Author's response to reviews

Title: Effect of Ganciclovir for the Treatment of Severe Cytomegalovirus-Associated Pneumonia in Children without Specific Immunocompromised Status

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Author's response to reviews: see over
Dear Professor Hans Helmut Niller and Professor Klaus Hamprecht

I am really grad and highly appreciated when I received your answers. Thank you for your precious comments and edits. I have revised our manuscript as your advice and some information were added to clarify our ideas.

First, I would like to revise as Professor Hans Helmut Niller comments and edits:

**Major Essential revisions:**

1. **Question 1:** The topics of premature birth and intrauterine infection are completely omitted. Would you please provide data, or at least discuss the topics. If intraurine infection was involved, how many cases of pneumonia were due to primary infection was involved, how many cases of pneumonia were due to primary infection of the mother during pregnancy, how many due to CMV-recurrence?

   **Answer:**
   
   I add results for children background in table 1. I also add the discussion about this topics in to first discussion paragraph (page 9).

   In our study, twenty six (60.5 %) patients were the first-child and thirty six (83.7%) patient were born with vaginal delivery, twenty three (53.5%) patients using exclusive breast fed and twenty five (58.1%) were term infants. As discussed in paragraph 1, I could not find out when these baby were exposed to CMV, so I could not conclude the infection were congenital or acquired.

2. **Question 2:** The nutritional statuses of the examined children, as compared to their aged cohorts, are not given and not discussed. Would you please present this data?
Answer: Thanks for your advice and comment. I had put this data on table 1 and discussed in the first discussion paragraph (page 9).

3. Question 3: The rapid test for influenza is not insufficient quantity to exclude co-infection with influenza viruses. Would you please re-examine the nucleic acids for influenza virus with RT-PCR and possibly parainfluenza virus, in addition?
Answer: Thank you for your comments. I know that the sensitivity and specificity of influenza rapid test is not enough to rule out influenza infection. But because of budget limitation, I could not perform RT-PCR for influenza virus and parainfluenza virus. But next time when I plan to do randomized control trial (RCT), I will apply more molecular test for these virus.

4. Question 4: Figure 3 Should show each individual case in the form of a dot graph, in addition to the histograms. Figure 1 and 2 would possibly also benefit from such a individual graphic representation.
Answer: Thanks for your advice and comment. I had changed the figure 3 as you recommended. And figure 1 and 2 were changed in to table 3.

Minor revised:

1. Question 1: Besides plasma and tracheal aspirates, did the authors also sample urine for quantitative CMV-PCR?

Answer: Thank you for your advice. I did not do CMV-PCR in urine. Next time when I do RCT, I will apply it as your advice.

2. Question 2: Were anti-CMV-enrich Immunoglobulin addionallly to GCV used for therapy in some cases?
Answer: No, no patients were used intravenous immunoglobulin additionally to GCV. I add this information in materials and methods paragraph (page 5)
3. **Question 3:** There are numerous spelling mistakes. I labeled them mostly in the accompany PDF. Please correct.

**Answer:** I had already correct all spelling mistake as you suggested.

**Discretionary revisions:**

1. **Question 1:** Reference 8 in paragraph Patients and case definitions: Would you please list the WHO criteria for severe pneumonia within the text?

**Answer:** Thank you for your advice. WHO criteria for severe pneumonia were listed in the material and methods, patients and case definitions paragraph (page 3).

2. **Question 2:** Paragraph Clinical symptoms and laboratory data, middle of page 7: GOT an GPT name AST and ALT in table 1 which are currently the preferred names.

**Answer:** AST and ALT is more common use than GOT and GOT, as you pointed out I changed them in middle of page 7 and table 3

3. **Question 3:** Discussion part, end of page 9: CMV-DNA copies number in Plasma might also stem from apoptotic cells carrying or replicating CMV

**Answer:** I agree with you that CMV-DNA copy number in plasma might also stem from apoptotic cells carrying or replicating CMV. So I deleted this sentence; (Plasma have no cells). Instead, I changed the sentence as follows; The CMV-DNA copy number in plasma, which indicates the amount of free CMV-DNA circulating in the bloodstream and the active CMV replication, is considered to be useful to monitor the clinical course of CMV infections; in discussion paragraph (page 10).
4. **Question 4:** References: The authors might also cite Siret and David. Arch Pediatr 2002, 9: 499-502, for a case of CMV-pneumonia in an immunompent infant.

**Answer:** Thanks for your advice. I added Siret and David in my reference as reference 8

**Secondly, I would like to answer and revise as Professor Klaus Hamprecht comments and edits:**

**Major compulsory revisions**

A. The following questions related to the study design and demographics arise

1. **Question 1:** Are the infants ranging in age from 45-85 days p.p., term infants or are there also preterm infants? It would be helpful to know the individual gestational ages and birth weights.

**Answer:** Thank you for your suggestion. I put their gestational ages of my patients in table 1.

2. **Question 2:** Are the infants congenitally or postnatal infected with CMV? In both cases severe CMV-associated pneumonia can be found, but during postnatal CMV infections pneumonia will arise most frequently late (4-8 weeks after birth)

**Answer:** It is difficult to know whether these infants were congenitally or postnatal infected with CMV. Because the time we met these patients were median 45-85 days. Thus I suspected they acquired CMV infection after birth as you suggested. I add this discussion on first paragraph discussion (page 9).

