Author’s response to reviews

Title: Pathway mapping of leukocyte transcriptome in influenza patients reveals distinct pathogenic mechanisms associated with progression to severe infection

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Author’s response to reviews:

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Sydney, December 2019

Rebuttal letter: MGNM-D-19-00071
Dear Editor,

We hereby submit, for your consideration, our revised research article (version R2) titled “Pathway mapping of leukocyte transcriptome in influenza patients reveals distinct pathogenic mechanisms associated with progression to severe infection”. We have addressed all the reviewers’ requested changes, which are highlighted in blue in the revised manuscript attached. Thank you for your attention to our paper. We look forward to your response.

Best Regards
Yoann Zerbib

Review BMC Medical Genomics

Reviewer reports:

Henk-Jan van den Ham (Reviewer 1): Thank your for the responses to my questions, this clarifies most of the issues that I was concerned with. My major comments (1-7) have been addressed adequately, but there are a few matters in the text that do require your attention (i.e., minor comments) following on comments 1-4 and 6.

1. The PCA shows that there is a predominant disease signature, which is good. Note that the text says ‘age’, which is not shown in the supp figures. I think the wording could be polished a bit more; and the comment on other viruses should be moved to the description of the cohort as one of the exclusion criteria.

Author response:
We thank the Reviewer for the positive comments. We have reworded the main text as follows.
“Patients infected with other viruses than influenza were excluded from the study” (page 5)
“There was no viral coinfection observed in the cohort.” (page 8)
“Unsupervised principal component analysis (PCA) was performed using normalized log2 gene-expression levels (Additional Figure 1). Based on two principal components, the analysis showed a separation in the gene expression between severe influenza, moderate influenza and healthy control subjects. We note that gender didn’t seem to be associated with a separation in gene expression profile (Additional Figure 1B).” (page 10)

2. The days after onset do differ slightly between the groups (though not significantly so), and thus represent a potential confounder. Please mention this matter in the discussion, as it is something that confounds many other studies.
Author response:
In response to the Reviewer’s comments, we have added the following clarifications:
“Also, patients arrive in hospital with different delay after onset. Although we found that the
time elapsed since symptom did not statistically significantly impact on gene-expression levels,
we cannot confidently exclude its potential confounding effect. Therefore, this issue should be
clarified in future studies.” (Page 19)

3. The reply to the question implies that the physicians always agreed on the classification of the
patients? If this is the case, please state so. If not, please explain how conflicting severity
assignments were dealt with.

Author response: We rephrase as follow to clarify:
“Patients assignment matches perfectly between the 2 experts.” (Page 9)

4. I see, would be good to compare the results of the WGCNA paper in the discussion. Currently
there is no reference to this specific paper anywhere in the manuscript.

Author response: As requested, we made the comparison with the WGCNA.
“These results were consistent with an unsupervised analysis previously published by our team
[13]. In that analysis, a weighted gene co-expression analysis (WGCNA) was performed to
identify disease modules associated with infection severity. The results of that analysis are in
keeping with the main findings of the current paper, which include (1) the neutrophil module
displayed the highest increase in modular expression as influenza severity progress from
moderate to severe form, (2), cell cycle module was upregulated, (3) immune response module
revealed broad downregulation in gene expression of key genes involved in innate and adaptive
immunity. Overall, the pathway analysis presented in this paper extends the previous WGCNA
analysis by providing additional insights on the biological pathways associated with severe
influenza infection.” (Page 15)

6. Interesting comparison, considerable overlap. A little bit more background & context on
this paper (either in results or in discussion) and a citation would make this more understandable
to the readers that are not familiar with the paper.

Author response: As requested, we add details on the external dataset as follow:
“External validation
We also performed a gene-based comparison with an external dataset (GEO 111368) [12]. This
external dataset has a similar study design to ours, which included 109 adult patients with
laboratory-confirmed influenza infection and 130 healthy participants. We found strong
similarity in the upregulated genes between the two studies: 17 upregulated genes were shared
between two studies (70%) (Although only 3 (15%) downregulated genes were shared between
the two studies). Notably, the upregulated genes encode proteins involved in neutrophil functions
and the downregulated genes encode proteins involved in the immune response. Among the most
differentially expressed genes, neutrophil activation and reduced immune response were the
dominant biological themes found in both dataset (additional table 5),” (Page 13-14)
Reviewer 2 (Reviewer 2): PEER REVIEWER ASSESSMENTS:

REQUESTED REVISIONS:

My only remaining concerning is concerning the absence of error bars in the diagrams. While it is good to now have the p-values and see significance, I still would like to see variability, which would be indicated by error bars.

Author response: To address reviewer’s concern about the variability and the error bars in the diagrams, we have improved Figures 4 and 5 by plotting the mean expression of each gene in each group (moderate, severe and healthy control) and adding errors bar for each figure. Accordingly, we have updated the Figure 4 and 5 legend as follows:

“Figure 4. Histogram of neutrophil-related significant changes in gene expression between severe influenza, moderate influenza illness and healthy controls. Y-axis shows normalised log2 expression levels. * indicate p<0.001, adjusted for multiple testing by Bonferroni method. ns denotes non-significant. HC denotes healthy control
4A. Genes encoding proteins involved in neutrophil extracellular trap formation. Expression differences are shown for (the strongest regulated) probesets of the individual gene.
4B. Genes encoding proteins involved in neutrophil migration. Expression differences are shown for (the strongest regulated) probesets of the individual gene.
4C. Genes encoding components of neutrophil granules. Expression differences are shown for (the strongest regulated) probesets of the individual gene.
Neutrophils-related genes were upregulated in patients with severe influenza illness compared to moderate influenza illness and healthy control subjects.” (Page 29)

“Figure 5. Histogram of MHC class II significant changes in gene expression between severe influenza compared to moderate influenza illness and healthy controls. Expression differences are shown for (the strongest regulated) probesets of the individual gene. Y-axis shows normalised log2 expression levels. * indicate p<0.001, adjusted for multiple testing by Bonferroni method. ns denotes non-significant. HC denotes healthy control
MHC class II were downregulated in patients with severe influenza illness compared to moderate influenza illness and healthy control subjects.” (Page 29)