Author's response to reviews

Title: Impact of maternal HIV-1 viremia on lymphocyte subsets among HIV-exposed uninfected infants: Protective mechanism or immunodeficiency?

Authors:

Fatima Kakkar (fatima.kakkar@umontreal.ca)
Valerie Lamarre (Valerie_Lamarre-pediatrie@ssss.gouv.qc.ca)
Thierry Ducruet (thierry.ducruet@gmail.com)
Marc Boucher (boucherhsj@aol.com)
Silvie Valois (cmis@recherche-ste-justine.qc.ca)
Hugo Soudeyns (hugo.soudeyns@recherche-ste-justine.qc.ca)
Normand Lapointe (normand_lapointe@ssss.gouv.qc.ca)

Version: 3 Date: 14 April 2014

Author's response to reviews: see over
Dear Editor,

Thank you for having our manuscript entitled “Impact of maternal HIV-1 viremia on lymphocyte subsets among HIV-exposed uninfected infants: Protective mechanism or immunodeficiency” reviewed by Drs. Chahroudi and Palm. We appreciate their detailed review of our manuscript, and have addressed each of their comments and suggested revisions. Below are our responses.

Reviewer 1:

1. The differences found in CD4 and CD19 percentages between groups are relatively small and there is no evidence provided that these differences are clinically relevant. The methods section states that this cohort of HIV exposed uninfected patients were followed frequently in the first 2 years of life, then annually until age 5, and every 2 years thereafter. I wonder if the authors could provide clinical outcomes data for these patients – ie, were there increased infectious disease diagnoses / hospitalizations / mortality seen in infants born to mothers in the highest viral load group?

Response: Unfortunately data on infectious diseases outcomes and associated morbidity was not collected systematically and recorded at all clinical visits, as this cohort was established to monitor for perinatal HIV infection and drug toxicity, and not specific clinical outcomes. While we provided annual follow-up until age 5 and every two years thereafter, some medical visits (especially during acute illness) would occur at other hospitals closer to the patients homes, or if minor, through walk-in-clinics in the patient’s neighbourhoods. Retrospectively we are unable to track this information; we are however working on collecting this data prospectively through our provincial health records database with patient consent.

2. The lymphocyte subsets studied were very basic. Do the authors have further specimens available to look more closely at CD4+ and CD8+ T cell subsets (ie, naive vs memory)? Was the HIV specificity of the CD4+ and CD8+ T cells ever assessed in this population? It would also be interesting to note whether the activation status of CD4+ and CD8+ T cells differed between the maternal viral load groups (immune activation might be predicted to be higher in infants born to mothers with higher viral loads).

Response: We are very much interested in looking at more specific T cell subsets, immune activation and HIV specific immune response among these different groups of infants. Unfortunately we do not have banked specimens on which these specific tests can be run. We are therefore planning to study this prospectively through a recently funded study by the Canadian Institute of Health Research entitled “Immunological abnormalities among HIV-exposed uninfected Infants”. With approximately 50 such infants born at our center each year, we anticipate sufficient recruitment with preliminary
results in 5 years. In the interim, we hope the publication of the results from this study could stimulate additional studies in this domain.

3. The middle viral load group (ie, VL 50-1000) does not appear to be particularly informative. As the authors state, this may be related to viral loads reported to be < 500 (from the early time period of the cohort) that were actually < 50 (if a more sensitive assay had been available for use). Could the authors reanalyze this group using available true values (ie, discard all subjects with reported VL <500)?

**Response:** All patients with viral load <500 (n=12) were removed from the analysis, without changing the significance of the results seen. The results as presented now in Tables 1, 2 and 3 and Figure 1 exclude these patients, and the main text has been amended to reflect these changes (page 7 lines 11-13)

4. It would be helpful to know the range of viral loads in the > 1000 group.

**Response:** A new table was created (Table 1) which show the ranges of viral load in the different groups, as well as maternal characteristics within each group in response to this question and one raised by Reviewer 2. The range reported for patients with VL> 1000 copies/ml is 2500 to 10 433 copies/ml, and this has been added to the text (page 8, line 8)

5. In the abstract the authors state “These differences persisted until 6 months of age”. Were the lymphocyte percentages studied in these patients after 6 months of age? Were any differences seen?

**Response:** After 6 months of age, lymphocyte subsets were not routinely tested (only biochemistry, haematology and HIV serology at 18 months). However we plan to do this prospectively in the new study described in response to point 2.

6. There was a wide range of antiretroviral regimens provided to these infants to for PMTCT (from no ART to triple therapy). For how long were the ARV regimens used? Did the authors compare lymphocyte percentages at 2 months (either while on ART or soon after discontinuation) in the different ART groups? They did control for infant ART exposure in the adjusted analysis, but I wonder if there was an effect of individual regimens on the immune cells examined.