B. **Question relating to nutrition of the infants**

3. **Question 3:** Are all infants feeded with breast milk? It’s important to know how the infants were exposed to CMV prior GCV treatment.
Answer: No, not all. 23/43 (53.9%) patients were fed with exclusive breast milk and 15/23 (34.9%) were apart breast milk with cow milk and 5/43 (11.6%) patients had formula nutrition (table 1). So I concluded they acquired CMV during and/or after birth as I discussed on first paragraph discussion (page 9).

C. Questions to the virological monitoring:

4. Question 4: Did the infants shed CMV in to urine at the treatment start and did the authors analyze also urine following GCV treatment?

Answer: Thank you for your comment, we did not check the CMV viral load in urine. Next time when I do RCT, I will do as your suggested.

5. Question 5: It would be very interesting to see the individual viral loads in blood and in tracheal secretions before and during antiviral treatment.

Answer: Thanks for your edit. It look really professional. I changed figure 1 as you suggested.

6. Question 6: The figure 1 and 2 can be replaced by table. Instead of these a new figure including the individual VL courses under therapy should be given

Answer: Yes, thank you for your edit. It look really great. I changed figure 1,2 as you seen in to table 3 as you suggested.

D. Question to the role of CMV in pneumonia of the infants.

10. Question 10: The diagnosis of CMV pneumonia in stem cell transplant recipient had been defined exactly (Ljungman et al., 2008) with respect to clinical symptoms, x-ray, quantitative CMV PCR or viral culture from tracheal secretions. How the diagnosis of CMV pneumonia was defined in this study?
Answer: Thank you for your question. The diagnosis of CMV pneumonia in current study made as described in the method paragraph (page 3): (1) severe pneumonia case as defined using WHO criteria (The young infant age less than 2 months had severe chest in drawing or fast breathing (60 per minute or more). And if the child age 2 months up to 5 years had chest indrawing) [8], (2) CMV-DNA detected in tracheal aspirate and blood plasma specimens with more than 5×10^3 copies/ml[9], (3) no co-infection with bacteria or viruses other than CMV.

E. Question to the outcome of the GCV treatment

1. **Question 1:** In table 1 is shown, that one infant died. In which way CMV involved?

   **Answer:** In table 1 is shown, one patient died. As we discuss in discussion paragraph. He was died because of acquired infection with *Burkholderia cepacia* at the second weeks of treatment therapy not because of CMV infection. I add this information to results paragraph outcome (page 8).

2. **Question 2:** Was there any evidence of treatment failure in this fatal case or any other case?

   **Answer:** Thank you for your interesting question. There was no evidence of treatment failure in this fatal case or and other case.

**Minor essential revisions:**

A

1. **Question 1:** Did the infants have primary CMV infections? See page 6: 70% of all infants had CMV IgM. Did they show low avidity?
Answer: In Page 6, 29 patients was positive for CMV-IgM (67.4%). We don’t know when these patients exposed to CMV so that we could not explain why only 70% patients was positive for CMV-IgM. Some patients might expose to CMV early at their life at the time of hospitalization the CMV-IgM become negative or they exposed to CMV just before their hospitalization and had no time to develop IgM antibody the CMV-IgM become negative. This can be answered by an antibody avidity assay but in NHP, the microbiology still have not done this test.

2. Questions 2: To the role of co infections: Rapid test systems based on immunochromatography for FLU are normally not sensitive enough to exclude H1N12009 and seasonal FLU.

Answer: Thank you so much for your comment. As you mentioned influenza rapid test could not rule out influenza. But because of budget limitation I could not conduct it. Next time when I do RCT for CMV treatment, I will apply molecular test for influenza, parainfluenza.

3. Question 3: The data on ADV and EV subfamilies should be shown

Answers: Thank you for your comment. I did PCR for Adv, but I did not do PCR for EV. I also did not do Adv subfamilies analysis, because of our budget limitation.

4. Question 4: What’s about hMPV

Answers: Thank you for your comment. PCR for HMPV were not able to perform at our institute at that time. Now it has been developed, so I will consider it in the future RCT.

B.
1. **Question 1:** Did some infants receive donor breast milk? Was the serology of the donor mother known?

**Answers:** No, no patients received donor breast milk.

2. **Question 2:** Or receive the infants formula nutrition? In these case CCVMV had to be excluded

**Answers:** Only 5 patients had formula nutrition. It is difficult to known when those patients exposed to CMV. But as I answered in Professor Han Helmut Niller question 2. I concluded all of the infants including those patients fed with formula milk acquired CMV during and/or after birth.

C.

1. **Question:** With which rationale the authors changed the treatment regimen with respect to GCV concentrations given by Kimberlin et al?

**Answers:** I follow the regimen on the Robert F. Pass in Principle and Practice of Pediatric Infectious Diseases (Reference 13)

Thank you for your time and we look forward to hearing from you soon.

Your sincerely,

Doan Thi Mai Thanh