CMV VIRAL LOAD AS A PREDICTOR OF THE CLINICAL COURSE OF CMV-ASSOCIATED PNEUMONIA IN HIV INFECTED AND UNINFECTED INFANTS

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Thesis presented in fulfilment of the requirements for the degree of Master of Medicine (Paediatrics) in the Faculty of Medicine and Health Sciences at Stellenbosch University

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship thereof (unless to the extent explicitly otherwise stated) and that I have not previously, in its entirety or in part, submitted it for obtaining any qualification.

Signature: Dr A. Lakhan Date

Abstract

Background

Lower respiratory tract infections is one of the leading causes of death in children under 5 years of age. In the advent of HIV, much of these lower respiratory tract infections are attributed to opportunistic infections such as Pneumocystis jiroveci pneumonia (PJP) and Cytomegalovirus (CMV). Short of a lung biopsy, diagnosing CMV pneumonia has proven difficult. Some studies suggest that blood CMV viral load might be of benefit in making the diagnosis of CMV-associated pneumonia. There is currently a scarcity of literature with regards to classifying severity of CMV-associated pneumonia and whether the CMV viral load can be used as a predictive indicator of severity and outcome of CMV-associated pneumonia.

Objectives

To determine whether CMV viral load is a positive predictor of severity and outcome of CMV-associated hypoxic pneumonia.

Methods

This was a retrospective descriptive study done in the Paediatric Intensive Care Unit (PICU) in Tygerberg Children's Hospital, Western Cape, South Africa. Study participants were identified from the National Health Laboratory Service (NHLS) database as those patients in PICU less than 1 year of age with a positive cytomegalovirus viral load between 1 January 2014 to 31 December 2015. Patient clinical, radiological, and biochemical data was collected and analyzed.

Results

A total of 87 patients were included in the study. Twenty-seven of these patients were HIV-positive, 3 of which were on antiretroviral therapy. Comparisons were made between two groups (CMV VL ≤ 4 and > 4) based on the median blood CMV viral load in our study of 4.0 (IQR 3.3-4.79). There was no difference in severity of disease based on $Pa0_2/Fi0_2$ ratio and x-ray findings between the two groups. The mortality in the two groups was similar with a 90% survival rate. (p=0.37). Patients with higher CMV VL required a longer duration of high frequency oscillation ventilation (HFOV) (p=0.005), yet the mean length of PICU stay between the two groups was not statistically different. (p=0.43). Patients who had CMV viral load > 4, had an increased incidence of PJP co-infection (p=0.018), lower CD4 counts (p=0.0001) and higher HIV viral loads (p=0.049). All the patients with PJP-CMV co-infection were successfully treated and discharged.

Conclusion

CMV viral load alone cannot diagnose pneumonia and showed limited utility in predicting the course and outcome of CMV-associated pneumonia in young infants. The association between CMV VL, PJP co-infection, lower blood CD4 count and HIV viral load suggests that CMV-associated pneumonia occurs in more immune suppressed young infants but the lack of disease severity being associated with the CMV VL limits the usefulness of the test in diagnosing CMV-associated pneumonia and the need for antiviral therapy.

Opsomming

Agtergrond

Lae lugweginfeksies is een van die hoofoorsake van dood in kinders onder 5 jaar. Met die aanslae van MIV, kan baie van hierdie infeksies toegeskryf word aan opportunistiese infeksies, soos Pneumocystis Jirovecii Longontsteking (PJL) en sitomegalovirus (SMV). Buiten'n longbiopsie, is dit moeilik om SMV-longontsteking te diagnoseer. Sommige studies stel voor dat bloed met 'n SMV viruslade mag bydrae tot 'n suksesvolle diagnose van SMV-geassosieerde longontsteking. Huidiglik is daar'n tekort aan navorsing aangaande die klassifisering van SMV-geassosieerde longonsteking se ernstigheid, sowel as antwoorde rondom maniere hoe die SMV viruslade mag bydrae tot 'n voorspelling van ernstigheidsgraad en uitkoms van SMV-geassosieerde longonsteking.

Mikpunte

Om vas te stel of SMV-viruslade 'n positiewe voorspeller is vir die graad van ernstigheid en uikoms van SMV-geassosieerde longontsteking.

Metodes

Hierdie was 'n retrospektiewe beskrywende studie, afgelê in die Pedriatiese Intensiewe Sorgeenheid (PISE) by Tygerberg Kinderhospitaal in die Wes Kaap, Suid Afrika. Deelname aan die studie was vasgestel deur pasiënte te identifiseer (op die Nasionale Gesondheidslabrotorium Diens databasis) wat in die PISE opgeneem is, minder as een jaar oud is, en gediagnoseer is met 'n positiewe SMV-viruslade tussen 1 Januarie 2014 en 31 Desember 2015. Kliniese, radiologiese, en biochemiese data is ingesamel en analiseer.

Uitslae

87 Pasiënte is in die studie ingesluit. Van hierdie, is 27 getoets as MIV-positief, waarvan 3 op antiretrovirale-terapie was. Twee groepe was onderskei (SMV VL ≤ 4 en > 4) gebaseer op die mediaan bloed SMV-virale lade in ons studie van 4.0 (IQR 3.3-4.79). Daar was geen verskil in ernstigheid van die siekte gebaseer op $Pa0_2/Fi0_2$ verhouding en X-strale nie. Die sterftekoers in die twee groepe was soortgelyk, met 'n 90% oorlewingssyfer. (p=0.37). Pasiënte met 'n hoër SMV VL het langer behandelings van hoëfrekewensie ventilasie (HFV) benodig (p=0.005), tog het die gemiddelde lengte van PISE-opname tussen die twee groepe nie statisties verskil nie (p=0.43). Pasiënte met 'n SMV VL van > 4 het 'n groter kans gestaan van 'n PJP mede-infeksie (p=0.018), laer CD4-tellings (p=0.0001), en hoër MIV-tellings (p=0.049). Al die pasiënte met PJP-SMV infeksie was suksesvol behandel en ontslaan.

