Reviewer’s report

Title: Infection by H1N1 pandemic virus and Tnf 308, Tnf 252, Tnf 238, Tnf 376, Il1Beta, IL6, Il8, ccl1 in Mexican population: Case-Control Study

Version: 2 Date: 24 April 2012

Reviewer: Chung Yan Cheung

Reviewer’s report:

The MS by Morales-García et al. presented a study attempting to identify if the different polymorphisms of various cytokines are associated with essentially 2 outcomes: a) infection and b) severity of infection with the 2009 H1N1 pandemic virus in patients from Mexico. The research question is well defined in the MS by the authors.

The methods used appear to be appropriate in general, however more description is needed for the way in which the data were analyzed e.g. what statistical test(s) was/were used to test for significance? Which software(s) was/were used? … etc.

I believe the study does have information to contribute to scientific knowledge in the field of infectious diseases, however I have concerns regarding the data itself, and its presentation in the MS in its current form (please see below). I cannot find any major problem in the standard of reporting, and there is no data deposition issues associated with the manuscript.

As I have major concerns regarding the data and its analysis (detailed below), at this juncture I cannot make judgment as to whether the discussion and conclusions are well balanced and adequately supported by the data. Therefore I am unable to decide on acceptance or rejection until the authors have responded to the concerns details below.

- Major Compulsory Revisions

1. In the methods, the authors described “A control group was formed by healthy volunteers, who had contact with patients infected with influenza A/H1N1”. Being “healthy controls”, I would be grateful if the authors can explain why some have disease symptoms as described in table 1: eg 42 control patients have fever. I am also unsure as to why it is indicated to be 82.4 %? As there are 245 control patients, then if my mathematics is correct, it should be (42/245) X 100 = 17%! Indeed, I have found this discrepancy in other data in table 1 as well.

2. If the inclusion criteria for the control group are indeed “healthy volunteers, who had contact with patients infected with influenza A/H1N1”, I am concerned that this may not be appropriate to address whether there are association between the gene polymorphisms and infection with A/H1N1. Whether one get infected with A/H1N1 or indeed any viruses is dependent on numerous factors ie.
timing of contact with the patient, titre of virus exposure, nature of contact etc. These factors need to be controlled for and there are no indications in the MS in this regards. Furthermore, how do the authors know whether the control subject has not been infected with A/H1N1 before or at a later time?

3. It is not clear from the MS as to the group of patients that are used for comparison to the fatal cases of A/H1N1 in order to determine the risk of death due to A/H1N1 infection. I suspect that non-fatal cases of A/H1N1 were used. If this is the case, the authors will need to define the inclusion criteria used and to show the demographic and other clinical data for the two groups separately before it is possible to assess whether the analysis is valid.

4. I think the entire MS needs some language corrections before being accepted, for example in the abstract:

i) I am not sure what is meant by “The molecular bases are very complex”? Does the authors mean “The molecular basis are very complex”?

ii) “Whether these genes are more affected by genetic polymorphism” does not seem logical, does the authors mean “Whether the pro-inflammatory response is affected by genetic polymorphism of cytokine genes”?

There are similar cases throughout the MS as well as grammatical errors and the flow of the MS is fragmented in places. I suggest that the authors seek professional help in identifying them.

- Minor Essential Revisions

5. There is currently no TNF +252 polymorphism as mentioned in the MS, surely the authors meant LTA +252 (rs909253).

6. The probe for detecting LTA +252 (rs909253) is not present in table 5

7. In the introduction, it was not apparent as to why the authors focused on particular polymorphisms as there are others that were not investigated by the authors e.g. there are 10 known polymorphisms for TNF and the authors only investigated 3 of them. It was only in the discussion that it becomes apparent. Therefore in order to make the MS more logical, the author may want to shift some of the description of the different polymorphisms to the introduction.

8. Did any of the patients received treatment with anti-viral drugs? if so this should be mentioned with time of administration and whether there are variation in dosage.

9. blood samples were taken from the subjects to quantify TNF levels, however the authors did not state when these samples were taken? It is important to know this in order to determine whether the analysis is valid.

10. At page 8 paragraph 5, the authors mentioned that “after adjustment for the concentration of cytokines, levels of PaO2 and admission to the ICU”. The authors should try to explain how they made the adjustments for these
parameters in order to assess whether it is valid to do so.

11. The authors stated in the Method that the patients were tested with PCR for A/H1N1, I wonder if there is viral load data available for these patients? if so they should be presented as well to aid assessment of the study.

Discretionary Revisions

12. As some readers of BMC Infectious Disease may not be very familiar with genetic association analysis, some additional description or explanation in layman’s terms regarding the ways the data was analyzed may be useful.

13. As the authors mentioned in discussion, this is a small study in terms of genetic association, and the authors may want to consider making the full set of raw data available online for others to perform meta-analysis in the future.

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

Quality of written English: Not suitable for publication unless extensively edited

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 interest.