Letter

Real-time reverse-transcription PCR in the diagnosis of influenza A (H1N1)v in intensive care unit adult patients

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Published: 7 December 2009
This article is online at http://ccforum.com/content/13/6/428

Critical Care 2009, **13:**428 (doi:10.1186/cc8164)

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Regarding the interesting article on novel influenza A (H1N1)v infection in intensive care adult patients with severe respiratory failure recently published in *Critical Care* [1], we should like to make the following comments.

The authors found that real-time RT-PCR for novel influenza A (H1N1)v virus in nasopharyngeal swabs on intensive care unit (ICU) admission was negative in four patients (12.5%) who later had a positive PCR result in respiratory secretions obtained at intubation, and concluded that 'a negative PCR result at admission should not exclude influenza A (H1N1)v due to the presence of false negative results in at least 10% cases' [1]. We of course understand what the authors meant. In our opinion, however, this assumption is formally inaccurate, and is somewhat misleading, as it might be inferred that real-time RT-PCR gives at least 10% of false negative results in patients with overt symptomatic influenza requiring ICU admission.

The negative results reported by the authors cannot be considered true false negative RT-PCR results, as samples were not tested in parallel by a different assay yielding a positive result – in fact, RT-PCR, which was used at participating centers, is currently the standard method for the diagnosis of influenza. Optimal sensitivity of RT-PCR and rapid antigen tests is achieved when upper tract respiratory specimens are collected within the first few days after the onset of symptoms, as appeared to be the case for the above-mentioned patients. Inappropriate sampling or specimen processing or suboptimal sensitivity of the PCR assay used most probably accounted for the negative results. No speculation on this matter can be made because the microbiological information given to the readers was rather scarce.

The possibility of false negative RT-PCR results for influenza A (H1N1)v in severely ill patients requiring admission to ICUs is a very important issue that must be further investigated.

Authors' response

Alejandro Rodríguez, Josep-Maria Sirvent, Lorenzo Socias, Sergio Martínez-Cuéllar and Jordi Rello, for the H1N1 SEMICYUC Working Group

We are grateful to Gimeno and Navarro, since this allows us to clarify diagnosis in intubated patients.

We reported that one out of six patients intubated with primary viral pneumonia had initial negative nasopharyngeal RT-PCR for (H1N1)v on ICU admission and later become positive (one patient required three samples) in respiratory secretions [1]. We agree that RT-PCR is clearly preferred to a rapid diagnostic test. In another study, however, four false negative results and two equivocal results were observed with the Center for Disease Control (H1)v assay [2]. The pretest probability of disease is an important issue. Our findings are consistent with further series of critically ill patients [3]. In pneumonia, viral load in the nasopharynx is lower and is concentrated in lower respiratory secretions, which should be the preferred specimen. The presence of epithelial cells is required and bronchoalveolar lavage should be the ideal technique. Unfortunately, bronchoalveolar lavage is not feasible in many patients due to severe hypoxemia and concerns to avoid generation of aerosols. Tracheal aspirate,

ICU = intensive care unit; PCR = polymerase chain reaction; RT = reverse transcriptase.

still suboptimal, is a reasonable alternative to nasopharyngeal swabs, provided that a specific laboratory protocol is followed.

Our findings alert us to suboptimal performance of RT-PCR for diagnosis of influenza A (H1N1)v pneumonia, which is important in the decision-making process. Only 25% of our patients started antiviral treatment within 48 hours of influenza onset. In high-risk groups (for example, pregnant women), pneumonia patients or critically ill patients during a pandemic, negative results from RT-PCR should not exclude this cause. The antiviral treatment should be maintained until the clinical diagnosis is confirmed by a new RT-PCR sample.

Competing interests

The authors declare that they have no competing interests.

References

1. Rello J, Rodríguez A, Ibañez P, Socias L, Cebrian J, Marques A, Guerrero J, Ruiz-Santana S, Marquez E, Del Nogal-Saez F, Alvarez-Lerma F, Martínez S, Ferrer M, Avellanas M, Granada R, Maraví-Poma E, Albert P, Sierra R, Vidaur L, Ortiz P, Prieto Del Portillo I, Galván B, León-Gil C; the H1N1 SEMICYUC Working Group: **Intensive care adult patients with severe respiratory failure caused by influenza A (H1N1)v in Spain.** *Crit Care* 2009, **13:**R148.
2. Ellis J, Iturriza M, Allen R, Bermingham A, Brown K, Gray J, Brown D: **Evaluation of four real-time PCR assays for detection of influenza A(H1N1)v viruses.** *Euro Surveill* 2009, **14:**pii 19230.
3. The ANZIC Influenza Investigators: **Critical care services and 2009 H1N1 influenza in Australia and New Zealand.** *N Engl J Med* 2009, **361**. [Epub ahead of print].