

REVIEWER #1

14th May 2013

University of the Witwatersrand MMed Dissertation

Student name: HA Diar

Student number: 9301192V

Title of Dissertation: Factors associated with cytomegalovirus (CMV) infection in neonates

Supervisor: Prof S Velaphi, Neonatology Department, Chris Hani Baragwanath Academic Hospital

** Overall**

A well written piece of work addressing a problem that is experienced in all neonatal units in South Africa. The work presents a clear picture of the condition in the newborn and addresses the mode of transmission and outcomes. [\[comment\]](#)

** General**

This research report is satisfactory with respect to literary style and presentation. The candidate is acquainted with the methods of research and has chosen an appropriate topic for the dissertation. [\[comment\]](#)

The layout is neat and the language and the grammar are good. [\[comment\]](#)

Specific Comments

Although retrospective, this study has strength in the use of the case controls. The case control should be indicated in the title: Factors associated with cytomegalovirus (CMV) infection in neonates: A case control study.

✓ As pointed out by reviewer #2, in the true sense this is NOT a case control study, hence I did NOT amend the title as suggested; please see reviewer 2 comments

Abstract

page v

This is clear and presents the findings of the study, one or two points maybe added to the existing abstract.

- ✓ It would be useful in the results to include actual numbers before the percentages e.g. 'Most patients have actual number before the percentage (91%) was of low birth weight.
 - amended in abstract with actual numbers before the percentages
- ✓ The number of CMV confirmed cases and the number of negative cases are not indicated.
 - amended in abstract with number of CMV-negative and CMV-positive cases
- ✓ Besides the low platelets, the raised direct bilirubin may be a point worth indicating.
 - The raised bilirubin (and raised liver enzymes) was NOT statistically significant and were therefore omitted from the abstract

Chapter 1

✓ Introduction page 1

The introduction was clear and to the point highlighting the background to the problem of neonates with CMV infection. [\[comment\]](#)

Chapter 2

✓ Literature Review

Page 3

- ✓ This was a systematic approach addressing the epidemiology of congenital cytomegalovirus followed by the 'Microbiology' of CMV. This technically should be the VIROLOGY of CMV.
 - [amended in text section 2.2 page 4](#)
- ✓ Clinical characteristics associated with the disease are presented, the laboratory abnormalities are indicated, the diagnosis and predictors of poor outcome in the infected babies presented. [\[comment\]](#)
- ✓ In the section 2.4 on the laboratory abnormalities the studies quoted defined thrombocytopenia as $< 100,000 \text{ cells/mm}^3$. [\[comment\]](#)

Chapter 3

page 12

✓ The hypothesis, aims and objectives were clear. [\[comment\]](#)

3.2 page 13

✓ This addresses the study design, population procedures, data collection, capturing and analysis and definitions. The pp65 antigen test was reviewed. A note was made on the ethical aspects of the study. [comment]

Chapter 4

Results

Page 17

✓ The results are well presented with good figures and tables giving a clear explanation of the results. [comment]

✓ The clinical details and prevalence was calculated. The maternal and neonatal details were given in tables. The laboratory results were given, followed by the HIV PCR and the outcomes in neonates. These were well done. The low platelet counts are highlighted in the positive babies. The maternal characteristics of the two groups studied were given in a table. The HIV status stands out in the CMV positive infants. The indications for testing are given. [comment]

- ✓ It is not clear why babies with neurological lesions such as calcifications or microcephaly were not seen. Intracranial calcifications may be the clue to the diagnosis. Table 4.10 indicates the head circumferences of the babies studied and there was no significant difference between the CMV positive and CMV negative babies.
 - amended please see text page 21
 - Microcephaly was present in 17% of congenital CMV-neonates. Screening cranial ultrasounds for intracranial calcifications were performed on 19 (79%)

of the congenital CMV-infected neonates. All the patients with microcephaly had normal cranial ultrasounds. Intra-cranial calcification was detected in 1 (4%) of the congenital CMV-infected neonates in the absence of microcephaly.

✓ Neonatal outcome and the comparison between the two groups of babies are made. Not unexpectedly the CMV infected babies were more likely to die before discharge.
[comment]

- ✓ The logistic regression was interesting raising the male gender at greater risk of dying.
 - The logistic regression was REMOVED from the text, as suggested by REVIEWER #2
- ✓ There was no reference to the diagnostic test specifically the pp65 which would have been interesting.
 - Although this reviewer raised a valid point about the pp65 antigen test, its importance in this study is doubtful as not all patients had pp65 tests performed and also due to the limitations mentioned below.
 - Of the 24 neonates with congenital CMV (urine shell-vial positive), 14 had a pp65 antigen tests done, with 10 having positive tests. The four CMV negative newborns' with negative pp65 antigen tests were likely false negative results.
 - The pp65 test was not performed locally. Late dispatching and therefore late receiving of specimens may have led to these false negative results as specimens were not analysed within 24 hours of sampling.

