# Cytomegalovirus pneumonia co-infection with pneumocystis jiroveci pneumonia in HIV exposed infants admitted with suspected pneumocystis jiroveci pneumonia

Dr A.O Ogugua

MBBS

Department of Paediatrics and Child Health,

University of the Free State

A research thesis submitted in partial fulfillment of the requirement of degree of
Masters in medicine (paediatrics) in the School of Medicine

University of the Free State

December 2010

## Declaration

This thesis is my original work and has not been presented for a degree or other awards in any other university.

Signature_____

Name: Dr. A. O. Ogugua

Student number: 2008006363

Date_____

**Supervisors' approval**

This thesis has been submitted to the graduate school with our approval as university supervisors.

Signature_____

Dr. A.E Kappos.

Department of Paediatrics UFS

Date _____

Signature_____

Prof. S.Brown.

Department of Paediatrics UFS

Date_____

Signature_____

**Dedication**

To my late mother: Izualor, for inculcating values and fundamentals of life in me.

To my wife: Eunice, for being the shining light in my life.

To my children: Obiemeka, Chibuzo and Chidera, for making our life meaningful; they are our pride.

To all HIV infected infants in South Africa: There seems to be light at the end of the tunnel. Remain hopeful!

**Acknowledgment**

This task was accomplished through important contributions made by various individuals and groups who devoted precious time and energy towards the production of this piece. To them all, I owe big gratitude.

My greatest thanks go to my project supervisors, Dr. A.E Kappos and Prof S. Brown, who not only played the part of mere supervision but actively participated in the run-up to and the execution of the research. Their wealth of experience was brought to bear by ways of constructive criticisms, invaluable suggestions and above all, painstaking attention to details especially during vetting of the manuscript. Words alone are not enough to express all they did to ensure that this piece of work saw the light of the day. May God Almighty continue to guide and bless them in all their endeavours.

My special thanks also remain evergreen to Dr Dominique Goedhals of the Department of Virology, at the Universitas Hospital, for her unwavering zeal and enthusiasm for the project, ensuring that correct specimens were collected and analyzed. She was outstanding in this regard.

I also owe a great deal of gratitude to all the staff of the Physiotherapy department, at Pelonomi Hospital, notably to A. du Plessis and T. Matobela for their vital role of specimen collection.
In the same token, I wish to thank all NHLS staff at Pelonomi hospital and the Department of Microbiology at Universitas hospital, whose contributions assisted me in many varied ways in the course of this research.

I will ever remain grateful to the architects of this work: Yolandi Goosen, the research assistant at the Department of Paediatrics, for taking charge of this work from the beginning to the end. She was relentless, dedicated and thorough in her work. Keep it up Yolandi!

Dr. Jacques of the Statistics Department, at UFS, did all the statistical calculations and analyses and to him, also, I express my deepest gratitude.

My special thanks go to all parents who gave consent for their babies to participate in this huge learning exercise. It has yielded some remarkable results and truly, I have enriched my knowledge for better care of these babies.

Finally, the bulk of the gratitude goes to my Head of Department, Prof. A.Venter for his fatherly role and for giving me the opportunity to really understand the meaning of research. This has opened my eyes, clearing all the myths surrounding research work. I hope to undertake more research projects in future with much enthusiasm.

# Table of contents

## Abstract

**Background:** Cytomegalovirus (CMV) and pneumocystis Jiroveci (PJP) are common causes of severe pneumonia in infants with human immuno-deficiency virus (HIV). Both CMV and PJP are associated with high mortality but the prevalence and effect of CMV pneumonia co-infection with PJP in our environment is poorly understood.

**Aim:** To establish the prevalence of CMV pneumonia co-infection with PJP in HIV exposed and / or infected infants presenting with suspected PJP and the effect of such co-infection on disease outcome.

**Design**: A prospective descriptive study.

**Setting:** Paediatric wards, Pelonomi Regional Hospital, Bloemfontein.

**Subjects:** Eight infants aged 2-6 months who were HIV exposed and / or infected admitted with suspected PJP from 1st July 2009 to 1st August 2009 and from 1st October 2009 through to 1st December 2009.

**Methods**: Blood and sputum specimens were collected using aseptic techniques. These were venous and arterial blood samples and non invasive induced sputum samples respectively. A diagnosis of probable CMV pneumonia was assumed if the peripheral blood CMV viral load was greater than 10,000 copies / ml together with a positive CMV result on induced sputum using shell vial culture (SVC) test. PJP was diagnosed by indirect immuno-fluorescencce test using induced sputum specimen.

**Main outcome measured**: Prevalence of CMV Pneumonia / PJP Co-infection in HIV exposed infants presenting with signs and symptoms resembling PJP, and the effect of such co-morbidity on disease outcome. As a secondary outcome, the influences of bactrim prophylaxis, anti-retroviral therapy (ART) and CD4% lymphocytes on the prognosis of these infants were also considered.

