are usually left out. As all surrogate parameters (GM, PCR) and BAL culture are subject to reduced sensitivity by underlying antifungal treatment this has a major impact on the low sensitivity you describe for BAL PCR. Therefore I would include the sentence about antifungal therapy into the results section.

II. Introduction:
1. In the paragraph “ Typical features of IFD…… “ you cite several publications for serum GM performance (Pazos C, Moragues, Verweij ) in several sentences. I would recommend combining these single statements into something like “sensitivity for Serum GM ranges from XXX to XX % (Citations) as this makes the statement more concise. Furthermore as you reflect on Serum GM I would also recommend mentioning BAL GM as it is acknowledged as a microbiologic criterion in the EORTC/MSG criteria and its sensitivity is probably higher than Serum GM sensitivity (Luong et al, Chest 2010)

2. In the paragraph “Recently, molecular diagnostic methods for detection …..” I would strongly suggest leaving out the term “recently” as BAL PCR is used and reported on, although not externally validated since 1993 (Spreadbury et al, Tang et al). I would however advise mentioning that the major problem in PCR diagnostics is the missing external standardization and the wide plethora of different methods (real-time, nested, etc) and that harmonization efforts are ongoing in order to help integrating PCR into the next revision of the EORTC criteria (White et al, J Clin Microbiol 2010)
II. Material & Methods:

1. Disease definitions:

I don’t understand the sentence “Besides BAL, the choice of further sampling techniques used during bronchoscopy was at the pulmonologist discretion”. What does that mean? And why did you cite the Stolz Chest 2007 article in that regard? Please elaborate.

2. In the paragraph “Severe neutropenia was defined as an absolute neutrophil count of …..” after this statement, which is important for immunocompromised individuals you write about nonspecific interstitial lung disease, organizing pneumonia, respiratory bronchiolitis, alveolar hemorrhage, etc. However I do not see the relevance to the actual topic of your manuscript, especially as these clinical conditions are not presented in the patient characteristics tables and not elaborated on later in the results or discussion section. I would recommend leaving that out as it doesn’t yield additional information.

3. In the paragraph “Patients were examined, treated, and followed up…” I would recommend providing the numbers of how often CT was performed in order for the reader to understand the population you are describing as the classic radiologic criteria for defining IA (nodules, halo-sign, air crescent sign, etc) are often not seen on conventional X-Ray.

III. Results:

III. 1. In the paragraph “A total of 191 immunocompromised….” You mention that the vast majority of patients required bronchoscopy due to fever and cough. However, only “Fifty-five percent of patients presented infiltrates in the Chest X-Ray. Do you perform BAL even when no infiltrate can be detected in your institution? or did the remaining 45% have infiltrates in CT? 

2. In the paragraph “ Bronchoalveolar lavage was performed in all cases”. I would leave that sentence out as this is a prerequisite for this study.

3. In the paragraph “ Therefore, there were 53 Patients with IFD” I would disagree with that statement. You had at least 11 patients with IFD (proven&probable acc. To EORTC/MSG, see my comments on the abstract). ?

IV. Discussion:

1. In the paragraph “The low sensitivity of the assay in our study…” you correctly (at least from my point of view) identify the explanation why you have a low number of cases (11 proven/probable) because of the low pre-test-probability. However that by itself not the explanation for the low PCR sensitivity (0 % in proven, 36 % in proven& probable cases according to Table 2) but might be the explanation for the reduced sensitivity when taking the possible cases into account (as more cases are included which do not suffer from IA). I would elaborating on that.

2. You state that one of the main reasons for negative PCR and therefore low sensitivity in the proven&probable patients is underlying antifungal therapy. This is very important, I would recommend citing other papers showing influence of
underlying antifungal therapy on surrogate parameter performance (Marr et al, 2005; Lass-Floerl et al 2005).

3. you state that “in the second case, the patient was on cyclosporine, had a suspicion of fungal infection and a positive aspergillus culture in BAL, thus fulfilling only the “possible” criteria. This is not true. Host Factor = CSA, Infiltrate = radiologic abnormality, BAL culture = microbiologic criterion # Patient would have been classified as “probable” without histology based on EORTC 2008 (see de PAuw et al CID 2008)

4. Were autopsies performed ? (basically the earlier studies recruited their higher number of “proven cases “ ususally based on autopsies. I would add this information

5. In your final sentence you state that “your results” suggest a limited clinical applicability of Aspergillus PCR in the BAL for detection of IFD in a population of immunosuppressed patients”. However, I do not agree with that statement the way it stands now. Firstly I would recommend changing IFD to IA as your PCR detects only aspergillus species. Secondly (based on your table 2) sensitivity for probable cases would be 50 % (4/8 cases) which I find acceptable, taking the amount of antifungal therapy nowadays prior to BAL sampling into account.

V. Tables:

1. In your table you list your possible patients, which, according to the EORTC Criteria (de Pauw et al CID 2008) fulfill the definition of Host criteria and lower respiratory tract disease (imaging, etc). However in your possible group you have several patients who have a positive aspergillus culture. According to table 2 of the de Pauw classification (CID 2008) of the EORTC these should be classified as “probable” increasing your total number of probable cases (“ Recovery by culture of a mold (e.g., Aspergillus, Fusarium, Zygomycetes, or Scedosporium species”, Table 2, de Pauw 2008, CID)

Minor essential revisions:

. you mentioned you had Serum GM for 105 patients, do you also have data on BAL GM ? Or is that not performed in your institution.

I would mention in your limitation paragraph, that the low-pretest probability of your study design lead to a limited number of proven/probable cases (11 total)

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.