Author’s response to reviews

Title: Galactomannan testing of bronchoalveolar lavage fluid is useful for diagnosis of invasive pulmonary aspergillosis in hematology patients

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Author’s response to reviews: see over
Dear Dr Koutsos,

Re: MS 1032021729503600 – Galactomannan testing

Thank you very much for giving us the opportunity to submit another revised version of our manuscript to your journal.

We would like to thank both referees for their instructive comments. Our reply to each specific comment is given below, and I hope that we will have sufficiently answered all questions.

Yours sincerely,

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Referee 1 (Alessandra Pasqualotto)
Thank you for the helpful comments. Our replies are listed in order below:

1. It has always been difficult to culture out Aspergillus spp. (the sensitivity of culture as a method for diagnosing invasive pulmonary aspergillosis or IPA has always been low), and we are also somewhat skeptical (although this is an EORTC criteria) that the appearance of “hyphae that are compatible with Aspergillus spp.” on histopathology equates with IPA. However we note that many authors, including Johann Maertens in the CID paper, use this definition and hence we have similarly adopted it. We think that “invasive mould disease” is too wide a term to use in this study, especially since galactomannan is relatively specific for Aspergillus spp.

2. We have included Johan Maertens CID paper as part of our references and discussion, although we would like to point out that this paper was only published after we had submitted our first revision.

3. We believe that we had misunderstood the reviewer’s previous comment. By stating that positive serum GM results were “not repeated”, we had meant that the patient was not subjected to an immediate second blood draw for re-confirmation of the results. Since GM tests are only done twice weekly at our institute, the subsequent blood draws are “on schedule” on the morning of the actual test day. In our laboratory, all GM results that are 0.7 or higher are confirmed by re-testing of the original serum sample. All BAL samples are tested twice as well if the GM results are 0.5 or higher. This is now reflected in the Methodology.
a. As to the choice of single static result of GM OD 0.7 for serum cut-off, this is specific to our institution, and was based on several older publications. We note Maertens 2004 BJH paper where his group recommended a static cut-off result of 0.8 or higher, but note that FDA’s cut-off is 0.5 or higher.

b. Dynamic cut-offs (i.e. consecutive samples where serum GM OD results are 0.5 or higher) are not quite feasible from the clinical viewpoint because of the fact that tests are done twice weekly at our institution and not more frequently.

c. We presume the reviewer referred to Table 2: we showed the “highest GM value” in the serum to highlight the difference between BAL and serum GM results. If the patient had several negative results (in practice, only 3 negative at most, as we took into account only serum GM results within two weeks of bronchoscopy) and one positive result that was confirmed on re-testing, we would accept this as a positive GM result fulfilling the EORTC-MSG microbiological criteria.

4. We had performed bronchoscopy for 10 cases and 52 controls without complications. This coupled with previous publications on BAL GM suggest that our conclusion that this procedure is safe and useful is not unqualified. We have removed the statement on “delay in the performance of bronchoscopy for up to 6 days.”

5. We have changed this sentence to read “…a higher cutoff for BAL as compared to serum galactomannan testing is necessary…”

Referee 3 (Emilie Frealle)

Thank you for the instructive comments. Our replies are listed below:

1. We feel that the reviewer has misunderstood the study methodology and apologize for the misperception. The whole study is prospective, and not just the BAL GM detection (unlike previous published studies where stored BAL samples were tested “prospectively” and data collected retrospectively). We recruited all cases and controls and obtained consent for the study from all subjects.

2. We thank the reviewer for the comment on “possible IFD”. Patients with “possible IFD” and no final diagnosis pose an interesting problem for studies such as these (in clinical practice, most would of course have been treated with antifungal agents). Such patients can have fungal infections (and an autopsy will be needed to verify these) – which in turn can be aspergillosis or some other mould disease – or the pulmonary lesions could be due to something else. We submit that it would be more rigorous to exclude such patients from a diagnostic study (although they might be included for a therapeutic study) because of the very uncertainty involved in the diagnosis. If the BAL GM results in such patients are positive (i.e. GM OD > 1.0), how can we be sure that these are not false-positive? Likewise if the BAL GM results are negative, how are we sure that these are not false-negative results? For the record, of the 4 patients that were excluded, two had high BAL GM results (> 1.0) and were treated as for IPA, and recovered. Two had low BAL GM results – both were prescribed
antifungals initially and only one was discontinued, but both recovered from the febrile episode. It is not easy to make sense of these results and include them in the analysis.

a. On another note, we agree that the 100% sensitivity is an overestimation, but this is because of the small sample size rather than anything else. We have highlighted this as a limitation of the study (on another note, similar high sensitivities have also been described for BAL GM and serum GM testing in past publications).

3. We are addressing IPA rather than IFD (since the diagnostic test in question is galactomannan testing, which is fairly specific for aspergillosis) in this study. As such, we feel that patients with possible IPA, but for which some other pulmonary diagnosis was ultimately made, can and should be part of the control group. That is one of the few reliable ways to determine the specificity of the test (short of autopsy studies). The mucormycosis patient in particular is an excellent demonstration of the specificity – and shortcoming – of the BAL GM test. A negative GM test does not preclude IFD caused by non-Aspergillus moulds.

a. There is a risk that the subjects with possible IPA (for which some other diagnoses was ultimately found) could have double pathology, i.e. IPA + an alternative diagnosis. But we judged this risk sufficiently small that it was worth including such subjects because of their potential value for determining the differentiating capability of the test. In any case, there were only 4 such cases.

4. We worked on total BAL (in the text this is described as “BAL fluid”), and subsequently followed the manufacturer's guidelines in determining the GM result.

5. There were 9 probable and 1 proven IPA’s by EORTC-MSG criteria (this is now included in the text). If we used IFD (rather than IPA – where culture or some other form of identification is required) criteria, there would be 3 proven IFD’s and 7 probable IFD cases.