Author's response to reviews

Title: Humoral and cellular responses to an unadjuvanted monovalent H1N1 pandemic influenza vaccine in hospital employees.

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Author's response to reviews: see over
Dr. Philippa Harris  
Editor BMC Infectious Diseases

We appreciate the comments from reviewers and acknowledge the opportunity to reply to their questions regarding our manuscript entitled "Humoral and cellular responses to an unadjuvanted monovalent H1N1 pandemic influenza vaccine in hospital employees". MS: 4307880411020510.

Below you will find the point-by-point reply to the reviewer’s questions and comments. In addition, we had corrected the manuscript following the reviewer’s suggestions and we are sending the revised version. A professional English editor corrected the manuscript.

Reviewer 1: Massimilliano Fabbiani

In this manuscript Herrera et al. investigate humoral and cellular responses to vaccination with a unadjuvant monovalent H1N1 pandemic influenza vaccine in healthy subjects. Despite 4 years has passed from the pandemic spread of the virus, the H1N1 serotypes is still circulating and it is included in the current formulations of trivalent vaccine. Many data are available on the humoral response to H1N1 influenza vaccination but few data are available on cellular immune response.

Results of the study are interesting since many details on both humoral and cellular responses to the vaccine are reported. As consequence, the results can contribute to better define the immune response to influenza vaccines. The article is well written, data are reported clearly and well discussed.

Some minor issues should be addressed before publication:

MINOR ESSENTIAL REVISIONS
Q1: Section Methods, “Antibody detection”: Please define in this section the criteria for seroprotection and seroconversion.

A1
We appreciate the observation; such definitions were set in fact in the results section in our previous version of the manuscript. Now, following the recommendation, we have added a paragraph to the Methods section called "Antibody detection" (lines 166-176).
Q2: Line 283: “After vaccination. The seroconversion (66.7%) and seroprotective (48.3%) rates increased”. The proportion shown in the text does not correspond to those the table 2, where a seroconversion rate of 48.3% and a seroprotection rate of 66.7% reported. Please clarify.

A2. Thanks for pointing out our error; we had inverted the numbers of those data in the text. We have already corrected the error in the main text (line 282), because the values in table 2 were correct. Seroconversion was 48.3% and seroprotection was 66.7%.

Q3
Lines 308-311: please show the number of subjects with and without anti-HA1 antibodies prior to vaccination.

A3
As we mentioned in the manuscript text, the anti-HA1 antibodies were only assessed in a subgroup of volunteers. Therefore, the number of subject with anti-HA1 antibodies was 9 and the number of subject without anti-HA1 antibodies was 13. Those numbers are mentioned in the text (line 302 and 304) and in figure 2 legend.

Q4
Line 312-313: the authors state that post vaccination GMT did not significantly differ in the groups. Please show the p values. If at least a statistical trend is not observed, no firm conclusions on the effects of anti-HA1 antibodies prior to vaccination can be drawn. In such case, this section appears purely speculative and should be removed.

A4
The observation is right, we did not find in fact a significant difference between groups, the p value was p=0.14 and this information has been added to the revised manuscript (line 304). We agree with the reviewer that consequently not a firm conclusion can be drawn based on these results and we removed part of a paragraph on this regard. The text removed from our previous version of manuscript is the following: “This result was presumably derived from seasonal vaccination, although this phenomenon did not reach statistical significance (Fig. 1c). These results suggest there is a possible immunosuppressive effect of prior seasonal vaccination on the humoral response to A/H1N1 vaccination “. Instead, we have added a sentence saying that we cannot conclude that pre-existing antibodies to the seasonal vaccine played a role in the induction of antibodies against the pandemic A/H1N1 vaccine (line 305, 307). However, we prefered not to remove the complete section because we are presenting data, which are part of our findings and it is an observation that deserves to be investigated with a higher number of subjects.
Q5
Lines 354-357: was this decay statistically significant? Please report the p value.

A5
No, the drop was not statistically significant and we have added the word “trend” and the p value (p=0.45) (line 348).