Chapter 5

Discussion

page 33

-  CMV is said to be the most common cause of congenital infection worldwide and specifically so in the USA. I am not clear if this applies in the South African setting. Could it more likely be HIV or syphilis?
 - amended please see page 32/ paragraph 1/ sentence 1
-  The prevalence and haematological presentation are discussed. The point raised was the nature of the selection of patients could result in an underestimate due to the majority being asymptomatic. [comment]
-  The poor growth of the baby is discussed. [comment]
-  The hepatosplenomegaly and the thrombocytopenia are raised along with the increase in direct bilirubin. This point may be important to raise in the abstract as well.
 - Not statistically significant, therefore NOT mentioned in abstract
-  On page 35 second paragraph - reference is made to microcephaly in this study which was not indicated in the results. This requires clarification
 - amended/ clarified in text please see page 21-22

The antenatal HIV prevalence and the transmission rates of HIV and CMV are discussed. It would appear that the presence of HIV co -infections increase the HIV transmission rate. [comment]

✓ The mortality related to male gender is presented. A combination of CMV infection in the boys with HIV would seem to be a strong predictor of mortality

- Removed logistic regression as suggested by REVIEWER #2

✓ The Strengthens and Limitations are presented. [comment]

✓ The corrections are of a minor nature and can be checked by the supervisor. [comment]

REVIEWER #2

Title: Factors associated with Cytomegalovirus (CMV) infection in neonates

Student: Hitesh Diar

Degree: MMed

Description of the dissertation

This is a retrospective review of hospital records of neonates at Chris Hani Baragwanath Hospital identified via laboratory records as having a cytomegalovirus (CMV) test requested. The study also compared infants who tested positive for CMV with those who were negative. The majority of tested infants were of low birth weight and preterm. The study identified the presence of hepatosplenomegaly, thrombocytopenia and infant HIV exposure or infection as predictors of CMV positivity (in those tested). The only significant predictor of mortality was being male.

Originality and importance

- There have been 4 recent studies looking at the association of HIV and CMV in infants, including the benefits of antenatal antiretroviral therapy in preventing CMV transmission. The report offers no obvious benefits of recognising risk factors for CMV in the study setting.

Acceptability for award of degree

The Literature review is well written, clear and covers relevant major issues.

- The Methods section is a bit skimpy on detail but satisfactory.
- The Results section could have been presented more concisely.
- The Discussion section requires substantial revision.
- The Conclusion and Recommendations sections require a complete re-write.

The candidate has a number of revisions to make. I am, therefore, recommending award of the degree, subject to substantial amendments, undertaken to the satisfaction of the Head of Department.

MAJOR COMMENTS

My major criticisms of the work are as follows:

✓ 1. Definition needs to be provided for each of clinical features, such as hepatomegaly or splenomegaly, and laboratory findings, e.g. thrombocytopaenia.

- amended in text page14 under section 3.2.5 Definitions

a. ✓ For clinical findings, indicate if this was based completely on what appeared in the patient record, or if you set a filter (eg. only accepting a description of a liver palpable 2 cm (or more) below the intercostal margin as hepatomegaly rather than a simple note stating "hepatomegaly" in the patient notes. If the former approach was used, then this needs to be clearly acknowledged as a study limitation.

- amended in text page and page14 section 3.2.5 Definitions and, page37 section 5.1.1 Strengthen and Limitations

b. ✓ For laboratory results, provide a reference for normal (e.g. thrombocytopaenia)

- amended in text page 14 under section 3.2.5 Definitions

✓ 2. The study is described as a case-control study? This is incorrect. There was no matching of cases with controls. Instead, the candidate merely compared clinically suspected CMV infants with positive and negative results in a sub-analysis of the primary data (infants tested for CMV).

- REMOVED: "case-controlled" as suggested by this reviewer

✓ 3. There are basic errors with data presentation that have to be corrected throughout the report, such as:

- it is inappropriate to provide percentages to two decimal points when there are less than 100 patients .in the study, e.g. 45.66% vs. 46% Categories that are not exclusive

- these have been amended in text as well as tables

✓ 4. The candidate creates an excessive number of tables that could easily have been consolidated into a few more meaningful ones. It is also unnecessary to present tables for

CMV positive infants first and then the full cohort later. Nevertheless, I am-willing to ignore this deficiency and not insist on a change.

- removed original tables 4.7, 4.8 and 4.15 as suggested by this reviewer

✓ 5. The technique of logistic-regression appears flawed. However, insufficient reporting of methodology prevents my establishing this conclusively. I recommend that this section be removed. You have not done this well enough (provided sufficient detail) for me to understand your results. There is too little detail in the methods section and your results are not plausible. A logistic regression is not a requirement for a Master's research report

- Removed logistic regression as suggested by Reviewer #2

✓ 6. The discussion is weak and does not highlight important study findings. It often provides data from different studies, without "pulling together" all the facts to offer an explanation for the study's finding(s). Please provide some sub-headings to assist the reader (and highlight themes). The candidate should have considered and discussed. [COMMENT]

a. ✓ Why a comparison between CMV tested infants who have positive and negative results important? What information does this disclose? Why did you not do a proper case-control study instead, choosing matched controls from the general neonatal population at Bara?

- Explained this in the text on page (and below)
- This study was based on a clinical impression/ hypothesis that neonates admitted to the Unit with congenital CMV likely have specific characteristics. Since the study population was made up of neonates suspected to have congenital CMV (and were tested to prove/ disprove this suspicion), those neonates that tested CMV negative were used as "controls" for comparison. This seemed plausible due to the time constraints imposed on clinicians in the Unit. The manual filing system also points to the issue of the time constraints as the majority of files either take a long time to locate or cannot be found at all. Hence, this study approach was utilised.

b. ✓ The low positive rate among infants tested (low sensitivity), and if this indicates "over-testing" for CMV in the unit.

- Based on the study findings, in the presence of suggestive clinical signs (hepatosplenomegaly and/ or persistent thrombocytopaenia and/ or persistent jaundice) testing for congenital CMV and HIV in HIV-exposed neonates would be warranted. The major impact of this study will be on the issue of “over-testing” whereby testing will be more focussed on at-risk neonates.

✓ c. Why being male was a predictor of mortality. [removed]

d. ✓ Major discrepancy in files procured between CMV positive and negative.

- The clinical information in the patient hospital files is not transferred and stored to an interactive database where this information could be easily retrieved. The filing system is done manually where the files are arranged according to the patient’s date of birth in individual file-boxes. This filing system is quite inefficient and cumbersome.
- The patient files are also being utilised by other study groups to retrieve information. The handling of these patient files by more than one/ two individuals leads to files being lost, damaged or misplaced. Hence, some of the CMV-negative files could either not be retrieved or incompletely retrieved.

e. ✓ Why the frequency of clinical signs differ in different settings. [see text page 34 paragraph 3]

- This represents the spectrum of disease manifestations based on different study populations due to disease severity and/ or geographic location and/ or available resources for testing and therapy.

✓ 7. The prevalence discussion needs to be re-written. You cannot establish prevalence in this kind of study (retrospective review) in my view. Further, I have offered reasons (below) why the argument is faulty. [amended in the text as INCIDENCE]

✓ 8. Add more limitations [amended in the text page section, as suggested below]

- Incomplete record availability
- Major discrepancy in files procured between CMV positive and negative

- Guessing why CMV test was ordered (indications were guesses at best). Depending on clinical report of clinical signs, e.g. presence of hepatomegaly
- No report of clinical care (e.g. ICU) or therapy, so difficult to comment on outcomes (compared to elsewhere)
- Late HIV testing - resulting in an underestimate of HIV transmission and positivity status
 - The main limitation of this study is it's retrospective nature. The inability to retrieve all the patient records for the congenital CMV-negative neonates had led to the major discrepancy in files procured between CMV positive and negative neonates. The indications for performing CMV testing in these patents were not always specific and detailed in the patient records. The HIV-exposed neonates were only tested for the presence of HIV infection at six weeks of life. This may have resulted in an underestimate of HIV transmission and positivity status as HIV may have been acquired much earlier than six weeks of life. The clinical information in the patient hospital files was not transferred and stored to an interactive database where this information could be easily retrieved. The filing was done manually where the files are arranged according to the patients' date of birth in individual file-boxes. The patient files were also being utilised by other study groups to retrieve information. The handling of these patient files by more than one individual leads to files being lost, damaged or misplaced. Also, the storage of files is done at a facility not within the Neonatal complex. Hence, some of the CMV-negative files could either not be retrieved or incompletely retrieved.

✓ 9. Neither of the two main conclusions offered are valid. [amended in the text on page 38 section 6.0 Conclusions]

- Your study did not (cannot) describe the prevalence of symptomatic CMV in HIV-exposed neonates-vat you describe this as "high" and claim this as a major contribution of the study.
- You cannot claim that you identified "specific characteristics" that distinguish neonates with congenital CMV infection from those without CMV, as-your-study design did not allow this (not a case- control study). All you can say is that you found a difference in some findings between CMV tested infants who were positive and negative. The value of knowing this is questionable (see earlier comment).

✓ 10. The two recommendations offered are weak and cannot be substantiated by the study results, i.e. they do not relate in any way to your results. I am left wondering about the value of the study.

- amended in the text on page 38 section 6.1.1 Recommendations

✓ 11. The candidate should include the study questionnaire/ data collection sheet (see Appendix A) and ethics clearance form (see Appendix B). The absence of a questionnaire prevented me from adequately interrogating the validity of the methodology and results.

MINOR COMMENTS

Abstract

- ✓ Case-control study - incorrect (see earlier comment) [removed]
- ✓ Indicate number of participants in first sentence of results section [amended]
- ✓ Add number of patients in second sentence of results section - there were x CMV positive and y CMV negative infants [amended]
- Unsure if results refer to all infants or to "cases" only [refer to congenital CMV positive patients only]
- ✓ Present percentages as an integer (no decimal points) [amended]
- ✓ Provide measurement unit for platelet count [amended]
- ✓ Give % not just p-value for any comparisons [amended]
- ✓ Conclusion is too non-specific. Improve by stating what are the implications of the study results. [amended]
- ✓ Do not understand the purpose-of having a preface, introduction and then a literature review. Suggest combine these three. [removed Preface only]

Introduction

- ✓ **Page 1** - what is the difference between the two statements - "Congenital CMV infection has been reported..." and "Co-infection with CMV and HIV ... ". The one statement suggests a 23% incidence and the other 50%, but they appear to be referring to the same.
 - [Removed "Co-infection with CMV and HIV has been shown to occur in up to 50% of infants born to HIV-infected mothers"]

Literature review

✓ I would have liked to have seen a discussion about the link between HIV and congenital CMV. However, this is not an essential change.

- [this was done on page 1 section 1.0 Introduction]

Methods

✓ Medians should be presented with their interquartile ranges (IQR), not ranges.

- [amended in text page 15 section 3.2.6 Data capturing and analysis and tables 4.7, 4.8, 4.9]

✓ Need detail on file extraction - where records kept, and why situation of missing files arose.

- [see text page 37 section 5.1.1 Strengths and Limitations]

✓ Explain why a 5-year study period was chosen - not any longer or shorter? [elaborated in the text page 13 section 3.2.2 Study sample]

- Firstly, the urine shell-vial culture and pp65 antigenaemia tests were both available from January 2004. Secondly, I felt that a five year period will provide one with enough numbers to achieve the study objectives.

Results

- ✓ **Page 17-** Major discrepancy in files procured between CMV positive and negative (86% vs 42%). Explain why in discussion and in limitations [see text page 37 section 5.1.1 Strengths and Limitations]
- ✓ **Page 18** - Table 4.1. Was indication for testing explicitly stated in records, or did you infer this? Presume latter- then cannot claim these were the indications- only An association. [in discussion with Supervisor left it as INDICATION]
- ✓ With only n=24, change percentage to single integer here and everywhere else (not two decimal points) [amended to single integer]
- ✓ **Table 4.1** should include all participants and have columns for positives and negatives [amended to include columns for CMV-positive and CMV-negatives]
- ✓ Inappropriate to just record p-values [amended in the relevant text and tables]

✓ **Page 18** section 4.1.2 Incidence not prevalence - these are new cases (in neonates) so should be incidence

Should say:

✓ (1) minimum incidence (since not all infants were tested)

- [removed prevalence]

✓ (2) How was range obtained? Inappropriate. More appropriate to provide 95% confidence interval to what is a point estimate.

- [removed "range" and amended to interquartile range as suggested by this reviewer]

• ✓ **Page 19** Tables 4.2 & 4.3 should include number of tests done each year Again incidence, not prevalence. [amended]

• ✓ **Page 22** - In methods section indicate how gestational age was assessed in the unit

- [It was not stated clearly in the hospital bedletters' how gestational age was assessed therefore, omitted]

• ✓ P-value to two decimal points adequate (3 acceptable for HIV)

- [amended]

• ✓ **Page 26** - Table 4.8 title: were these indications or merely associations?

- [left as INDICATIONS as per discussion with Supervisor]

Discussion

• ✓ **Page 32-33** You cannot calculate a "prevalence" based on your study design. At best you have a "minimum incidence." The whole discussion around this need to be changed.

- [amended to INCIDENCE]

✓ **Page 32-33** - Comparison of results with Schaub's study. Your interpretation of the difference is wrong. Difference was who was tested, not what test was used.

- [amended on page 32-33 section 5.1 Discussion]

Conclusion and Recommendations

✓ **Page 39-** Cannot conclude that "prevalence" was high based on your methodology.

- [amended to INCIDENCE]

✓ Cannot conclude that males were 23 times more likely to die- methodology flawed. Offer an explanation why this was the case, if true.

- [removed]

References

✓ This section is generally well done. Suggest correct deficiencies in references 4, 7, 10, 12, 15, 20, 25, 26, 28, 31, 38, 41. [amended]

✓ Add reference normal ranges for thrombocytopaenia etc., in Methods [amended]

✓ Add reference for New Ballard score in Methods [omitted]

Detailed criticisms

<u>Page/line</u>	<u>Original</u>	<u>Correction needed</u>
<u>Abstract</u>		
✓ page v/6	Who are not CMV-infected	Were
<u>Literature review</u>		
✓ page 3/18	Is more likely to be of the reactivation or recurrent type	What does this mean? [removed]
✓ page 4/14	Occurs in the newborns	Remove 'the'
✓ page 8/5	DB Abbreviation	direct bilirubin
✓ page 12/3	Identity	Distinguish
✓ page 12/9	hospital	Hospital
✓ page 12/ 13	Demographic, clinical characteristics	demographic, features/ characteristics, clinical characteristics
✓ page 12/ 17	Those who are CMV-uninfected	Were

Methods		
✓ page 13/6	At Chris Hani	To
✓ page 13/5	Study population (The study population: all neonates at Bara)	Study sample
✓ page 14/14	Signs were determined from	extracted from
✓ page 15/4	A normal distribution of either maternal or neonatal continuous and/or discrete variables was considered if these variables had p-values ≥0.2 using the Shapiro - Wilk's W test.	Explain this? Don't understand What you're saying [removed]
Results		
✓ page 19	The majority of neonates with congenital CMV infection (79%) were born to HIV-positive mothers result.	Provide numerator and denominator (19/24) not just %, since you do not indicate how many participants had this Presume not all 24 (728) had HIV result (full cohort) [Yes]
✓ page 20	<18 18-34 ≥34	Explain why this categorization. [see text]

✓ page 20	0 0-3 >3	Category 0 indicated in two groups. Why not 0,1,2,3,+, (categories not helpful) [amended]
✓ page 21	Table 4.5	Categories again not exclusive [amended]
✓ page 22		Indicate how defined increased AST, ALT & GGT (what was normal?) [see text: methods]
✓ page 23	Among the 24 patients	Provide % positive
✓ page 23	Table 4.7	Remove table, incorporate into text
✓ page 23	Table 4.8	Remove table, incorporate into text
✓ page 23		Describe timing of death (day of life)
✓ page 24	Table 4.9 HIV	? missing (% not 100) Wrong %
✓ page 28	Table 4.11	Misaligned columns (move values up)
✓ page 29	Table 4.12	Percentage not equal to 100
✓ page 30	Table 4.14	remove denominators

<u>Discussion</u> ✓ page 33 This represented a subset of a pool of neonates suspected to have CMV infection within the first three weeks of life, based on clinical and/or haematological and/or biochemical indices	Explain this (unclear) [re-phrased]
page 34	Check reference [amended]
✓ page 34 These might be related to the time of acquiring infection or whether it was a new infection or a reactivation	Explain this (unclear) [re-phrased]
✓ page 36 Doyle (P=0.008) ²	Meaningless to provide a p-value to describe incidence
✓ page 36 HIV- infected (67% v/s 42%; P<0.001)¹	What being compared to what? [see text]
✓ page 38 "These HIV-exposed (perinatal)	Why is this a limitation? [see text]
✓ 39 'the odds can be as high "	Suggest delete(d)