Reviewer's report

Title: The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study

Version: 2
Date: 21 March 2014

Reviewer: H Rogier van Doorn

Reviewer's report:

This paper describes the correlation between detection of EV71 by real-time PCR in blood with demographic and clinical data. The questions posed by the authors are well defined and straightforward.

Major compulsory revisions:

The testing methods used require more detail, i.e. there is no reference or description of the real-time PCR for EV71 detection.

Only in the discussion does it become clear that only one timepoint per patient was used; this should be made clear already in the abstract, described in detail in the methods section (with a table with samples collected per day of illness) and be reflected in the way the results are presented in the abstract and body of text. The authors should also try to describe how these timepoints were chosen, or whether they used residual specimens from the haematology/chemistry labs. This is my main concern with the findings and conclusions of this paper. It is not unlikely that this causes considerable bias in the results, e.g. severe patients more likely to be sampled later (or come to the hospital later, or be transferred to the tertiary centres where the authors enroled their patients, etc) as they stay in the hospital longer, whereas mild patients would be expected to be discharged earlier. The authors should add a careful multivariate/logistic regression analysis of their findings to correct for this.

Minor essential revisions:

There are a lot of language and grammatical errors and the manuscript may benefit from additional review for this particular purpose (I have not made any attempts to point these out one by one, as my English is not perfect).

Essential revisions, point by point (most have to do with my major concern stated above)

No line numbering was included, please change for later reviews

As stated above, the results section of the abstract does not properly reflect the sampling method. A sentence as "two thirds of viremia occurred in within the first three days of infection" only makes sense if there is a denominator and if we know how many samples were collected before and after day 3.

No association between severity and viremia was found, but if severe patients
were sampled later and viremia only occurs early in disease than this may have been an artefact. The risk of complications increased if viremia was detected after day 4, but is the chance of sampling after day 4 not also higher in severe patients? A thorough multivariate analysis is required.

The background section can be more to the point, e.g. in third and fourth paragraph: 'the professionals', 'several health policies', 'made some success', 'still did not identify', 'have been investigated for a long time, but no conclusion', 'still unclear'. Better to describe well what we know, and what assessment of viremia may add.

In the methods section 'patients and clinical data collection', 'sampling and laboratory testing' should be grouped together for clarity. Explain that 1 blood sample per patient was collected for viremia assessments and how these timepoints were chosen. Similar for swabs: Was EV71 detection by PCR/culture done on throat swabs taken on admission? Were multiple swabs taken?

Reference the grading system used.

In the results section:
Age is better expressed as median, than mean.
Classify the patients by grade first and then further explain in more detail, particularly for the grade 3 and 4 patients (as now only grade 1 and 2 are further detailed)
Define 'acute stage', HFMD patients usually progress to severe disease within 72 hours, but samples up to day 7 are included in this study.
Include how many samples were negative per sampling day (e.g. in a table).
The sentence "The peak... rapidly afterwards" cannot be understood in the context of single sampling timepoints and without knowing the numbers of negatives. Similar for the last paragraph on page 8, which should first be corrected for severity, day of illness etc. I have commented on the conclusion of the paragraph of page 9 in the abstract section.

The discussion and conclusion should be rewritten entirely after the extra analyses have been done. The authors should take care to choose their wording so as not to suggest longitudinal sampling and kinetics thus not use terms as "peak of viremia", "at day 4, viremia halved" etc.

We do not have information on how many patients were sampled at day 7 and therefore cannot interpret what it means that no viremia was detected.

The authors discuss the value of viremia in EBV/CMV vs dengue/noro/hanta viral infections and state that EV71 behaves more like the latter, which basically is a description of the difference between chronic and acute viral infection.

The authors do not describe the possible pathophysiological role of viremia/haematogenous seeding or retrograde axonal transport in spread to the CNS.
Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests