Author's response to reviews

Title: A Prospective Study Comparing Quantitative Cytomegalovirus (CMV) Polymerase Chain Reaction in Plasma and pp65 Antigenemia Assay in Monitoring Patients after Allogeneic Stem Cell Transplantation.

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Version: 2 Date: 25 September 2006

Author's response to reviews: see over
To the Editor,
BMC Infectious Diseases

Rome 25 September, 2006

MS: 1806109335104434
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Dear Editor,
Enclosed please find our revised manuscript in light of the reviewers' and editorial comments. We provide a cover letter giving a point-by-point response to the concerns. I look forward to hear from you soon, and I thank you in advance for your kind attention.

Best regards

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Editor:
1) Ethical approval and patient consent was obtained: see patients and study design, page 5
2) Definition of CMV disease was given: see page 5, written in red

Review 1

1. For the data that is presented on page 7. Looking at the detection by antigenemia vs. COBAS and the correlations between antigenemia and COBAS - the data should also be assessed using only the samples in which the patient is not on therapy.

Response: correlations between antigenemia and COBAS has been assessed using only the samples in which the patient is not on therapy (see page 7, Correlations between pp65 antigenemia and Cobas CMV PCR assays, written in red)

1bis The rate of decline in antigen and DNA, as the authors state later in the paper, is not the same. So this could influence the results, and what would helpful is to know when the patient is not on pre-emptive therapy how well do the DNA and Ag agree.

Response: in tables 1, 2 and 3 every sample with a dot indicates when the patient is not on pre-emptive therapy

2. Would analyze all data, and then those samples collected on therapy when assessing the sensitivity of the PCR and antigenemia, the authors compare the tests to each other. It would be helpful to see the data defining any positive test, either antigenemia or PCR as the gold standard and then comparing the antigenemia and PCR tests to this gold standard.

Response: all data have been analyzed and comparisons have been made has indicated by the reviewer (see page 7, Detection of CMV by pp65 antigenemia, and by the Cobas CMV PCR assays, written in red)

3. In the discussion, there needs to be some comment made about the difficulties in interpreting the "clinical" sensitivity of the PCR assay when pre-emptive therapy was based on antigenemia. It may be that with the antigenemia test the additional positives just lead over to treatment and do not actually prevent disease. The design of this study can not assess that possibility. The three patients with disease leads one to believe that there may be patients that will have disease that are PCR negative, as have been shown in the literature. However, a discussion of the limitation of the study design needs to be included.

Response: comment to this regard has been made, see Discussion, page 10, written in red

4. Figure 1, Both the antigenemia and the PCR should be log transformed when analyzing the correlation between the two tests.

Response: antigenemia and the PCR values have been log transformed (see Figure 1)
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The criteria on page 5, - In the method/section - criteria for the diagnosis of CMV disease the sentence is a little confusing as written, is it a positive biopsy or a positive culture or are we doing PCR on the biopsy material? Not clear as written. Also is infection defined as a positive antigenemia or as a positive antigenemia or PCR?

Response: Infection and disease have been written and reported on page 5 and written in red:

Criteria for the diagnosis of CMV infection and disease

Cytomegalovirus infection and disease were defined according to published recommendations (27). Active CMV infection was defined as the detection of pp65 antigen in leukocytes and/or presence of CMV DNA in plasma. CMV enteritis was defined as the presence of gastrointestinal symptoms, findings of macroscopic mucosal lesions on endoscopy, and demonstration of CMV infection (by culture, histopathologic testing and immunohistochemical analysis) in biopsy samples taken from colon. Detection of CMV by PCR alone was considered insufficient for the diagnosis of CMV intestinal disease.

2. Patient 0138.1 who developed CMV disease, as could the authors provide some comment as to why the PCR antigenemia was positive weeks after therapy was stopped. Details would be helpful.

Response: details have been reported on page 9, section Patients with CMV disease, and written in red.

2bis. Also similarly do the authors have an explanation for the disconnect between the antigenemia and PCR as the antigenemia is coming down from weeks +49 to +63, the PCR is actually increasing quite dramatically.

Response: an explanation for the disconnect between the antigenemia and PCR has been reported on pages 11 and 12, written in red.

Review 2

1a) "CMV disease" and "CMV organ localization" are used with the same meaning, which is not (see results, clinical outcome, p 7). "CMV organ localization" is the pathological demonstration of CMV infected cells in tissues of a given organ, while "CMV disease" refers to biopsy-proven organ involvement in the presence of specific clinical symptoms.

Response: we agree with the referee about the definition of CMV disease (see Criteria for the diagnosis of CMV infection and disease, page 5, written in red).

Cytomegalovirus infection and disease were defined according to published recommendations (27). Active CMV infection was defined as the detection of pp65 antigen in leukocytes and/or presence of CMV DNA in plasma. CMV enteritis was defined as the presence of gastrointestinal symptoms, findings of macroscopic mucosal lesions on endoscopy, and demonstration of CMV infection (by culture, histopathologic testing and immunohistochemical analysis) in biopsy samples taken from colon. Detection of CMV by PCR alone was considered insufficient for the diagnosis of CMV intestinal disease.
1b) Correlation between antigenemia and plasma PCR appears gross, rather than fair-good or good. In particular, none of the dots in Fig. 1 appears even close to the regression line. The Authors should revise their statistics reporting r-e2 coefficient instead of r.
Response: statistic has been revised by reporting r-e2 coefficient instead of r, and results are reported on page 7, written in red.

1c) The results of this study point to a different kinetics of antigenemia and viral DNA in SCT. This observation has been reported in SCT and other transplant settings as well, indicating a better adherence of viral DNA levels in blood to the level of active viral replication. Although the Authors mention this aspect, they should develop this line of discussion (also by carefully revising the reference list which is missing key papers) instead of forcing a correlation between the two virologic parameters.
Response: the line of discussion suggested by the reviewer has been added, page 11, lines 6-10, written in red. References have been added (n 32,33,34).

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1a) Any data not significantly different means equal. Thus, sentences like "pp65-positivity was earlier, although not significant than plasma PCR" (abstract) does not stand and must be removed.
Response: the sentence has been removed from the abstract.

1b) Was the study powerful enough to reveal all possible differences? Please, report how the sample size was calculated.

1c) Kappa is sometimes 0.49 and sometimes 0.48; r is sometimes 0.496 and sometimes 0.503.
Response: the kappa value has been modified, page 7, written in red.

1d) What about the outcome of the 3 pts with CMV organ localization? Why was the virus searched for in biopsy? Specific symptoms or occasional finding in biopsy performed to evaluate intestinal GVHD? Differentiating between bystander positivity and potential cause of disease is mandatory.
Response: detailed case reports are reported in section “patients with disease”, pages 8 and 9.
1e) Acyclovir as "antiviral" prophylaxis? ACV is specific for HSV and VZV. It has also a moderate effect on CMV, but is not a pan-virus drug!
   Response: has been modified in acyclovir for herpes simplex virus infections, page 5, written in red.

1f) Kaplan-Meier is mentioned in M&M and no results are reported. Delete.
   Response: KM has been deleted from M&M.

1g) I'm not sure that the small numbers of pts in each group can support the analysis of incidence of positive pp65 and plasma DNA according to type of transplant.
   Response: even if the number of patients is small, we believe that the incidence is related to time and not to sample size.

Discretionary Revisions (which the author can choose to ignore)
1a) Antigenemia and plasma PCR have been determined using commercially available and well established methods. Skipping the detailed description of the two techniques would shorten the paper without impairing the message.
   Response: the section dedicated to virological assays have been reduced.