Gevolgtrekking

SMV-VL alleen kan nie longontsteking diagnoseer nie. Dít dui op ‘n beperkte bruikbaarheid in die voorspelling van die verloop en uitkoms van SMV-geassosieerde longontsteking in jong kinders. Die assosiëring tussen SMV VL, PJP mede-infeksie, laer bloed CD4 telling, en MIV VL stel voor dat SMV-geassosieerde longontsteking meer gereeld voorkom in jong kinders met verswygde-immuunstelsels. Die tekort aan siekte-ernstigheid assosiasie met die SMV VL beperk egter die bruikbaarheid van die toets in die diagnose van SMV-geassosieerde longontsteking en die noodsaaklikheid vir ‘n antivirale terapie.

Dedication

To my son, Wolf, you remind me of the goodness in this world and inspire me to be the greatest version of myself.

'For I know the plans I have for you, declares the Lord, plans to prosper you and not to harm you, plans to give you hope and a future'

Jeremiah 29:11

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Abbreviations

AIDS: Acquired immune deficiency syndrome
BAL: Bronchoalveolar lavage
cART: Combination antiretroviral therapy
CMV: Cytomegalovirus
CPAP: Continuous positive airway pressure
CT: Computed tomography
ECM: Enterprise Content Management
HFOV: High frequency oscillation ventilation
HIV: Human immunodeficiency virus
IPPV: Intermittent positive airway pressure
IS: Induced Sputum
LDH: Lactate dehydrogenase
MRSA: Methicillin-resistant Staphylococcus aureus
NHLS: National Health Laboratory Service
NP02: Nasal prong oxygen
PCR: Polymerase chain reaction
PICU: Paediatric intensive care unit
PIM2: Paediatric Index of Mortality 2
PJP: Pneumocystis jiroveci pneumonia
PMTCT: Prevention of mother-to-child transmission
RCWMCH: Red Cross War Memorial Children's Hospital
RSV: Respiratory syncytial virus
TB: Tuberculosis
VL: Viral load

Chapter 1: Introduction

In 2014 there were 2.6 million children globally living with HIV. Eighty-eight percent of these children were from Sub-Saharan Africa. [1] Lower respiratory tract infections are a common complication of HIV. [2] In 2015, the leading cause of death for children under 5 years of age in the post neonatal period was lower respiratory tract infections at 13%. [3]

Pneumocystis jiroveci pneumonia is usually the first manifestation of HIV disease in children under the age of 6 months.[4,5,6] Pneumocystis jiroveci pneumonia, also known as PJP, is an important AIDS defining infection and has a high mortality rate.[6,7,8] It is responsible for as many as one third to one half of all HIV-related deaths in African infants.[9] HIV co-infection negatively influences the course of pneumonia, especially in hospital settings owing to longer stays and higher mortality rates, as shown by one study.[8] Outcomes for PJP in children are generally poor.[4] Some authors have suggested that the outcomes are even worse when associated with CMV coinfection, whereas others have found no significant difference in outcomes in CMV/PJP co-infected children[4,10]

Human cytomegalovirus, also known as Human Herpesvirus 5, is an ubiquitous-herpesvirus.[11] The prevalence of cytomegalovirus infection is high in patients infected with HIV.[12,13] It can present in various forms ranging from asymptomatic virus shedding to retinitis, central nervous system or pulmonary involvement and gastrointestinal or wasting syndromes.[12] In one South African study it was found that CMV-associated pneumonia was three times more common in HIV-infected children than uninfected children.[14] It has been shown that prevalence of CMV infection peaks first at 6 months of age, presumably denoting congenital infection, and peaks again between 13 and 24 months, indicating acquired infection.[15] In younger children primary CMV infection causes pneumonitis, hepatomegaly and petechial rashes, whereas in older children and adolescence CMV may cause syndrome of fatigue, malaise, myalgia, headache, fever, hepatosplenomegaly, elevated liver enzymes and atypical lymphocytosis. Despite signs and symptoms of CMV infection being diverse – most cases are still subclinical. [16] Symptoms of CMV associated pneumonia include coughing, shortness of breath, fever and weight loss. Signs include adventitious sounds on auscultation, hypoxaemia and requirement of ventilatory support.[17] As with most viral pneumonias x-ray findings are nonspecific and may include: unilateral or bilateral streaky densities, confluent mottled opacities, a diffusely granular appearance with air-bronchograms or show alveolar air-displacement.[17,18] There is also an overlap of findings on x-ray between those with CMV pneumonia and PJP making the diagnosis difficult.[19] Plain film radiographic findings of CMV infection may be very subtle and easily missed, thus computed tomography is a more reliable

modality, especially in immunocompromised patients.[17] CT findings include diffuse ground-glass opacities and consolidations, atelectasis, air-trapping, and rarely reticular and nodular patterns.[17]