**Results**: The prevalence of CMV pneumonia alone was 50%, and that of CMV Pneumonia/PJP co-infection was 12.5%, while PJP alone was 25%. All patients with either CMV Pneumonia alone or PJP alone survived and were discharged home. Clinically, CMV pneumonia / PJP co-infection had a poor outcome since the affected patient died after prolonged mechanical ventilation and hospital stay, but this finding was not statistically significant ( C.I = 2.2%-47.1%, p = 0.49).

**Conclusion:** CMV Pneumonia/PJP Co-infection is prevalent (12.5%) in our setting and seems to carry a poor prognosis. CMV pneumonia alone is highly prevalent (50%) but under - diagnosed. Clinically, it seems that PJP alone has excellent prognosis. It is recommended that more research be carried out in this field over a longer period of time with larger sample population to achieve a conclusive result.

## Abbreviations and acronyms

1. ART: Antiretroviral Treatment.
2. CD4: Cluster of differentiation 4.
3. CD8: Cluster of differentiation 8.
4. CMV: Cytomegalovirus.
5. PCR: Polymerase chain reaction.
6. PJP: Pneumocystis Jiroveci Pneumonia.
7. WHO: World Health Organization.
8. UNICEF: United Nations Children's Fund.
9. UNAID: Joint United Nations Programme on HIV/AIDS.
10. AIDS: Acquired Immuno-deficiency Syndrome.
11. EDTA: EthyleneDiamine TetraAcetic acid.
12. BAL: Broncho-alveolar lavage.
13. HIV: Human Immuno- deficiency Virus.
14. UFS: University of the Free State.
15. SVC: Shell vial culture.

## Chapter One: Introduction/ literature review

The emergence of human immuno-deficiency virus (HIV) infection in this era, as a precursor to acquired immuno-deficiency syndrome (AIDS), has been described differently in many quarters as a "plague", epidemic and man-made evil with the obnoxious aim of bringing the world's population to extinction. The World Health Organization (WHO) and other agencies across the globe are working relentlessly, in many ways, in their quest to stem this pandemic and its devastating consequences.

HIV is the virus responsible for weakening the immune system, particularly the cluster of differentiation 4 (CD4) lymphocytes causing their gradual depletion and ultimately leading to the development of AIDS.

Paediatric HIV is mainly vertically transmitted. In an effort to curb this phenomenon, the programme of prevention of mother to child transmission (PMTCT) was initiated. This includes the administration of highly active antiretroviral therapy (HAART) to the mother, elective cesarean section, early nevirapine use by the baby up until breast feeding is stopped, and replacement feeding. 30% of pregnant women are HIV positive at booking. Without interventions transmission rate ranges from 25-48%. This figure could be reduced to 0% (that is 100% reduction) with appropriate interventions (1).

The burden of this disease is enormous. It is estimated that about 33.4 million people (adult and children) live with HIV world - wide at the end of 2008 and that about 67% of this (24.4 million) live in sub-saharan Africa. An estimated 1.4 million Africans died from HIV in 2008. About14.1 million children were orphaned by this epidemic in 2008 and an estimated 1.8 million children were living with HIV as at 2008. Paediatric new HIV infection rate stood at 13% over the same period (2).

South Africa has the highest number of people living with HIV in the whole world with about 5.3 million of the population infected, including 220,000 children below the age of 15 years in 2008. The prevalence of HIV in South Africa was 17.5% in 2008 with children below the age of 15 years accounting for 4.2% of this figure. 310,000 people died of AIDS in 2008 in South Africa (2)

Children with HIV/AIDS are highly susceptible to different kinds of infections which are the most important cause of death in this group. Severe respiratory infections are particularly reported to be the most common cause of death in HIV/AIDS patients. These include: bacterial (Strep. Pneumonia, Mycobacterium), viral (CMV=30-40%) and fungal (PJP=38%) (3). Infants below 6 months of age are the worst affected, due to their poor immune system and other practices like replacement feeding instead of breast milk etc.

Pneumocystis jiroveci, previously known as Pneumocystis carinii, is the organism responsible for PJP; a common opportunistic infections in HIV exposed and / or infected infants. The pneumocystis genus isolated in humans is known to be a unicellular fungus named Pneumocystis jiroveci after Dr. Otto Jirovec who (together with his team) isolated it in humans (4).

PJP in the immune-compromised is a serious medical condition and life-threatening. In the healthy host, infections are usually subclinical. The disease occurs almost exclusively in severely immune-compromised hosts including those with congenital or acquired immunodeficiency disorders, malignancies and organ transplanted patients. HIV affects the CD4 cells causing their gradual decline. This predisposes the individuals to many opportunistic infections of which PJP is one of the most serious. Normally, CD4 counts are higher in infants and young children than in adults and decline over the first years of life. Therefore, children may develop opportunistic infections at higher CD4 counts than adults. PJP is most commonly seen in infants when the CD4 count declines to below 750 cell/ul (15% of lymphocytes) under the age of 12 months, necessitating early introduction of prophylaxis in this age group irrespective of the CD4 count (5).