**Response:** A description of the antiretroviral regimens provided to the infants for PMTCT was provided in the main text (page 6 lines 12-17). In summary, all infants received 6 weeks of ART for prevention of mother-to-child transmission as soon as it became available. This resulted in different neonatal treatment regimens over time. From 1994-1997, infants at our center received AZT alone, from 1998-1999 AZT in combination with 3TC, and from 1999-2007, AZT, 3TC and Nelfinavir. Following the FDA recall of Nelfinavir in 2007, the neonatal regimen changed again from 2007-2010 to a combination of AZT and 3TC. We do not have lymphocyte counts on the infants while on ART; the closest measure is the 2 month lymphocyte count at which point all infants had discontinued treatment.

A supplementary table has been included which shows more detailed results of our multiple linear regression models for the outcomes of interest (CD4 and CD19), and the
statistical significance of all variables (including infant ARV) included the final models. The results show that while increasing numbers of ARVS used in the infants has an effect on absolute and relative CD4 counts at 2 months of age, at 6 months this effect is only significant on the relative CD4 count. The number of ARVs used in the infants had an effect on relative but not absolute CD19 counts at 2 months of age, and no effect on CD19 (relative or absolute) at 6 months of age. We have therefore adjusted for infant ARV, given that the overall effect on lymphocyte counts is not clear.

We have chosen to present this table as supplementary information (available as online) for the interested reader, and not in the main text, so as to avoid overloading tables 2 and 3 where the primary analysis of interest (lymphocyte means according to maternal viral load groups) is reported.

7. Page 4, line 13 references listed are “18-120” – I think the authors meant “18-20”.

Response: Page 4 line 13 has been corrected to «18-20»

8. Page 10, line 20 please spell out “UU” (unexposed, uninfected)

Response: This sentence is now on page 11, line 8 and has been corrected to “unexposed uninfected”.

Reviewer 2

1. Major limitations to the study are represented by the absence of absolute T-cell count. It is neither clear if with absolute number the result still remain significant. Thus new statistical analysis reporting absolute cell count numbers should be performed.

Response: We have now included the results for absolute CD4, CD8 and CD19 T cell counts in the manuscript and the former Table 1 has now become Tables 2 and 3 to accommodate these changes. The results section in the text has also been modified to reflect these changes. The differences in infant CD4 count remain significant when comparing absolute or relative CD4 at 2 and 6 months of age. While there remain differences in absolute CD19 count, these are only statistically significant in percentages at 2 and 6 months, not in absolute count. The results section in the text has been corrected to reflect these changes.

- Page 9 line 1: Tables 2 and 3 are listed
- Page 9 lines 2-9: Wording has been changed to include absolute CD4
- Page 9 lines 19-20: Two sentences have been included to describe absolute CD19 results
- Page 9 lines 22-23 and page 10 lines 1-3: Wording has been changed to include absolute CD8 results

The discussion has also been modified

- Page 11, lines 11-14: Includes discussion on the relevance of CD19 changes seen (percentage vs absolute count).
2. Furthermore poor information on the status of the mother are reported.

Response: We have included a new table (Table 1) reporting the status of the mothers within the different groups, specifically describing the range of viral load at delivery, CD4 count at delivery, anti-retroviral drug regimens used, maternal age, and gestational age of infants. These are also described on page 8, lines 6-16.

3. Did you observe any incidence of vascular malformations or other side effects in your study? Is there any relation with Retrovir exposure as reported by other authors

Response: Previous work from our team in collaboration with others has not shown significant adverse effects from the use of combination ART in neonates (Bitnun et al, abstract to be presented at the Canadian HIV AIDS Research meeting, 2014) or comparing monotherapy with Retrovir to combination antiretroviral therapy, (Kakkar et al, abstract presented at the Canadian HIV AIDS Research meeting, 2012), However, data on vascular malformations or other congenital malformations has not been assessed systematically, though given the small size of our cohort and that treating team has not changed over time, we can say anecdotally that we have not observed this effect. We are now working to study long term health outcomes among these children in a new prospective national (Canadian) cohort study, funded by the Canadian Institute of Health Research (Principal Investigator: Dr. Helene Coté), and anticipate preliminary results in the next 5 years.

We hope these responses and revisions have sufficiently answered the questions raised, and strengthened the manuscript for publication in *BMC Infectious Diseases*. We thank you for your consideration, and look forward to your response,

Sincerely,

Fatima Kakkar, MD, MPH

Corresponding Author
Department of Pediatrics & Infectious Diseases
CHU Sainte-Justine
Montreal, Quebec, Canada
Email: fatima.kakkar@umontreal.ca