Definitive diagnosis of CMV pneumonia requires histologic confirmation on lung biopsy. [12] In our setting, routine lung biopsy in all children admitted with hypoxic pneumonia does not only carry risk but it is also unfeasible. Without lung biopsy, accurate diagnosis of CMV pneumonia is difficult. Therefore, less invasive investigations are preferred. Monitoring of CMV viraemia by qualitative and quantitative polymerase chain reaction (PCR) is widely used in solid organ and bone marrow transplant recipients for the diagnosis and treatment of CMV infection and disease. Signs and symptoms of pulmonary disease coupled with detection of CMV in BAL fluid or lung tissue is considered internationally accepted criteria for diagnosing CMV in transplant patients.[20] This criteria, however, is not useful in the setting of HIV infection as BAL fluid is reported to lack utility in diagnosing CMV associated pneumonia in HIV-infected patients.[21] Quantifying CMV viral load in non-bronchoscopic bronchoalveolar lavage specimens is useful in diagnosing CMV pneumonitis in infants and is more predictive than plasma viral load.[22]

There is existing evidence that the degree of CMV dissemination in the blood, which is measured by CMV load values, is a significant risk factor for progression from CMV infection to symptomatic disease.[23] A Kenyan study on HIV-infected children reported a peak in serum CMV viral load values soon after primary CMV infection and in advanced HIV disease.[24] An autopsy-based study in HIV-infected adults reported that a cutoff of > 4.0 log copies/mL has a specificity and positive predictive value of 100%, respectively, in predicting CMV disease.[25]

Hsiao et al. showed, in a cross-sectional study on children in the Western Cape, that 76% of their patients who had CMV pneumonia (based on a positive CMV PCR on NPA, IS or BAL) also had CMV viral load logs of ≥ 4.1 copies/ml. Interestingly, of those who had CMV pneumonia, 89% of them were HIV infected. [26]

As quantitative CMV PCR is best used to rule in, rather than to rule out CMV disease in HIV-infected individuals at high risk [21,25], it is possible that patients who did have CMV pneumonia were missed. Due to these limitations, lung biopsy still remains the gold standard in diagnosis in CMV pneumonia. There is currently no data comparing confirmed CMV pneumonia on lung biopsy and CMV viral load. It has been shown that CMV load is more useful in assessing the response to antiviral treatment and to determine antiviral treatment endpoints rather than severity of disease and outcome in patients after solid organ transplantation.[27] In a retrospective descriptive study done in a Western Cape PICU, prior to the implementation of

treatment for CMV, it was shown that CMV pneumonia was a contributor to the high mortality rate of the cohort and it's has since been recommended that all immunocompromised infants being ventilated for severe pneumonia be treated empirically for CMV and PJP infection.[28]

Some suggest that the primary role of ganciclovir is in the prevention of CMV infection, however, it is still a very effective treatment for CMV infection. [29] Valganciclovir is a safe and useful alternative oral therapy. [30] Foscarnet and cidofovir have been recommended for patients with ganciclovir-resistant virus. [31]

Chapter 2: Research Justification

Research Question: Is CMV viral load a good predictor of CMV-associated pneumonia severity and outcome?

There is currently a scarcity of literature with regards to classifying severity of CMV-associated pneumonia and whether the CMV viral load can be used as a predictive indicator of severity of disease and disease outcome.

Null hypothesis:

The null hypothesis of this study is that the CMV viral load does not influence the severity and outcome of CMV-associated hypoxic pneumonia.

Primary Aim: To determine whether CMV viral load is a positive predictor of severity and outcome of CMV-associated hypoxic pneumonia.

Severity of disease is determined by three factors:

1. Pa02/Fi02 ratio on admission and 24 hours after admission. A Pa02/Fi02 ratio of ≤ 200 (suggestive of acute respiratory distress syndrome) is indicative of severe lung disease.
2. Chest x-ray findings. Diffuse alveolar pattern changes is suggestive of severe lung disease.
3. Length of invasive ventilation required. This includes HFOV and IPPV.

Factors differentiating outcome would include:

1. PICU mortality
2. Morbidity: Supplementary oxygen dependency 28 days after admission

Secondary Aim:

1. To identify the risk factors influencing outcome of CMV-associated pneumonia.

Chapter 3: Material and Methods

Setting

This was a retrospective descriptive study done in the Paediatric Intensive Care Unit (PICU) in Tygerberg Children's Hospital, Western Cape, South Africa. The PICU, a ten-bed unit, serving a population of approximately 2 million persons and admits children aged 2 weeks to 13 years of age. The Tygerberg Children's Hospital is situated in the G Block of the Tygerberg Hospital and is part of the larger Tygerberg Hospital, which is the academic training hospital of the University of Stellenbosch. This hospital serves the immediate surrounding areas, providing primary and secondary health care to children, as well as tertiary care to all paediatric patients in Metro East, and the Northern and Eastern rural districts of the Western Cape.

Population

Study participants were identified from the National Health Laboratory Service (NHLS) database as those patients in Tygerberg PICU (Ward A9) who were less than 1 year of age and had a positive cytomegalovirus viral load between 1 January 2014 to 31 December 2015 and had been admitted to the PICU with hypoxic pneumonia. No formal sample size was calculated since all objectives are descriptive in nature. The sample size was determined by the number of infants admitted to the PICU with hypoxic pneumonia, the number of infants with hypoxic pneumonia who had a blood CMV viral load performed, logistical constraints and availability of eligible files within the sampling period.

Inclusion criteria

- Patients less than 1 year of age admitted to paediatric intensive care unit with hypoxic pneumonia.
- Admission period from 1 January 2014 to 31 December 2015.
- Patients with a positive CMV viral load.

Exclusion criteria

- Incomplete or missing information
- CMV viral load not done or lower than detectable levels
- Acute respiratory distress syndrome not related to lung disease

Outline of procedure

Study participants were identified from the National Health Laboratory Service database as those patients in Tygerberg PICU (Ward A9) who were less than 1 year of age and had a positive cytomegalovirus viral load between 1 January 2014 to 31 December 2015. A formal request was sent to the NHLS to extract this information.