Cytomegalovirus is a member of the herpes family of viruses. In most people with fully functioning immune systems, initial infection with CMV may cause only a mild upper respiratory tract illness, but afterwards it is kept dormant. If the immune system is destroyed as in the case of people with advanced HIV disease, it can be re-activated. The average CD4 cell count of people at the time they develop the first episode of CMV is below 30cells/$mm^3$ (6). When the host immune system is immature (such as in the very young) or compromised (such as in AIDS), infections can be associated with disease and the target organs includes the lungs and retina amongst others.

Babies acquire CMV through vertical transmissions: - In-utero (30-40%).

- Perinatal (57%).

- Breast milk (53%) (7).

Studies have shown that HIV infected women have a higher rate of CMV infection and viral shedding and therefore exhibit higher transmission rate (7). The progression of CMV infection to diseases like pneumonia is common with clinical manifestation of CMV pneumonia occurring in 25-40% of AIDS patients before the introduction of HAART (8).

The intensity of CMV pneumonia is related to the severity of immune-suppression (9). Cell mediated immunity is the most important immunologic factor in controlling CMV infection. Therefore, patients who lack this type of immunity are completely susceptible to CMV disease. CMV-specific $CD4^+$ and $CD8^+$ lymphocytes play a major role in immune protection after primary or re-activation of latent disease. Patients who do not have CMV – specific $CD4^+$ or $CD8^+$ cells (such as HIV infected infants) are at higher risk for CMV pneumonitis (10). This could explain why HIV patients are targets of CMV and PJP and why this carries a very poor prognosis if both infections co-exist in one individual with HIV infection.

In children with AIDS, persistent pneumonia is a major problem. In a study of 307 paediatric AIDS patients, disseminated CMV was found to be a common cause of persistent pneumonia (19%) (11). In a clinical and pathological study on the cause of death in 75 AIDS patients (as quoted in the above report), with emphasis on the role of pulmonary disease, CMV was the most prevalent pathogen and was isolated in 44 (58.6%) patients. CMV was shown to have caused significant disease in 21 (28%) of patients of whom 5 (6.6%) died of CMV pneumonia. In another study contained in this same article (11), out of 54 patients who died with HIV infection, 57% had histological evidence of CMV infection in the lung at autopsy. One half of the patients with evidence of CMV pneumonia were thought to have died of respiratory failure, although other causative organisms such as Pneumocystis jiroveci where also found in most of the patients.

In an interim report on a prospective study on the incidence of CMV infection among 74 HIV infected and uninfected infants hospitalized in the Western Cape Province, it was found that 48% of patients with severe pneumonia had CMV infection, 38% had probable CMV pneumonia and 12% had PCP/CMV co-infection resulting in a total of 31% in-hospital mortality (12). The study further revealed that CMV was an important, yet under-recognized pathogen in infants presenting with severe pneumonia in high HIV prevalent settings. The study had a few limitations especially in the area of CMV diagnosis. Here nasopharyngeal aspirates were used which have been reported to have a very low sensitivity and high chance of specimen contamination. However, it was still a useful work as it sensitized health workers, like us, to the incidence of an important, yet significantly under-diagnosed, condition.

Another study published by the institute of Child Health London (2001), in which a total of 85 infants were enrolled, showed that amongst infants with PCP, 79% were born to mothers tested for HIV. The study found that infants with a dual diagnosis of PJP and CMV were more likely to be ventilated and receive corticosteroids than those with PCP alone (13).

According to a study published in 2001 in the South African Journal of HIV medicine, CMV pneumonia had a high prevalence in HIV-1 infected South African children in Johannesburg and contributed to mortality of between 32% and 38.8% as confirmed by lung biopsy specimen (14). Another study, referred in (14), documented 48.8% prevalence of PJP on postmortem lung biopsies in HIV children with severe LRTI. The study found no clinical or laboratory tests able to distinguish between children with or without PJP.

In a prospective observational study to evaluate the outcome of 31 HIV positive infants after 3 months of age admitted to Steve Biko Academic Hospital in Pretoria, South Africa, with clinical PJP and using tracheal aspirates for the diagnosis of PJP and both tracheal aspirates and blood specimens for the diagnosis of CMV pneumonia, it was found that the mortality rate in PJP positive infants was 23.1% and PJP negative infants 22.2% and that the prevalence of PJP/CMV pneumonia co-infection was high. Infants who died, 7/31 (22.5%), irrespective of whether they were PJP positive or negative had CMV pneumonia and all PJP proven cases without

CMV pneumonia co infection survived. The average length of stay for PJP proven patients without co-infection was shorter. The study suggested that PJP, *per se*, was not necessarily a "bad" disease (15).

