Reviewer’s report

Title: In situ Molecular Identification of the Influenza A (H1N1) 2009 Neuraminidase in patients with severe and fatal infections during a pandemic in Mexico City

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Reviewer: Ana L Gutiérrez-Escolano

Reviewer’s report:

The aim of this study was to develop an in situ RT-PCR method for the detection of influenza A (H1N1) 2009 strains in archival material of paraffin-embedded human tissues. The method described is very sensitive, and specifically detect the neuraminidase viral mRNA in lung biopsies from patients who died from pneumonia caused by Influenza A (H1N1). This methodology can also provide evidence of the cellular type susceptible to A (H1N1) viral infection.

Major compulsory revisions

Authors should improve the manuscript’s grammar

Since the aim of this manuscript is to show that an in situ RT-PCR method is sensitive and specific for the detection of influenza A (H1N1) 2009 strains in paraffin-embedded human tissues, material and methods section must be clearly described. Therefore some suggestions were addressed as follows:

1. In the Materials and Methods, “Primers section”, it is stated that: “…pre-validated neuraminidase specific primer sequences were selected….”. An explanation of the pre-validation or a reference must be included for both neuraminidase and #2-m primers. Moreover, the size of the amplicons corresponding to the neuraminidase and #2-m genes should be stated in this section.

2. In the Materials and Methods, “In situ RT-PCR section”, it is stated that the reverse transcription was performed with 3 #g of random primers. Please indicate the nature of these primers. The amount 3#g of primers is correct? In this section, it is also indicated that the PCR was performed with a primer mix, please be more specific. The amplification of both neuraminidase and #2-m cDNA was performed in the same PCR reaction?

3. In the Materials and Methods, “Sequencing of in situ RT-PCR products section”, the conditions for the PCR to amplify the cDNA obtained from the solution over the tissue are referred as traditional in vitro PCR. Could the authors be more specific? In this paragraph it is also stated that: “…as expected, sequences corresponded to the segment between the middle part and the 3’ end of the NA gene”. However no information about the size and the region of the NA RNA that was amplified was previously described. I recommend to include this information as suggested above and to remove this paragraph from the Materials
and Methods section.

4. In the Materials and Methods, “In vitro duplex amplification technique section”, it is not specified the nature of the duplex PCR products amplified. Moreover, when describing the reagents used it is indicated that “400 nM of each primer, 2.5 units of Taq DNA polymerase (Invitrogen, U. S. A.), and specific primers were used”. Can the authors explain which primers are used in the reaction and which products were amplified?

5. In the Results section (“Clinical features of patients with Influenza”), it is mentioned that “a total of 8 samples obtained from influenza-positive patients were analyzed using In situ RT-PCR for influenza A (H1N1) 2009”. The type of samples should be described.

6. In Table 1, the type of “Conventional methods” that was used to determine that all the samples were positive to Influenza A (H1N1) should be indicated. Since it was corroborated that all the patients were positive to Influenza A (H1N1), why is it stated in the title of Table 1, “…influenza-like illness patients”? Also consider changing the word “characteristics” for “data”.

7. In the Results section (“Expression levels of mRNA neuraminidase A (H1N1) influenza gene in patients with influenza”), the results reported correspond just to 3 out of the 8 patients analyzed. Why the authors did not showed the amplification obtained form all of them. The number of negative samples must be increased. Why the positive samples were not included in the assay?

8. In the Results section (“NA A (H1N1) influenza gene can be detected in biopsies using in situ RT-PCR”), the information in this paragraph is difficult to understand as it is written.

9. In Figure 3 and Table 1, the results obtained from a single Influenza A (H1N1)-negative-patient is included, although it is referred that two negative results, besides the “non related cells control” were included.

10. In Figure 3, the signal indicated by arrows in the samples corresponding to patient P03 are difficult to observe.

11. Figure 3 legend is not complete.

12. The signal detected in a macrophage is an isolated result or it was observed in macrophages from the other samples analyzed? How the authors determined the cell type?

13. A phylogenetic analysis of data presented in Table 2 should be included to better support some of the ideas analyzed in the discussion section.

Level of interest: An article of importance in its field

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

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interest