Retrieval of folders and data abstraction were done by the principal investigator. Patient clinical data was collected retrospectively from their patient files: either from the hard copy patient files or from the electronic patient files on the Enterprise Content Management (ECM) system. Radiological data was collected from the Tygerberg iSite Enterprise System and interpreted by the study supervisors. All other blood results were collected from the National Health Laboratory Service data base.

Each patient enrolled in the study was assigned a unique patient identifier number and a paper based case report form was used to collect the data from the laboratory and radiology databases and clinical records. The nutritional status was determined by the standard deviation classification for weight for age by the World Health Organisation. This data was then entered into an electronic database. Patient names, hospital numbers and physical addresses were not entered in the electronic database.

Variables collected: Age, sex, HIV status etc. (see Appendix A)

Definitions:

- **Hypoxic pneumonia:** a pneumonic process requiring oxygen support (eg. Nasal prongs, continuous positive airway pressure, intermittent positive pressure, high frequency oscillation ventilation)
- **PIM2 score:** scoring system for rating the severity of medical illness for children, one of several ICU scoring systems.

All data was handled and managed according to Good Clinical Practice (GCP) requirements and ethical standards. The database (checked for accuracy and completeness) were stored on a laptop computer, which was kept in a locked cabinet in a locked office. Copies were used to perform calculations and analysis. The database was backed up on a daily, weekly, monthly

and 6 monthly basis onto a storage disk. The paper based case report forms were stored separately. All paper documents were kept in a locked cupboard.

Statistical Analysis

Data analyses were performed using the STATISTICA data analysis software system. Numerical data was presented as means and standard deviations. Categorical data presented as numbers and proportions. T-tests were used to compare numerical data. Comparison of proportions was used to compare categorical data. ANOVA (Analysis Of Variance) was used for the determination of difference between groups. AUC (area under the curve) was used to predict the cut-off levels for variables of interest. Significance was set at $p < 0.05$.

Ethics

The research Protocol "The role of cytomegalovirus in children with hypoxic pneumonia admitted to a paediatric intensive care unit" (S16/03/046) was approved by the Human Research and Ethics Counsel (HREC) of Stellenbosch University.

Chapter 4: Results

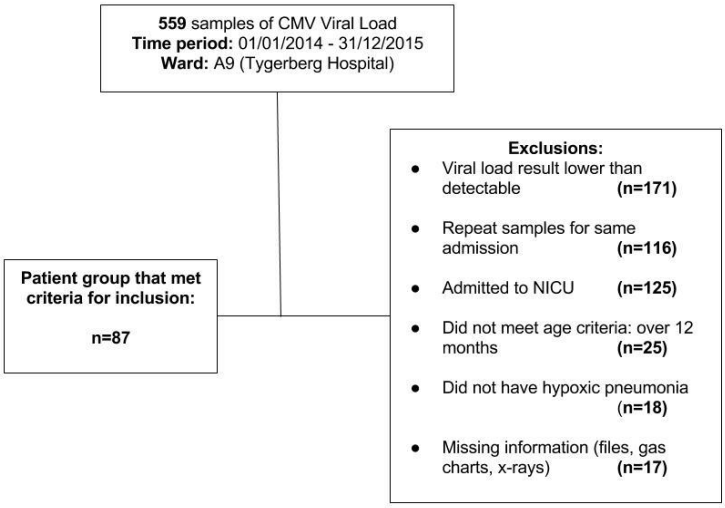


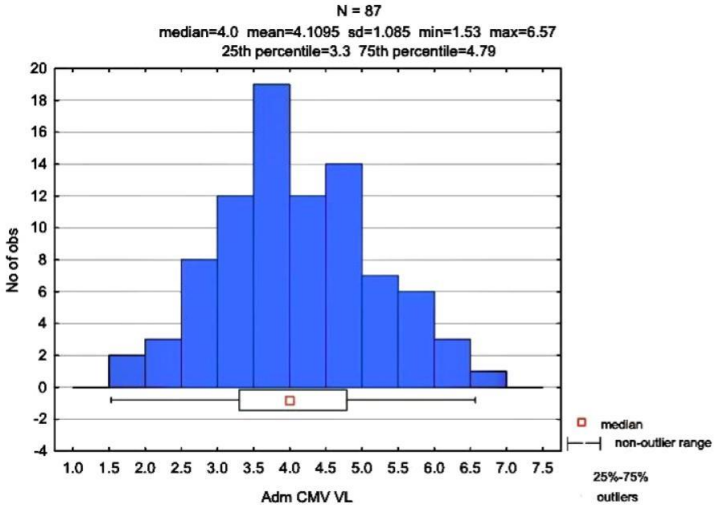
Figure 1. Patient recruitment diagram

A total of 559 CMV viral load samples from 1 January 2014 till 31 December 2015 were reviewed for inclusion in the study. One hundred and seventy-one of these samples were lower than detectable limits, 116 were repeat samples from the same patient during the index admission, 25 samples were from patients over the age of 1 year, 125 were samples taken from patients who were admitted in the NICU, 18 had been admitted to the PICU for other indications (post-operative care etc.) and not hypoxic pneumonia, 17 were excluded due to missing or incomplete information (files, arterial blood gas result sheets etc.). Eighty-seven patients met study definition and were included in the study. (Figure 1)

The mean age for the group was 3.9 months (IQR:2.2-4.8). Forty percent (n=35) were born premature at birth**.** The nutritional status of the group showed that 43% (n=37) were classified as being severely underweight for age, 41% (n=36) were underweight for age and 16% (n=14) were of normal weight for age. The mean and standard deviation for weight for age Z-score was -2.68 (IQR: -3.0 - -0.83). Thirty-one percent (n=27) of the patients were HIV positive, of which 89% (n=24) were newly diagnosed with HIV at the index admission and not receiving antiretroviral therapy. Risk of mortality was assessed using the PIM 2 scores. The study group showed a mean risk of mortality of 24% (CI:18 to 29%).

