Reviewer’s report

Title: Gene expression profiling unveils a novel biomarker combination for the diagnosis of serious bacterial infections in children

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Reviewer: Ganjana Lertmemongkolchai

Reviewer’s report:

This is a very interesting study that could be applied to clinical problems. However, there are some concerns that need to be addressed.

Major compulsory revision:

1. Gene expression profiling is not the center of this study since it has no details on microarray analysis, characteristics of 12 patient samples plus 3 controls used for gene expression profiling. As the authors mentioned (Results: Page 9, last line in the first paragraph) that “A complete description of the microarray analysis is being reported separately”, therefore this term needs to be removed from the title and abstract of this manuscript to avoid any misleading. In addition, Affymetrix microarray analysis and microarray bioinformatics analysis (Materials and Methods: Page 16) are irrelevant to this manuscript.

2. The study setting, population and characteristics of study patients enrolled between 2004-2006 have been described previously (Reference 5) and the same numbers of samples were tested for biomarkers suggesting that these samples might be studied as left over samples. This raised the question of sample storage and integrity over the past 5 years.

3. Characteristics of cases vs. controls: Since all controls were HIV-uninfected (Page 14) but patients with serious confirmed or no detectable bacterial infection were 52 or 47 % HIV-infected (Table 1). The authors did not mention whether they did the analysis based on HIV status as well. Figures 2-3: it was the comparison between cases (confirmed serious vs. no detectable infection) vs. controls or survivor vs. non-survivor. It could have been more informative if the HIV status, previous antibiotics treatment and meningitis condition were also analyzed, particularly in the serious bacterial infection group. Moreover, the characteristics of control group should be inserted into Table 1 to ensure a fair comparison.

4. Page 9, first paragraph: The statement that “The fold changes in the controls compared to the cases were resistin: -5.88, p=0.06, neutrophil gelatinase associated lipocalin (NGAL) -4.19, p=0.002 and granulysin 3.14, p=0.002” indicated RNA over expression of resistin and NGAL but under expression of granulysin in the cases. However, Figure 3 showed that the concentration of all three proteins in the cases was significantly higher than the controls. The discrepancy between RNA expression and protein concentration needs to be
clarified or discussed.

5. The numbers of samples used in each assay were not equal (Figure 1). The authors need to explain the reason, how the samples were selected and how many samples were tested across all four says.

6. The combination of selected markers seems to increase the parameters such as NRI or AUC, compared to a single marker. However, the authors may need to discuss how the increasing number (eg. AUC from 0.80 to 0.90) would make any changes in terms of clinical management.

Minor essential revision:
1. Table 1: NBI was ‘no detectable bacterial infection’, wasn’t it? The word ‘detectable’ was missing from the table head.
2. Table 2: Many genes were not mentioned the text. They need to be clarified or removed from the table.
3. Table 3 was not mentioned in the text.
4. Table 4: table and text in the Results section was repeated.
5. All figure legends did not provide enough details and should be extensively rewritten (see Guidance for authors) including sample sizes analyzed in each figure.
6. Figure 6 is not novel, and may be removed. Figure 4-5 could be combined (panel A and B) to shorten the manuscript.
7. Abbreviation: SBI and CBI were confusingly used in the manuscript and it needs to be consistent.
8. Spearman’s correlation coefficient was mentioned in Statistical analysis (Page 19, line 3) but was not clear where it was used in this study.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.