Author's response to reviews

Title: CMV infection of liver transplant recipients: Comparison of antigenemia and molecular biology assays

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Version: 2 Date: 2 May 2001

PDF covering letter
Dear Dr. Carole Mongin,

I’m writing to you in reply of your email of 27/3/2001 concerning our submission of the manuscript ‘CMV infection on liver transplant recipients: comparison of antigenemia and molecular biology assays’.

As requested in your letter, we have now completed a revised manuscript, which we are now submitting for your appreciation.

As already mentioned in a previous letter, we read with great pleasure all comments made by the referees, to whom we are grateful as we think their comments have provided us with valuable insights as to improve our manuscript. Accordingly and as requested, I would like, to provide a point-by-point answer to the referees’ comments:

1) Kirsten StGeorge Review

- The rate of CMV disease among the patients in the article is indeed, very high, and does not reflect the true incidence among the units’ patients. As now mentioned in the Materials & Methods section (page 5) and the Discussion (page 14), the incidence merely reflects our concern that we had in such a small number of patients an equally representation of patients with and without CMV disease, so that comparisons could be meaningful. The true incidence of the disease among the units’ patients is now also stated in the discussion (page 14).

- The frequency of testing after hospital disclosure is now clearly mentioned in the materials and methods section. Frequency before disclosure was already clearly stated. No trial is made to homogenize what is not in its origin (whenever clinically relevant), as this reflects patients’ voluntary referral to the clinic due to occasional presence of symptoms at a time other than routine checks. In any case, the frequency of testing for each patient can be clearly inferred from figures 1 and 2.

- Assays descriptions have now been reduced according to the referee’s recommendations.

- Mention to the internal control in CMV Nuclisens was already present on the materials and methods. It was removed due to the reduction on the assays
description, but a reference to this, and to the respective results is now made on the results section.

- Prophylactic treatment was never administered to any of the patients. The error on materials and methods section has now been corrected. Also there is now a clear reference to the patients that were retransplanted and the respective reasons on the materials and methods section (page 6). The time of retransplantation is now also clearly shown on the graphs of figures 1 and 2.

- Dose and duration of ganciclovir therapy is now mentioned in materials and methods (page 7-CMV disease definition). Duration of therapy is now also clearly shown on the graphs of figures 1 and 2.

- Disease definition has been restated to clearly distinguish what is the general definition of CMV disease that was used as guideline, and what was our practical approach to disease identification. As the referee’s point’s out, the need for a quick intervention does not allow the everyday use of all implications of the general guideline. We believe the new text is now clearer in all its implications.

- The “claim” that 9 patients had reactivated CMV infection has been removed. It was indeed not our intention to claim it to be a fact, but rather to mention it as a possibility.

- The statement “antigenemia was the first test to become negative” has been reformulated to reflect the referee’s concern. The issue was again caused by an unfortunate sentence. It was our intention to state the opposite, i.e., antigenemia was never the last test to become negative.

- Table 3 was misleading due to the use of variable exponents. This even caused us to incorrectly read it. It has now been corrected, as well as all sections that referred to it. We apologize for the mistake.

- Tables 1, 2 and 3 have now been statistically analysed. This had not been done before, because we felt (has it is now obvious from the p values observed) that to make statistically relevant observations we will need to extend the present work beyond the presently included 10 patients. That is however the aim of another work that we are currently pursuing.
• Mention to other publications referring a higher viral load and an earlier onset of infection among patients who show CMV related disease is now included (page 15).

2) Paul Evans Review

• Table 3 was misleading due to the use of variable exponents. This even caused us to incorrectly read it. It has now been corrected, as well as all sections that referred to it. We apologize for the mistake.

  ➢ Sensitivity, specificity and positive and negative predictive values for all 3 tests used have been calculated and are shown on table 4. We thank the referee for pointing this to our attention. Although it seemed an obvious thing to do, we opted at first not to calculate these statistics due to the scarcity of patients studied. However, the results turned out to be quite interesting.

  ➢ The aim of the paper was never to compare the use of different clinical materials, but rather the use of different commercially available methods. Otherwise the same methodology should have been used on the various body fluids. For this reason, we do not feel a specific need to emphasize the different body fluids used by the different methods assayed, as this difference is inherent to the commercial kits tested. It is true though that this should be clearly stated in the materials and methods section. For this reason, we have rephrased this, and believe it is now clearly mentioned. Also, the different targets are now clearly mentioned not only on the materials and methods section, but also on the discussion (end of page 14), as a response to the referee’s minor point #1.

  ➢ The gene targeted by the cobas amplicor CMV monitor is now stated in the materials and methods section

  ➢ Repeated sentence on page 2 was eliminated. We apologize for the embarrassing mistake.

  ➢ Title has been corrected according to the referee’s suggestion.

  ➢ The text has been thoroughly checked against a spell checker. We hope all mistakes have been purged. Again we apologise for these mistakes.
3) Also, we would like to answer, as requested, the competing interests questionnaire.

- We hereby state that:
  - None of the authors has in the past five years received any fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this paper. The only exception being the partial funding of the work both by local branches of Roche and Organon Teknika, in the form of free kits donations, as clearly stated in the acknowledgments section of the paper.
  - None of the authors holds any stocks or shares in organizations that may in any way gain or lose financially from the publication of this paper
  - None of the authors has any other financial competing interests in relation to the publication of this paper
  - None of the authors has any non-financial competing interests in relation to the publication of this paper

4) Finally, and as requested, we include a list of the major alterations included in the new manuscript version:

- Title has changes according to a referees suggestion
- Abstract now includes a M&M section. Last Sentence of the results section was added.
- Second sentence of the M&M section added
- Second paragraph of page 6 was added
- Third paragraph of page 7 (section “CMV definition” of M&M was added)
- Description of the molecular biology assays was reduced to a minimum
- Added a statistical analysis section on M&M
- 3rd sentence of page 10 (“a similar control..”) was added
- Second sentence of the second paragraph (“According to the serostatus…”) was changed
- Last 2 sentences of overall test performance (page 11) were changed
- First sentence of page 12 (“as a result…”) was changed. Last sentence of the same paragraph was changed to include “although the difference did not reach statistical significance”.
- Second sentence of last paragraph of page 12 (“Again, this cut-off…”) was changed
- All text on page 13 has been added.
Page 14, first paragraph from 4th sentence on (“It must however be stressed…”) is new text. Next paragraph from sentence 5 to the end of paragraph (“This curious observation…”) is new text.

- Last paragraph of results is new text
- The sentence “Furthermore, despite its qualitative nature…” at the end of the conclusion section is new.
- New references added (18-22).
- Exponents on table 3 normalized to $10^3$.
- Table 4 is new
- Fig 2 has been changed to include duration of treatment, and retransplantation events

Hopping this will fulfil your expectations, and waiting for your reply,
Yours Sincerely,
José Cabeda.