	HIV + (n=27)	HIV - (n=60)	Total n (%)
Age (months), mean (SD)	4.1 (2.8)	3.8 (2.0)	
Males, n (%)	13 (14)	36 (41.4)	49 (56.3)
Black	20	40	60 (69)
White	1	1	2 (2.3)
Coloured	6	18	24 (27.6)
Indian	0	0	0
Other	0	1	1 (1.1)
Premature at birth	4	31	35 (40.2)
On ART	3	n/a	3 (3.4)
Discharged	24	54	78 (90)
Died	3	6	9 (10)

Table 1: Characteristics of children in study based on HIV status



Graph 1: Statistical analysis of CMV VL on admission

As per the statistical analyses of CMV viral loads done on admission (see Graph 1), the mean CMV viral load was 4.1 (SD 1.08) and the median was 4.0 (IQR 3.3-4.79). The authors decided make comparisons between two groups, where the CMV viral load log value was either ≤ 4.0 or > 4.0. Coincidently, there is literature differentiating between these exact groups where a CMV viral load log ≤ 4.0 and >4.0 are classified as CMV infection and CMV disease respectively.

		CMV VL <=4 (n=44)	CMV VL > 4 (n=43)	p-value
Normal weight	(n=47)	21	16	0.161
Underweight	(n=14)	5	9	0.113
Severely Underweight	(n=36)	18	18	0.464
Premature at birth	(n=35)	17	18	0.378
HIV positive	(n=27)	13	14	0.382
Discharged	(n=78)	39	39	0.374
Died	(n=9)	5	4	0.374
Oxygen on Day 28	(n=12)	5	7	0.254
		Mean (SD)	Mean (SD)	
Risk of mortality %	(n=87)	23 (21)	24 (26)	0.715
Length of Stay (days)	(n=77)	10.56 (8.43)	12.08 (8.49)	0.434

Table 2: Comparison of patient characteristics according to CMV VL

With regard to nutritional status, there were no significant differences between the two groups. Of the 27 patients who were HIV positive, 13 had CMV viral load log values ≤ 4 and 14 had CMV viral loads > 4.0 (p 0.38).

Outcome was based on PICU mortality (either discharged or died) and morbidity (still requiring oxygen support 28 days from admission to PICU. Outcomes were favourable as 90% (n=78) of the patients were discharged from PICU, while 10% (n=9) died while in PICU. There was no significant differences between the two groups regarding mortality. Similarly, there were no differences between the 12 patients who were still on oxygen on day 28 after admission, 7 were from the group with higher CMV viral loads (p=0.25). Length of PICU stay did not differ between the two groups (p=0.43). (Table 2)

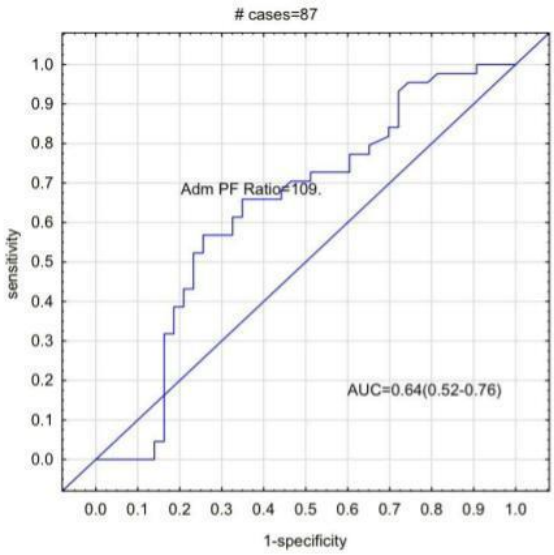
		Mean (SD)	CMV <=4 (n=44)	CMV > 4 (n=43)	95th CI	p-value
Blood Results						
CD4 Count Abs (cells/uL)	(n=25)	764.48 (762.20)	1097.84 (769.22)	422.83 (764.32)	-1001,9;-348.08	0.0001
HIV VL Log	(n=27)	4.987(3.753)	4.142(4.968)	5.771(2.002)	0.007;3.25	0.049
LDH (U/L)	(n=29)	1653.69 (1862.25)	2108.81 (1917.82)	1093.54 (1862.25)	-1821.33;-209.21	0.014
Neutrophils (10^9/L)	(n=85)	8.63 (7.65)	8.03 (7.29)	9.25 (8.05)	-2.05;4.49	0.461
Lymphocytes (10^9/L)	(n=85)	4.04 (3.29)	4.54 (4.13)	3.53 (2.04)	-2.4;0.38	0.153
Oxygen Support (Days)						
NP02	(n=74)	7.45 (9.40)	6.44 (9.12)	8.39 (9.11)	-1.94;5.84	0.321
CPAP	(n=50)	3.06 (2.26)	2.73 (2.28)	3.41 (2.27)	-0.29;1.65	0.167
IPPV	(n=77)	4.92 (4.74)	4.45 (4.77)	5.43 (4.79)	-1.06;3.02	0.341
HFOV	(n=38)	6.05 (4.30)	5.00 (4.30)	7.67 (4.45)	0.81;4.54	0.005

