Author's response to reviews

Title: Genotype distribution of human papillomavirus (HPV) in histological sections of cervical intraepithelial neoplasia and invasive cervical carcinoma in Madrid, Spain.

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Author's response to reviews: see over
Dear Sir:

Please find hereby attached for your consideration the review of our work entitled “Genotype distribution of human papillomavirus (HPV) in histological sections of cervical intraepithelial neoplasia and invasive cervical carcinoma in Madrid, Spain.”

Following your editorial Requests:

1. Informed consent was not required for this study since the results presented here come from HPV genotyping routinely performed, as an adjunct to the cytological and histological study, in an anatomical pathology laboratory. The detection and genotyping was done in clinical setting and in order to protect patient confidentiality the identifiers of personal data were always deleted. The study was supervised by the ethical committee of our hospital. The name of the committee is: “Comité Ético de Investigación Clínica (CEIC)”. Hospital General Universitario Gregorio Marañón. C/ Doctor Esquerdo, 46 - 28007 Madrid.

2. Competing interests: The authors declare that they have non-financial competing interests.

3. Authors' contributions: BGE participated in the design of the study, global data acquisition, analysis and preparation of the first draft. EMR revised critically the first and final draft, participated in the data acquisition, and conceived the desing of the study, EAF conceptual and planning of the design of the study, surgical pathology data acquisition and analysis. All authors read and approved the final manuscript.

Answers to the reviewers and changes made in the manuscript:

R1.Q1: The Introduction needs much more references. For example, the classification of HPVs, the number of mucosal types, the available vaccines, the variation of oncogenic potential, etc, must be supported with references.

A.A1: We have added seven references to the manuscript. Some paragraphs in the introduction have been expanded. All changes are marked in yellow.

R1.Q2: Second paragraph: Co-infection is known to increase the risk of CIN but not cervical cancer. It is more accepted that co-infection is a marker of immunity. However, in cervical cancer samples, co-infection is rarely detected. Even if a woman is infected with multiple types (showing a potential problem in term of immunity), only type 16 or 18 for example cause cancer. The impact of co-infection should be better explained with references.

A.A2: That's true. Follow-up studies suggest that the presence of multiple types does not influence the course of HPV infections. We added the following reference: “Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM.: A 2-year prospective study of
human papillomavirus persistence among women with a cytological diagnosis or atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis 2007; 195:1582-9.”.

R1.Q3: I suggest reducing the Introduction regarding statement that goes outside the focus of the study. For example, there are statements about the impact of the vaccine on the potential genotype replacement which I think is inappropriate, because this is not the scope of the study but also because it is not really supported by the literature.
A.A3: We think that these paragraphs are necessary to keep them because they justify our work. For example in our environment it is important to know how many cases are related to the 58 genotype since current vaccine would not been preventing these infections.

R1.Q4: I do not understand the forth paragraph of the Introduction.
R1.Q5: The introduction must describe the general epidemiology of HPV in Spain (Spain is well known to have low prevalence of HPV in women with normal cytology). This should be presented.
A.A4-5: We have added the information requested and provided two more references relating to the prevalence of HPV infections in Spain.

R1.Q6: Can you give more details about the “stratified and not random consecutive samples”? What is it exactly?.
A.Q6: We want to say that this is a longitudinal and retrospective study in which we have collected ALL the diagnoses cases consecutively since January 2005 to July 2010 and not taken randomly. We have rewritten this paragraph.

R1.Q7: The Method section should provide the details about the analysis. For example, different methods were used to estimate % in tables 2 and 4. This should be explained. 95% confidence intervals should also be provided for estimates (percentage).
A.Q7: We added the following paragraph: “Two methods were used to estimate the frequency of HPV positivity: percentages referred to the number of lesions infected by one or several genotypes and percentages referred to the total number of virus detected in each kind of lesion and in the total of them.”

R1.Q8: Results about the % of HPV positivity are given in this section but this is an important result and it should be presented in the Result section (not in the method). And this % is estimated at less than 47% (533\1137). It would have been interesting to provide % of HPV positivity for the total number of abnormal specimen (1137) stratified according to lesions (Benign, CIN1, CIN2-3, etc.). Also is 47% is comparable with other studies?
A.Q8: We have moved this information to the first paragraph of the results section.

R1.Q9: It is stated in this section that Inform consent was not required because that HPV genotyping is routinely performed. Then you described in the next section how the detection and genotyping was done. I don’t understand? Are detection and genotyping done in clinical setting? Or is because these data were collected in another study? Need more details.
A1.Q9: The detection and genotyping was done in clinical setting and the Informed consent is not necessary in the protocol of our hospital. However, our study was supervised by the ethical committee of our hospital in order to preserve the anonymity of the patients.

R1.Q10: ... the Result section is written as an output and not as a manuscript. Authors don’t need to repeat all the data presented in tables. Only essentials should be presented in the Results section. It must be considerably reduced as most of the information is presented in tables.
A.Q10: We consider it necessary to specify each of the results as we have done in order to the rigor of the data.

R1.Q11: Table 2 et 4: replace “genotipe” by genotype. Replace n# by N and define in the footnote.
A.Q11: Done

R2.Q1 and Q2: 1. The number of included benign lesions and invasive cervical cancer cases are too small (19 and 7). 2. The majority of samples consist of CIN 1 cases, which is well known to regress spontaneously, especially in young women. Therefore, the more important, CIN 2-3 and ICC cases, are in minority.

A.Q1 and Q2: As we say in the manuscript we have collected all of the cases from our service between January 2005 and July 2010. It was not our intention to make a selection of cases that were representative. We have made a retrospective study of real casuistry in that period of time. The low number of invasive carcinomas, 7, is in accordance with the low incidence of this cancer in our country: 2.7 / 100,000 women-year.

R2.Q3: How do authors explain cases of ICC with the presence of only HPV 11. It has been confirmed, that cervical cancer cases are in 99,7% high-risk HPV positive. It would be interesting to know, what kind of histological subtypes were those HPV 11 positive cervical cancer cases?

A.Q3: This case is unusual one. Recently it has been related with the verrucous carcinoma. “Mod Pathol. 2012 Jun 8. doi: 10.1038/modpathol.2012.91. Comprehensive analysis of human papillomavirus prevalence and the potential role of low-risk types in verrucous carcinoma.” Our case was a certain verrucous pattern.

R2.Q4: The conclusion about limited effectiveness of HPV vaccination based on this study is questionable.

A.Q4: We agree with the general view that the quadrivalent vaccine (6, 18, 11, 16) can protect 70% of HPV infections whereas there is a possible cross-reactivity with genotypes 31, 33 and 45. However, we want to alert the risk in cases of infections with HPV 58 that it is high in our community and that such a vaccine would not protect it.

R2.Q5: There are also some grammar and spelling mistakes.

A.Q5: We have reviewed the manuscript and made corrections that are highlighted in yellow.

The work now consist of 13 pages and 4 tables (abstract included). Our current address, telephone and fax number are:

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We thank you for your attention and remain expectant for your response.

Yours faithfully,

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