Q6
Lines 398-401: was this increase statistically significant? Please report the p value.

A6
Yes, our results showed a significant increment in CD8$^+$ T cell proliferation in response to pandemic and seasonal specific peptides post-pandemic A/H1N1 vaccination (p<0.05) (Fig. 3 and 4a). The p value was added in the manuscript (line 385). “Among individuals with positive proliferative responses prior to and after pandemic vaccination, the percentage of positive subjects increased to 70.8% (p<0.05) for the pandemic A/H1N1 serotype and to 43% (p<0.05) and 47% (p>0.05) for the seasonal H1N1 and H3N2 serotypes, respectively (Fig. 4b)”. The p value was also added (line 391-393).

DISCRETIONARY REVISIONS
Q7
Line 188: the reference provided (Ref 17) refers to the use of ELISPOT during tuberculosis. Please add a reference where this method is used to measure IFN-gamma-producing T-cells after influenza vaccination.

A7
We have followed your suggestion and added a reference where the ELISPOT assay was used to measure IFN-γ-producing T-cells in influenza. However, we also kept the former reference because we used the ELISPOT conditions that are described in that manuscript. (References 20-21)

Q8
Line 258: “46 of the subjects”, please add in parenthesis the percentage on the total.

A8
Regarding your suggestion we added the corresponding percentage and made a correction (45 instead of 46 subjects) because we noticed that one subject was wrong positioned in the group of volunteers with documented 2009 seasonal vaccine and moved that person to the 2008/TIV vaccine group. Therefore, 45 subjects (75%) were previously vaccinated with 2009/TIV, 5 (8.3%) with 2008/TIV and 50 (83.3%) with 2008 or 2009 TIV. We have added these percentages in the text: 45 subjects (75%) (2009/TIV) (lines 109, 255), 5 (8.3%) with 2008/TIV (lines 112, 257) and 50 subjects (83%) 2008/2009 TIV (line 276). The correct numbers are also shown in table 1.
Reviewer number 2: Zoltan Vajo

Overall, this is an important topic with a legitimate aim. However, I am little bit concerned about the study design and sample size. Selecting health care workers as study subjects is known to introduce at least some bias and decreases the ability to extend the findings to the general population. The interpretation of the results is even more complicated by the fact that some of the subjects received TIV and some did not-if I understand it correctly.

Comment to the reviewer:

The concern of this reviewer would make sense if the purpose of our study would be to represent the immune response to a vaccine in a whole population. However such is not the case and we established our specific aim since the title of the study and refer only to hospital employees. We performed the study in personnel of a third level hospital for respiratory diseases because these people are potentially exposed to more respiratory virus than open population and have been vaccinated with influenza seasonal vaccine without evaluation of the immune response before and after the new vaccination schedule. The study of immune cellular and antibody responses in such individuals, we believe, can contribute to the knowledge of the effect of annual seasonal or pandemic vaccination on the humoral and cellular immune response.

Q1
Introduction: The term “H2N3” seasonal vaccine” must be an error. Similarly, in the Methods section, what is the meant by “H1N2 seasonal vaccine?

A1 We have corrected these mistakes and added the vaccine composition for 2008 and 2009 influenza seasonal vaccines (line 109-114). We substituted the incorrect terms H2N3 and H1N2 for 2008 and 2009 trivalent vaccines (lines 255-258).

Q2
Methods: Please specify the NCBI website used

A2
We apologize for missing this information before and according to your observation we have completed the website information and have added it as a reference (reference 15).

Q3
There is no description of how the sample size was determined.

A3
To determine the sample size in our study, there were two considerations. First, at the time of the study, the vaccinated population at the INER –the hospital where the study was held- was of 500 workers, therefore with an α= 0.05 and a >99% of confidence interval the
sample size originally calculated for the study was of 217. However, this sample size was not possible to assess by flow cytometry analysis. Then, the sample size calculation was based on preliminary findings by our laboratory in which we observed a positive proliferative response of 3.8 (with SD = 4.0) to pandemic H1N1-peptide. Assuming a normal distribution of such specific response to the influenza vaccine, with an \( \alpha = 0.05 \) and 90\% of statistical power (confidence interval) and by using a two-tail t test, we calculated \( n=38 \) for our study. The sample size for antibody titers was based on the European recommendations for studies evaluating influenza vaccine for initial dose finding studies (at least 50 subjects per group, reference 19). Therefore \( n=60 \) was defined.