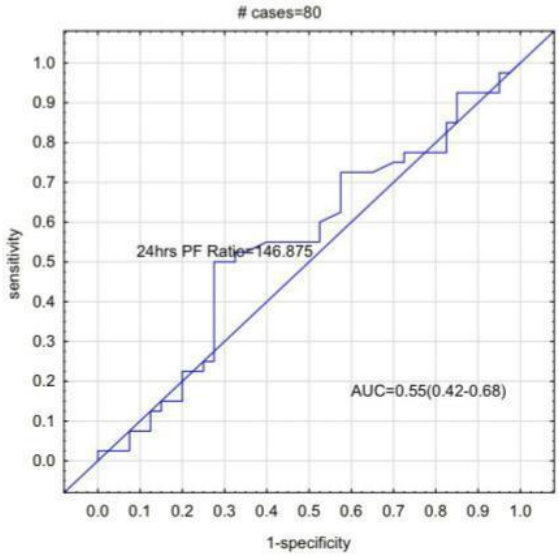
Table 3: Comparison of blood results and oxygen requirement according to CMV VL

As per Table 3, a comparison of CD4 counts and CMV VL showed that the mean absolute CD4 count in the group with higher CMV viral loads was significantly lower than in the group with lower viral loads. (p 0.0001). The converse was true for HIV VL (p 0.049). Patients with

higher CMV viral loads had lower mean LDH levels when compared to the group with lower CMV viral loads (p 0.014).

The only significant difference between those with higher and lower CMV viral loads with regards to duration of specific mode oxygen support needed showed that the group with higher CMV viral loads required a longer duration of HFOV (p 0.005).





Graph 2 and 3: ROC curves comparing PF ratio at admission and 24hrs, respectively, to CMV VL

Receiver operating characteristic curves comparing $Pa0_2/Fi0_2$ ratios on admission and 24 hours after to the blood CMV viral load log showed that on admission, the group with CMV viral load ≤ 4.0 was significantly different from the group with viral load > 4.0 when related to the $Pa0_2/Fi0_2$ ratio with a sensitivity of 0.64 (p 0.01), however, at 24 hours after admission the sensitivity is 0.55 (p 0.23).

	CMV VL <=4 (n=44)	CMV VL > 4 (n=43)	p-value
Normal (n=4)	3	1	0.279
Bronchopneumonia (n=43)	24	19	0.335
DIffuse Alveolar Pattern (n=36)	15	21	0.187
Interstitial Lung Disease (n=2)	1	1	NS
Lobar Pneumonia (n=2)	1	1	NS

Table 4: Comparison between chest x-ray changes according to CMV VL

NS=Not significant (sample size was too small to analyze)

There were no significant differences with regard to x-ray findings between the two groups. While 21 (58%) of the 36 patients who had a diffuse alveolar pattern on x-ray indicating severe

disease were from the group with higher viral loads, however, the difference between those from the group with lower viral loads was not significant (p 0.08).

	CMV VL <=4 (n=44)	CMV VL > 4 (n=43)	p-value
Rhinovirus (n=22)	10	12	0.527
RSV (n=17)	10	7	0.479
PJP (n=14)	3	11	0.018
Candida Albicans (n=7)	2	5	0.225
Klebsiella Pneumoniae (n=7)	4	3	0.719
Adenovirus (n=4)	2	2	NS
Parainfluenza (n=4)	3	1	NS
Metapneumovirus (n=3)	2	1	NS
Influenza A (n=3)	2	1	NS
Stenotrophomonas (n=3)	2	1	NS
Pseudomonas (n=3)	2	1	NS
Mycobacterium TB (n=2)	2	0	NS
Coronvirus (n=2)	1	1	NS
Moraxella Catarrhalis (n=2)	1	1	NS
Human Enterovirus (n=2)	1	1	NS
Acinetobacter Baumannii (n=2)	2	0	NS
Serratia Marcesens (n=1)	1	0	NS
Enterobacter Cloacae (n=1)	1	0	NS
Staphlococcus Aureus (n=1)	1	0	NS
E.Coli (n=1)	1	0	NS
MRSA (n=1)	0	1	NS

Table 5: Other organisms grown on Tracheal Aspirate (in order of frequency)

NS = not significant (sample size was too small to analyze), CMV VL = Cytomegalovirus viral load, RSV = Respiratory Syncytial VIrus, PJP = Pneumocystis jiroveci pneumonia, MRSA = Methicillin-resistant Staphylococcus aureus

Multiple organisms were cultured from tracheal aspirate on admission (see Table 5), in order of highest to lowest frequency. A significant finding was that those with higher CMV viral loads also had a higher incidence of PJP co-infection (p 0.008). Rhinovirus (n=22) and RSV (n=17) were two most frequently observed organisms, however there was no significance difference in relation to CMV viral load (p=0.28 and p=0.22 respectively). All those with PJP co-infection were treated for CMV and subsequently discharged from PICU.

	n (%)	CMV <=4	CMV > 4
Number	28 (32.12)	17 (19.54)	11 (12.64)
HIV positive	2	1	1
Oxygen dependant	0	0	0
Died	4	3	1
PJP co-infection	0	0	0

Table 6: Patients who were not treated for CMV

In the study group 28 (32%) were not treated treatment for CMV-associated pneumonia. Two of these patients were HIV positive, 1 from each comparative group, and no patients in either groups had PJP co-infection. A total of 4 patients of this subset died during PICU admission, with only 1 of the deaths being from the group with the higher CMV viral loads. The patient that died was HIV infected. No patients that were not treated for CMV-associated pneumonia required supplementary oxygen for greater than 28 days after admission. As the subset was so small, it was not statistically analyzed and is used for descriptive purposes. (Table 6)

Chapter 5: Discussion

The main finding of this retrospective review was that in patients with CMV-associated pneumonia, there was no obvious association between CMV viral load and the course and outcome from CMV-associated pneumonia. Patients with higher CMV viral loads required a longer duration of HFOV, however there was no difference in length of PICU stay or mortality. There was a relation to CMV viral load and PJP co-infection with more PJP co-infection occurring in patients with higher CMV VL. There was a 100% survival among those treated for CMV-PJP co-infection in this study.

While CMV viral load in the group with log values ≤ 4 was a positive predictor of a $Pa0_2/Fi0_2$ ratio on admission, the sensitivity was only 64% and at 24 hours after admission, it lacked utility as the sensitivity was only 55%. This illustrates that viral load was a poor predictor of disease severity in this study. This was not dissimilar to a prospective study by Cloete *et al.,* [32] from the PICU at Steve Biko Academic Hospital, Pretoria, RSA, which reported that of the patients with hypoxic acute lower respiratory tract infection with a $Pa0_2/Fi0_2$ ratio of <200, only 27% had CMV viral load log values > 4.0.

Ranieri *et al*. [33] described diffuse alveolar disease as a pathological hallmark of ARDS. Thirty-six (41.4%) of the study population had a diffuse alveolar disease pattern on chest x-ray while only 21(58%) were from the group with CMV viral load log values of > 4.0, which according to literature is classified as CMV disease. In a 9-year retrospective study by Gie, *et al*. [34] that the most common causative organism diagnosed in open lung biopsy on patients that had diffuse lung disease, was CMV. As there was no statistical significance difference between the two groups (viz. CMV VL ≤ 4 and CMV > 4) with regards to x-ray changes, and in particular, diffuse alveolar disease, we cannot confidently deduce that a CMV viral load > 4 equates to CMV-associated pneumonia or that it predicts the severity of disease.

The duration of HFOV needed, significantly differed between those with higher vs lower CMV viral loads, in that those with higher CMV VL needed a longer duration of HFOV, yet there was no difference in other ventilation modalities. Despite this fact, the length of PICU stay between the two groups showed no difference. There is a paucity of literature in our setting demonstrating the effect of the CMV viral load on length of ventilation, however, in Vietnam, Mai Doan *et al*. [35], showed that patients with CMV viral loads > 4 had a longer duration of oxygen support, however, the modalities of oxygen support were not stipulated. An audit of primary medical conditions in children admitted to the paediatric intensive care unit of Charlotte Maxeke Johannesburg Academic Hospital by Mopeli *et al*. [35] showed that children

who tested HIV PCR-positive had a significantly longer duration of ventilation than those who were exposed but uninfected but HIV exposed children were ventilated for 4 more days than those who were unexposed. As the HIV status in the Vietnamese study was not looked at, we cannot exclude how many of those that had longer oxygen requirements were HIV positive. We cannot, with confidence, say that there isn't a relationship between CMV viral load and duration of oxygen requirement as this could differ with HIV status. Another shortfall was that of the HIV negative children in our group, we were unable to quantify how many of them were HIV exposed. In addition, many of the patients in our study had multiple organisms on tracheal aspirate which may have influenced the mode of ventilation required.

There was a 90% PICU survival of the patients admitted to PICU with CMV-associated pneumonia with no difference in the two CMV viral load groups. It has been well documented by many authors in similar settings to our study that there is no significant difference in mortality between HIV infected and HIV uninfected children with pneumonia in PICU. [32,36,37] Our findings are consistent with those of Cloete *et al*. [32] where a multivariate analysis of the variables, including CMV viral load, that influenced mortality there was no single factor that influenced mortality. The survival in our study was akin to that of the study by Jeena and Githinji [37], who attributed better outcomes to treatment as opposed to studies done in the pre-cART and pre-ganciclovir era in 2004/5, where the mortality rate of CMV-associated pneumonia in HIV positive children was 72% at Tygerberg Hospital [28] and 55% at Red Cross War Memorial Children's Hospital [14]. It is worth mentioning that of the 27 HIV-infected children in our population group, 89% (n=24) were newly diagnosed with HIV at the index admission and had not been commenced on cART.

The PICU mortality for this study was 10.3% which was more than two times lower than the expected PIM2 mortality rate of 24%. Solomon *et al*. [38], based on a retrospective audit at the RCWMCH, showed the PIM2 score to be an appropriate mortality risk assessment score in the context of a PICU in South Africa (SA). The author did acknowledge, however, that further studies from multiple PICUs in SA, were needed to evaluate the use of the PIM 2 in the SA context as it could differ between PICUs. Regrettably, no such study had been undertaken in Tygerberg Hospital to determine whether the PIM 2 score was a valid assessment tool of mortality risk in that PICU. We therefore cannot postulate that, based on the PIM2 score, better outcomes were due to PICU practices itself or a lack of utility of the PIM2 score in our setting. An association between CMV VL and PJP co-infection was noted where the incidence of PJP co-infection was significantly higher in the group with CMV VL > 4. This was also observed by Zampoli *et al*. [14] in a prospective study in Cape Town that showed that CMV-associated pneumonia with PJP co-infection has a poor outcome. [4,10,14,28] In contrast, there was a 100%

survival of the patients in this study with PJP co-infection, albeit small number of patients with CMV-PJP co-infection. All of the co-infected patients were treated for CMV-associated pneumonia with ganciclovir. A decrease in mortality from PJP was also observed by Cloete *et al*. [32] in their study. One can conjecture that as poor mortality in these patients were seen in the pre-ganciclovir and pre-cART periods, the decline in mortality may be attributed to the availability and early use of ganciclovir and antiretroviral therapy. This is, however, pure conjecture and as no single factor is responsible for better outcome, many therapeutic interventions may contribute to it as well.

Of interest was of the 14 patients with PJP co-infection, 50% were HIV-uninfected. Only three of these patients were investigated for an underlying primary immune deficiency. One of the CMV-PJP co-infected patients who was HIV-uninfected had an open lung biopsy performed. This resulted in a final diagnosis of pulmonary interstitial glycogenosis. Five (71%) of those who were HIV-uninfected also happened to have been premature at birth. The association between prematurity and PJP in HIV-uninfected patients is well documented. The relationship between CMV VL and lower CD4 counts is worthy of being explored further. In our study where CD4 counts were significantly lower in patients with higher CMV viral loads. Many authors have described this relationship between CMV infection and low CD4 counts, where it has been postulated that the incidence of CMV infection is greater in those with lower CD4 counts. Our findings were similar to that of a study in India by Kannangai *et al*. [40]

An interesting finding in this study was the significant difference in mean serum LDH levels in the two comparative groups. While LDH was elevated in both groups, as would be expected based on literature, the mean LDH was significantly lower in the group with higher viral loads. There is a lack of literature demonstrating a relationship between serum LDH values and CMV viral loads in patients being treated for CMV-associated pneumonia. The higher serum LDH levels were not associated with CMV-PJP co-infection.

Study Limitations

The most important limitation of the study was that without lung biopsy it is difficult to diagnose CMV pneumonia and without it, actual proof of CMV-associated pneumonia is remains uncertain, as documented in previous studies. The measure of viral load does not equate to pulmonary disease and while the majority of our participants had multiple pathogens isolated from tracheal aspirates, we cannot exclude that these pathogens were not the causative agent of the hypoxic pneumonia. The second limitation was that of those patients who were HIV-uninfected, we were unable to document the number that HIV-exposed but uninfected. This maybe important as infants cared for by HIV-infected parents are most likely to be exposed to a different pathogen load. The literature also suggests that HIV-exposed uninfected infants might have some degree of yet unexplained immune deficiency making them more vulnerable to develop opportunistic infections. In addition, only a fraction of the patients with PJP co-infection had been investigated for a primary immune deficiency which had contributed to them developing CMV-associated pneumonia. As this was a retrospective study, there were many different management techniques employed which could influence our results.

Chapter 6: Conclusion

CMV viral load alone cannot diagnose CMV-associated pneumonia and showed limited utility in predicting the course and outcome of CMV-associated pneumonia. The obvious relation between CMV VL, PJP co-infection and CD4 count may suggest that the viral load is indicative of some underlying ill-defined disease process. This remains unclear from our study. As lung biopsy is not always feasible in our setting, a diagnosis of CMV pneumonia may need to be made by looking at multiple factors simultaneously, which include radiological changes, clinical presentation and laboratory investigation. The difficulty arises in patients who are HIV-uninfected as CMV is more difficult to diagnose. Despite the increase in HIV awareness and availability of PMTCT to exposed babies, the majority of the HIV-infected patients were newly diagnosed on admission to PICU. This may be suggestive of failed PMTCT, poor surveillance or a lack of insight into the disease etc. While there is an improvement in the total number of newly diagnosed people since antiretroviral therapy becoming more readily available, it seems as though those who are infected by vertical transmission account significantly in the group while there are modalities available to prevent transmission.

Chapter 7: Recommendations

1. All HIV positive and HIV exposed children admitted with hypoxic pneumonia should be treated empirically for CMV and PJP regardless of CMV viral load value.
2. In patients where CMV pneumonia is suspected, CMV viral load on BAL may prove more accurate in making the diagnosis in correlation with clinical and radiological findings. This hypothesis needs further investigation.
3. All HIV-uninfected children diagnosed with PJP infection should be adequately worked up for primary immune deficiencies unless there is a known reason for their immunosuppression.
4. All HIV-exposed infants are to be adequately placed on PMTCT and mothers are to be thoroughly counseled regarding the risk factors involved in increased transmission. There needs to be even more awareness surrounding HIV and every effort to destigmatize this condition.

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Appendix A (Case report form)

Study Number: _____

Race:

Black	White	Coloured	Indian

Sex:

M	F

Area of residence: _____

Date of birth: _____ Weight (on admission): _____kg

Date of admission to PICU (DD/MM/YY):_____/_____/_____

PIM Score: _____

NP02	CPAP	IPPV	HFOV
Duration: _____ days	Duration: _____ days	Duration: _____ days	Duration: _____ days

HIV Status:

Positive	Negative	Unknown

If positive, is patient on HAART:

Y	N	Unknown

CD4 Count (Absolute): _____
HIV VL Log: _____

Acute Phase Reactants (admission): CRP: _____ PCT: _____

Blood Culture on admission: _____

CMV Viral Load (Log): _____

Tracheal Aspirate: If yes, result:
_____

Y	N

Nosocomial infection during ICU stay: If yes, organism cultured: _____

Y	N

CXR:
_____

Treated for CMV:

Y	N

If yes, drug used:

Ganciclovir Duration: _____ (days)	Valganciclovir Duration: _____ (days)

Outcome:

1. Death	Date of death (DD/MM/YY): _____/_____/_____
2. Discharged from PICU	Date of discharge (DD/MM/YY): _____/_____/_____

Oxygen dependent:

Y	N

If yes,

In-hospital oxygen	Domiciliary oxygen