Author's response to reviews

Title: Twelve years' detection of respiratory viruses by immunofluorescence in hospitalised children: impact of the introduction of a new respiratory picornavirus assay

Authors:

Christine D Sadeghi (c.sadeghi@students.unibe.ch)
Christoph Aebi (christoph.aebi@insel.ch)
Meri Gorgievski-Hrisoho (meri.gorgievski@ifik.unibe.ch)
Kathrin Mühlemann (kathrin.muehlemann@ifik.unibe.ch)
Maria Teresa Barbani (mariateresa.barbani@ifik.unibe.ch)

Version: 5 Date: 23 December 2010

Author's response to reviews: see over
Dear Editor

Thank you for your suggestions and comments. Below we list the point-by-point responses to the concerns.

Response to Reviewer’s report:

1. Picornaviruses were the most common viruses missed with the DFA (see Section "Results: Rate of viral detection": last sentence). With the exception of ADV, all viruses had more positives with Luminex than with DFA. Respiratory picornaviruses were the most frequent viruses detected among the additional positives (57 from 78) with the Luminex, followed by six additional positives each for RSV and IFA. In addition, several samples were positive for COR or PIF4, two virus species that we did not search for by DFA.

2. With Luminex we found 10.2% of samples harboring two respiratory viruses and 0.8% containing three respiratory viruses. This is in stark contrast with the 0.8% of samples positive for two viruses found by DFA. Interestingly, in 81% of all samples with two viruses detected by Luminex (21/26), one of the viruses was a respiratory picornavirus (added in Section “Results: Codetection of respiratory viruses”). The role of double infections is addressed in the section "Discussion".

3. Concerning HMPV we agree with the reviewer and removed our remarks about the lack of a cyclic appearance from the discussion.

4. We decided to divide Figure 2 in two sub-figures. We added a sub-figure B with a different scale, as suggested, to decompress the graph for the low incidence viruses (ADV, IFB, PIV). Sub-figure A is the same as the previous Figure 2. We left this figure unchanged in order to compare the incidences of all viruses. HMPV is shown in detail in Figure 3.

Responses to Associate Editor’s comments:

1. The relative sensitivity of the DFA for the different viruses is an issue (see also response to Dr. Huemer). The additional positives with PCR were in more than 70% of cases respiratory picornaviruses and in the multiple infections more than 80% contained respiratory picornaviruses. The limitation of DFA is in the detection of respiratory picornaviruses mostly and it is questionable if this is a limitation or advantage (see Discussion section).

2. We agree that the sensitivity of DFA would be lower in an adult population. We added in the conclusion that our findings
concern a paediatric population.

3. About quantitative PCR for respiratory viruses: we think that the interpretation of results of quantitative PCR-Methods is sometimes difficult, even in homogeneous samples such as plasma. There is much discussion about at what viral load a therapy should be started. The interpretation and the comparison of quantitative results would be more difficult in respiratory specimens which are not homogeneous, not standardized and are taken from different people in multiple ways (different swabs or aspirates) and not always correctly. DFA is also the only method that allows assessment of the quality of respiratory samples.

4. About cost: In our laboratory, the cost of DFA is lower than the cost of PCR. Most of our staff are able to do both DFA and PCR tests. New workers are taught by more experienced staff so that the experience doesn't get lost. Preparing and staining samples for DFA is very easy and takes only a few minutes. The correct reading of the slides requires experience which, thanks to our large volume of DFA testing, we are able to maintain. Moreover in our experience PCR-Methods need skilled workers as well, as even with a standardized PCR there is always an important risk of contamination especially when you run many samples from many different children on the same plate. Additionally, in Switzerland, the price of a DFA for the patients (as reimbursed by the health insurance) is 6x less than that of a PCR method.

6. We are not preparing a separate publication on comparison of DFA and RVP as there have already been several such publications and a new version of the RVP is now available.

7. Our DFA for respiratory picornaviruses does not allow the differentiation between rhinoviruses and enteroviruses. We added this point in the Section "Methods".

8. Concerning Figure 2: see response to reviewer.

Responses to Editorial Requests:

1. Concern ethics committee approval: see section "Methods" of the manuscript

2. Informed consent: we benefited from an "exemption from obtaining informed consent" from the University Hospital Ethics Committee because of the retrospective study design. We analysed existing results of tests which were carried out during 12 years in the interest of hospitalized patients to diagnose their illness. In our hospital all hospitalized patients have to declare at the admission if their data can be
used for research studies in an anonymous way, and none of the patients whose samples were used in our study refused.

Yours faithfully,

Meri Gorgievski-Hrisoho