Q4

Results: In general, the result section is a mixture of results and discussion: I would recommended removing all explanation and references from the results section and remove them to the discussion part, which the manuscript currently does not have.

A4

Yes, the manuscript has not a result and discussion section separately, but these sections are presented as a combination of results and discussion. This kind of format is optional, as it is mentioned in BMC infectious Disease instructions for authors: “Results and discussion may be combined into a single section or presented separately”.

Q5

The part on the potential effect of prior seasonal vaccination is speculative and not supported by the findings-Why were the subjects divided into subgroups based on the presence or absence of pre-existing antibodies, and not for previous vaccination status? There were only 14 subjects who did not receive TIV.

A5

We appreciate the observation by the reviewer and recognize that our suggestion of the potential effect of pre-existing antibodies was speculative since not significant difference was observed. In consequence, we have removed the part of the paragraph with such speculative conclusion (please see answer 4 to reviewer 1) and we have added a sentence saying that a definitive conclusion cannot be drawn from these results (line 305 -307). Regarding the question of “why the subjects were divided into subgroup based on the presence or absence of pre-existing antibodies and not for previous vaccination status”, the rationale of our response is the following. As we mentioned in the manuscript, it has been reported that recent seasonal vaccination can decrease antibody responses to the pandemic A/H1N1 vaccine, but the mechanism of such phenomenon has not been explored (line added: 293-294). Therefore, we investigated whether the pre-existence of anti-HA1 antibodies played a role in the decrease of the pandemic A/H1N1 vaccine antibody responses; therefore we established our criteria of comparison on the titer of antibodies instead of if individuals were or not previously vaccinated.
The observation that only 14 subjects did not receive TIV is incorrect. We apologize for the lack of clarity and the mistake in the grouping of one of the participants (see answer 8 Reviewer 1). The correct numbers are shown in table 1 and in the main text; 45 subjects (75%) were previously vaccinated with 2009/TIV (lines 109, 255), 5 (8.3%) with 2008/TIV (lines 111, 256) and 50 (83.3%) with 2008 or 2009 TIV (line 275). In fact, only 10 subjects did not receive TIV. The subjects of study were randomly selected from the hospital workers, who intended to receive the pandemic influenza vaccine through a public institutional vaccine program (line 106-108); therefore, subjects with or without previous seasonal influenza vaccine were not equally distributed since hospital workers are permanently under institutional vaccine program.

Because of the small numbers of subjects without previous TIV vaccination (10/60) we did not see any statistical differences when we compared antibody titers, IFN-γ production, nor CD8 T cell proliferation; although they did not receive TIV, they were recently exposed to influenza patients and that could explain the similarity in their immune responses with the TIV vaccinated population.

Q6
The finding that the vaccine failed to meet immunogenicity responses required for licensing is surprising, to say the least.

A7
This is a good point, commented by the reviewer. Certainly that finding was not expected. However, we believe that the previous influenza vaccination (most of our studied subjects had received previous annual seasonal influenza vaccines) could affect the response to the virus, inducing a humoral response with higher antibody titers driven to the common epitopes rather to the specific epitopes of the new virus-vaccine (see reference 1 and 2 below). If that is the case, we propose that in populations occupationally exposed and previously vaccinated with the seasonal influenza vaccine, the increase of CD8+ T cell proliferation in response to specific epitopes could be a useful parameter to indicate protective immunity induced by vaccination. This proposal was set in our conclusion. Finally, we think the institutional vaccination programs are not the most important, but there should also be the evaluation of the impact of repeated vaccination.

Editorial Requirement
After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

Quality of written English: We have followed your advice and got assistance by a professional editing service. The abstract was modified and reduced to 250 words.