Several studies have shown that CMV could be isolated from the lung tissue of patients with proven PJP, but controversy remains whether it causes morbidity or contributes to mortality, as asserted by Tam (1999). In this paper, Tam (1999) stated that: "the importance of pulmonary CMV infection in HIV-positive patients is controversial as CMV is often found in broncheoalveolar larvages (BAL) of these patients without evidence of CMV pneumonia" (16). This statement is important considering the earlier report that 19% of patients studied developed pneumonia caused by CMV. It therefore presents a challenge for future research, including meta - analysis, in this field in order to resolve this controversy.

According to a retrospective study by Slogrove et al in Cape Town, South Africa (2010), the prevalence of PJP in HIV exposed but uninfected infants was 37.5% while that of CMV in the same sample population was 12.5%. CMV co-infection was excluded in all the patients with PJP. The study found that they all had mixed pathogens and prolonged hospital stay with a median duration of 38.5 (14-81) days. None of the infants had primary immunodeficiency. The outcome was good as there was no mortality in all the patients studied (17)

The development of PJP in apparently immune-competent term infants who were HIV unexposed is rare with only about 4 cases reported by the end of the year 2000. Few available documented cases seemed to suggest that those affected infants usually suffer from chronic diarrhea, severe malnutrition, zinc deficiency, necrotizing enterocolitis (NEC), and congenital CMV infection and therefore at risk of developing PJP. They recover very well with early and appropriate treatment, without mortality (18).

Some interventional measures by ways of prophylaxis and treatment have been instituted in most countries to reduce the burden of both the opportunistic infections and HIV/AIDS. Co-trimoxazole (bactrim) prophylaxis and ART have been successfully used in these regards. HIV exposed infants who are uninfected have greater morbidity and mortality than HIV unexposed infants and children. Children born to HIV positive mothers, regardless of their eventual HIV status are faced with

a high opportunistic disease burden, many of which can be prevented by timely initiation of co-trimoxazole prophylaxis. Prophylaxis with co-trimoxazole, apart from its effectiveness against PJP, also provides other benefits such as preventing infections from other respiratory, urinary and gastrointestinal pathogens and even malaria in some cases (19). A randomized controlled trial conducted in Zambia on HIV infected children showed that mortality could be reduced by about 50% and the number of hospital admissions significantly reduced by about 23% among HIV positive children using co-trimoxazole prophylaxis (19).

In a study conducted in Cape Town, South Africa in 2000, to investigate the incidence and associated features of PCP in African HIV-infected children, Zar et al found that co-trimoxazole prophylaxis reduced the incidence of PCP significantly. Out of the 59 children who received co-trimoxazole prophylaxis, only one (1.7%) developed PCP while fourteen out of the 92 (15.2%) who did not receive co-trimoxazole prophylaxis developed PCP (20). This study also supported the National Department of Health's recommendation that co-trimoxazole prophylaxis was effective and should be initiated in HIV positive infants from 4-6 weeks of age.

Highly active anti-retroviral therapy (HAART) could also exact a positive influence on opportunistic respiratory infections. Zar (2008) published the incidence rate of respiratory infections per 100 child years. Pneumocystis carinii pneumonia, bacterial pneumonia and other respiratory pathogens were studied. The incidence rates of PCP per 100 child years before and after HAART were 1.3 and 0.1 respectively while in bacterial pneumonia, there was a reduction in the incidence from 11.1 before HAART to 2.2 after HAART (21). The finding reinforced the perception that ART should be initiated early in HIV positive children to achieve a holistic care.

The incidence of respiratory infections in HIV positive children vary between developed and developing countries with incidence much lower in the developed countries. An estimated 1.4 million women with HIV give birth yearly in developing countries and all of their babies ought to have started co-trimoxazole prophylaxis by age 4- 6 weeks. In 2008, only about 8% of infants needing co-trimoxazole prophylaxis had commenced treatment by latest at two months of age (22). This prophylactic treatment had been the standard of care in developed countries for many years and this contributed, to a larger extent, to this variation in incidence of opportunistic respiratory infections between developed and developing countries.

Higher incidence of hunger, poverty, malnutrition and war in developing countries also made these infants highly vulnerable to these opportunistic infections especially PCP and CMV.

One important difficulty in establishing the absolute prevalence of CMV co-infection with PJP in HIV positive infants lies in the enormous problems encountered in confirming the diagnosis of both CMV pneumonia and PJP. Bronchoalveolar lavage (BAL) has a high sensitivity and specificity, but is highly invasive and therefore not routinely done in children. In the light of the above, the term probable CMV pneumonia is usually applied. A PCR specimen (induced sputum and blood) is usually used and even this has its own disadvantages (low specificity). However, efforts were made in this study to use CMV shell vial culture on induced sputum specimens which has a relatively high sensitivity (88.9%) and specificity (99.7%) and at the same time, is much less invasive (23). It is pertinent to note here that these tests need highly trained personnel and are quite expensive to perform.

In the light of the controversies and difficulties surrounding this area of study, the prevalence of CMV/PJP co-infection in HIV exposed/infected infants was still considered worthy of investigation as it aims to identify the burden of this disease, so that attention and resources could eventually be proportionately diverted to it, ultimately leading to reduction in mortality. Furthermore, CMV pneumonia increases mortality, worsens the prognosis of PJP in infants with HIV and increases the need for and duration of mechanical ventilation (13). If this co-infection is borne in mind, appropriate investigations and treatments could be instituted, and it could be shown to play a significant role in reducing mortality and morbidity in HIV infected/exposed infants.

The heavy burden of HIV and associated respiratory opportunistic infections in South Africa is equally borne by the Free State in general, and Bloemfontein in particular. According to WHO clinical case definition in December 2005, the prevalence of paediatric HIV infection in Bloemfontein was 14.5% (24). The majority of patients admitted to our health facility at Pelonomi hospital, Bloemfontein, present with respiratory signs and symptoms. According to data from our local statistics, close to 70% of admissions are due to lower respiratory tract infections (Pneumonia). Causative agents vary according to age of presentation.

Generally community acquired pneumonias are mostly due to bacterial causes (e.g. Strep pneumonia), especially in the setting of an undernourished or immune compromised paediatric population. The other potential causative agents, such as PJP and viral pneumonias (particularly CMV Pneumonia), especially in HIV infected infants, are suspected but very difficult to diagnose. Thus empirical antibiotic treatments are often initiated but are not always successful in managing this specific population of children. This lack of definite diagnosis of other causative organisms in HIV exposed/infected infants admitted with pneumonia sometimes leads to inadequate treatment of these specific patients with, at times, prolonged hospital stay and/or even mortality as a consequence. Heavy strain is also placed on the limited health resources.

The goal of this study was to determine the prevalence of CMV pneumonia co-infection in HIV exposed infants admitted with suspected PJP. It aimed at establishing the diagnosis of CMV pneumonia and/or PJP in HIV exposed infants and the effect of CMV pneumonia/PJP co-morbidity on disease outcome. It also aimed at identifying the role of antiretroviral therapy and bactrim (co-trimoxazole) prophylaxis in improving the condition of these infants.

The significance of this study rested in the fact that it created an awareness of the existence of other possible causes of severe pneumonia in the HIV exposed/infected babies which were mostly ascribed to bacterial aetiology with often not enough emphasis placed on others. The study established the existence of some causes of severe pneumonia (other than bacterial), alone or in combination, in infants that were HIV exposed/infected and also examined the role of ART and other treatment modalities in reducing mortality, limiting hospital stay, and obviating the need for mechanical ventilation. It, further, underscored the need to acquire a special diagnostic kit, locally, to promote effective and timely diagnosis of CMV pneumonia. This study attempted to establish the prevalence of CMV pneumonia co-infection with PJP in HIV infected infants and tested the research null hypothesis that CMV pneumonia co-infection with PJP in HIV exposed infants does not influence the outcome.

Knowledge of the prevalence of CMV co-infection with PJP would be used to inform the planning and distribution of diagnostic and health delivery services.

Results of the study could point to the need for the establishment of a diagnostic laboratory in Bloemfontein that screens for CMV which in the past was done at centralized laboratories with the resultant high costs of transport and delay in feedback. Most importantly, knowledge of previously undiagnosed factors like CMV pneumonia could improve the overall management of HIV exposed/infected infants admitted with suspected PJP making it more purposeful and cost effective

## Chapter 2: Subjects and methods

The study was a prospective descriptive study carried out in the wards of Department of Paediatrics and Child Health at Pelonomi Regional Hospital Bloemfontein from July 1st to 30th and from September 1st through to October 31st 2009 (over a 3 month period). It aimed primarily to determine the prevalence of CMV pneumonia co-infection with PJP in HIV exposed and/or infected infants admitted with suspected PJP and the effect of such co-morbidity on disease outcome. The further objectives of the study were to establish the diagnosis of probable CMV pneumonia and its co-infection with PJP in our setting and to evaluate the influence of ART (anti-retroviral treatment), CD4 percentage count and co – trimoxazole prophylaxis on the disease outcome of these patients. Inclusion criteria were age 2-6 months, HIV exposure and suspected PJP on admission, while the exclusion criteria included malignant conditions, chronic steroid medication, congenital immunodeficiency state and infants born to HIV negative mothers.

Study-specific enrolment forms to obtain consent for participation in the study were designed and issued to parents of all infants willing to take part. They were available during working hours and in the evenings in the wards. All communication was in the preferred language of the parents (Sotho, Afrikaans and/or English), through an interpreter when necessary. Details of the study were explained to the parents and a written information guide about the study in the appropriate language was dispensed. Parents were also given information on how to access the result if they wished to know the outcome of the research. No financial compensation was provided for participation and they could withdraw the participation of their child at any time. Hospital numbers were used instead of names to guarantee absolute confidentiality.

The empirical diagnosis of PJP was made using a combination of widely accepted clinical and biochemical (LDH) variables as explained later.

### Definitions of terms:

For the purpose of this study certain definitions were formulated; namely:

**HIV exposed:** This meant infants whose mothers were HIV positive during pregnancy. Babies delivered to such mothers we said to be HIV exposed.

**Prolonged hospital stay:** Those patients who were admitted to our facility for more than 10 days on treatment.

**Confirmed PJP:** Babies were diagnosed with PJP if they had positive PJP immuneflouresc-
ence culture on induced sputum specimen.

**Probable CMV pneumonia:** Babies were diagnosed with CMV pneumonia if their blood CMV viral load was above 10, 000 copies/ml with positive SVC culture on induced sputum specimen. The viral load cut-off was defined as the lowest level of viraemia which was considered a positive result for diagnostic purposes. A cut-off value of 10,000copies/ml for CMV viral load, which gives specificity and positive predictive value of 100% was used in this research as recommended in other studies (25). No absolute definite diagnostic test for CMV pneumonia exists and the strength of the diagnosis rested on weighing a variety of clinical and laboratory findings such as high risk patients, presence of signs of severe pneumonia, demonstration of CMV (antigen or its genome) in good quantity, together with the demonstration of CMV in alveolar cells as used in published studies (26).

**Severe pneumonia:** Infants presenting with:

- Cough with difficulty in breathing.
- Tachypnoea (respiratory rate > 50cpm).
- Chest in-drawing with or without inability to drink, cyanosis or head bobbing.

**Suspected PJP:** HIV exposed infants with:

- Hypoxia out of proportion to the clinical findings on consultation
- LDH >500 iu/l
- Plus signs of severe pneumonia.

## Specimens

Specimens used in the study were induced sputum and peripheral arterial or venous blood.

## Obtaining specimens:

**Induced sputum:** Induced sputum specimen collection was carried out as follows:

Patients were kept nil per os for 2 hours prior to the sampling to avoid aspiration and thereafter nebulized with 2ml of 5% saline for 5-10 minutes prior to chest physiotherapy (percussions, vibrations and shaking) in applicable postural drainage positions suitable for each patient. Induced sputum samples were obtained using Romsom suction catheters (finger tip control) size 6 (2.0mm) attached to a Thebe Medical aspirating tube – Suki (8ml) and Hospival 350 portable suction unit with suction pressure set between 10-20 kPa. Sterile suctioning technique, BSN medical dressing tray and Johnson and Johnson KY lubricating jelly were also used.

A nasal or oral route was used. The catheter was advanced until a cough reflex was elicited or resistance was met in the trachea. Suction was then applied whilst simultaneously withdrawing the catheter, collecting the sample. The procedure was repeated 2-3 times until there was a minimum of 0.5ml specimen in the Suki tube. Portion of the specimen was transferred to a viral transport medium and sent on ice to the laboratory for CMV shell vial culture and the remainder sent for PJP culture.

**Blood specimens:** Blood analyses were done for CMV viral load, HIV PCR, CD4 count and LDH.

8cc venous or arterial blood was collected with a butterfly "scalp vein" needle using universal infection control measure. Adequate haemostasis was allowed after each blood sampling. Of the 8cc of blood collected:

2cc was used for CMV PCR viral load testing,

2cc was used for HIV PCR testing,

3cc was used for CD4 count measurement and

1cc was used for serum LDH quantitative assay.

## Diagnosis:

**Diagnosis of CMV pneumonia:** Both blood and induced sputum specimens were used. Quantitative (viral load) and qualitative CMV PCR were done on blood specimens and shell vial culture done on induced sputum specimen. Blood CMV viral load above 10,000copies/ml with positive CMV shell vial culture (SVC) on induced sputum was used as a probable diagnosis of CMV pneumonia (25, 26).

## CMV quantitative assay (viral load):

Cytomegalovirus (CMV) viral load testing was performed by the Department of Medical Virology, NHLS Groote Schuur, on whole blood collected in an EDTA tube. The CMV R-gene$^{TM}$ kit (Argene) on the Rotorgene platform was used according to manufacturer's instructions. This is a real-time PCR technique which involves viral DNA extraction followed by amplification and detection using hydrolysis probes. The results are expressed as the number of viral copies/ml and the linear range of the assay is from 500 to 1 x $10^7$copies/ml.

Induced sputum samples transported in viral transport medium and on ice were used for CMV isolation using the shell vial method at the National Institute of Communicable Diseases, Johannesburg. The $D^3$ DFA Cytomegaolvirus Immediate Early Antigen Identification Kit from Diagnostic Hybrids (Pro-Gen Diagnostics) was used. Briefly, specimens were inoculation onto Hs27 cells (human foreskin fibroblast) and centrifuged for 1 hour at 2000rpm. Following incubation at 37°C for 24-48 hours, immunofluorescent staining for CMV immediate early antigen was performed.

## Diagnosis of PJP using induced sputum specimen (Principles of the test):

Indirect immuno-fluorescence method (also known as double antibody method) was used. The test was performed by the Department of Microbiology, University of the Free State (according to the package insert from the manufacturer-Axis-Shield diagnostics limited UK). It involved the use of pre-treated induced sputum specimen

with lysis solution in the ratio of 1:10 to clear it of saliva. The sputum was then centrifuged and washed. Pellets (sediments) were then placed on glass slides and fixed with acetone. The specimens were now enzyme-digested. Murine anti Pneumocystis jerovicci antibody (primary antibody) and fluorescently labelled antimouse antibody were added in turn after incubation, rinsing, wicking and air drying steps. Positive results were indicated by oocytes seen as medium bright to bright apple green on a fluorescence microscope.

## HIV infection using HIV PCR test:

HIV DNA PCR testing was performed by the Department of Virology, NHLS Universitas using EDTA blood samples. Testing was performed according to manufacturer's instructions using the COBAS□□AmpliPrep/ COBAS□□TaqMan□□ HIV -1 Qual Test kit form Roche Diagnostics. This is a real-time PCR technique using the COBAS□□AmpliPrep and COBAS□□TaqMan□□□equipment for extraction and amplification/detection.

## Statistical analysis

The goal of this research primarily was to determine the prevalence of CMV pneumonia co-infection with PJP in HIV exposed infants presenting with suspected PJP and the effect of this co-morbidity on disease outcome.

Using such variables as shell vial culture test from induced sputum specimen and CMV viral load test from blood specimen to define CMV pneumonia only; PJP immuno-fluorenscence test from induced sputum specimen to define PJP only and the combination of these three tests (SVC, CMV viral load and PJP immunofluorescence assay) to define CMV pneumonia/PJP co-infection, and choosing a confidence interval of 95% (critical alpha value of 0.05), statistical significance tests were carried out.

An Anova test and the Fisher's exact test were used to determine the statistical significance of CMV pneumonia/PJP co-morbidity on the above mentioned variables.

## Assumptions

Deliberate assumptions were made that the samples used represented the population of HIV exposed and/or infected infants with clinical PJP and that the induced sputum specimen collected were all from the lungs and not from or contaminated in the throat. Further assumptions were also made that viral load (>10 000 copies/ml) in the blood and positive CMV shell vial culture in induced sputum signified systemic CMV disease with probable pulmonary CMV extension

## Chapter 3: Results

During the study period (July 1st to 30th and from September 1st through to October 31st 2009), 13 infants between the ages of 2 – 6 months, admitted to our institution with clinical suspicion of PJP were enrolled for the study. This group consisted of six males and seven females; median age 3 months (range 2-6 months). Five specimens (38.5%) were excluded; two on grounds of error of specimen sampling, two on account of laboratory errors and the last one was lost in transit. Laboratory results can be viewed in table 1. The final specimens analyzed came from 8 (61.5% of total subjects recruited) subjects, 6 males and 2 females, with median age of 3 months (range 2-6 months). All were HIV positive and admitted with suspected PJP (Table 1).

CMV pneumonia/PJP co-infection was diagnosed in one (12.5%) patient while PJP only and CMV pneumonia only were diagnosed in 2 (25%) and 4 (50%) patients respectively (Figure 1). Two patients (25%) died; one with the co-infection above who was ventilated and the other had none of the infections but met the inclusion criteria (See below).

Patients were treated with high doses of intravenous co-trimoxazole (bactrim) and oral prednisone (for those that were severely hypoxic) until clinical improvement was achieved and only then were they switched to oral co-trimoxazole. A total of 21 days of bactrim and 14 days of prednisone treatment were given. No patient received Gancyclovir during this period. Six patients (75%) received bactrim prophylaxis in total: four of these six patients (67%) had CMV pneumonia only and the remaining two (33%) were PJP culture-positive (PJP only). The one patient with CMV pneumonia / PJP co-morbidity did not receive prophylaxis. This patient died. Fifty percent were started on ART in the hospital ward while the other half was referred to an ART centre. More details can be viewed in Tables 2 and 3.

Impact of ART on disease outcome was also examined. One patient with PJP only, had ART (in addition to other treatment modalities), improved and was discharged. All four (100%) of the patients with CMV pneumonia only, received ART, improved and were discharged. The one patient with PJP/CMV pneumonia co-infection did not receive ART and died.

Only one patient out of the total eight patients (12.5%) was ventilated while seven (87.5%) had prolonged hospital stay (more than 10 days on admission and treatment). Two patients (25%) died; one was ventilated and the other not. The latter had no laboratory confirmation of CMV pneumonia and / or PJP but had low CD4 % count of 6.3% and met the inclusion criteria (Table 4). The results also showed that artificial milk feeding (formula) was the main feeding choice (62.5%) while only 37.5% of mothers breastfed their babies (see Table 3). All 3 infants that were breastfed had CMV infection, 2 of them (67%) had CMV pneumonia only while 1 (33%) had CMV pneumonia/PJP co-infection. None of the infants that were on breast milk had PJP only.

All the patients diagnosed with CMV pneumonia only (n= 4) and PJP only (n= 2) improved and were discharged after prolonged hospital stay (median no of days = 30 days (range = 12 to 45 days). The 4 patients that had CMV pneumonia only had a median CD4% count of 8.2%% (range 12- 40%, average=18.8%). Two (50%) of this CMV pneumonia group were started on ART while in the ward. The only patient ventilated had CMV pneumonia/PJP co infection and was not on ART, received no bactrim prophylaxis and had a CD4% count of 2.3%. This patient had prolonged hospital stay (more than 10 days of admission and treatment) and ultimately died. The average CD4% count of the two patients (2/8, 25%) with PJP only was 4.8% and both of them were on bactrim prophylaxis. The outcome was good and they were discharged home, though both had a prolonged hospital stay (11 and 15 days respectively). Neither of them was ventilated. These results are summarized in Table 4. The group that improved (CMV pneumonia only and PJP only) had relatively higher CD4 % count than one of the two patients that died who had co-infection (average CD4% count 18.8% and 4.8% respectively versus 2.3%). See table 4.

The effect of CMV pneumonia / PJP co-morbidity on disease outcome/mortality was analyzed using Anova test and Fisher's exact test and showed no significant difference (p= 0.49). The role of bactrim prophylaxis in reducing PJP incidences and that of ART in influencing outcome also showed no significant difference (p= 0.07 for both cases). The clinical impression was that the prognosis of PJP alone or CMV alone was good and that that of PJP/CMV pneumonia co-infection was less favourable. See Table 4.

## Table 1: Cytomegalovirus pneumonia co-infection with PJP in HIV exposed infants.

## Laboratory results

| Nr | PJP PCR (sputum) | CMV qual (blood) | CMV Viral load(copies/ml) | CMV SVC(sputum) | CD4% count | LDH(u/l) | Age(months) |
|---|---|---|---|---|---|---|---|
| 1 | ------ | ------- | ----- | ------ | 35.1 | 779 | 4 |
| 2 | ------- | ------ | ------ | ------ | 18.2 | 770 | 3 |
| 3 | ------ | ------- | ------ | ------ | 19.0 | 883 | 5 |
| 4 | negative | positive | 58,95 | positive | 12.0 | 503 | 3 |
| 5 | ------ | negative | negative | ------ | 29.00 | 580 | 3 |
| 6 | positive | positive | 2,800 | positive | 4.4 | 663 | 6 |
| 7 | negative | positive | 12,600 | positive | 40.0 | 843 | 2 |
| 8 | negative | positive | 17,100 | negative | 6.3 | 554 | 4 |
| 9 | negative | positive | 380700 | positive | 10.0 | 1979 | 2 |
| 10 | positive | positive | 2980100 | positive | 2.3 | 1080 | 3 |
| 11 | positive | negative | negative | negative | 5.1 | 693 | 3 |
| 12 | negative | negative | negative | ------ | ------- | 2735 | 3 |
| 13 | negative | positive | 130100 | positive | 13.3 | 955 | 6 |

Key to table 1: ------- = Rejected or lost specimen

**Table 2: Drug treatments of the patients studied, their average CD4% count and the outcome.**

| | Bactrim prophylaxis | ART | Average CD4% count | Outcome |
|---|---|---|---|---|
| PJP only (n=2) | 2(100%) | 1(50%) | 4.8% | discharged |
| CMV only (n=4) | 4(100%) | 3(75%) | 18.8% | discharged |
| CMV/PJP (n=1) | 0 (0%) | 0 (0%) | 2.3% | died |

**Table 3: Frequency table showing the occurrence of the variables**

| Variable | Frequency (n) | Percent (%) |
|---|---|---|
| BP | 6 | 75 |
| FF | 5 | 62.5 |
| BM | 3 | 37.5 |
| SAIW | 4 | 50 |
| RA | 4 | 50 |
| VENT | 1 | 12.5 |
| PHS | 7 | 87.5 |
| DIW | 2 | 25 |

Abbreviations: BP= Bactrim prophylaxis, FF= Formular feed, BM= Breast milk, SAIW= Started ART in the ward, VENT. = Ventilated, PHS= Prolong -ed hospital stay, DIW= Died in the ward.

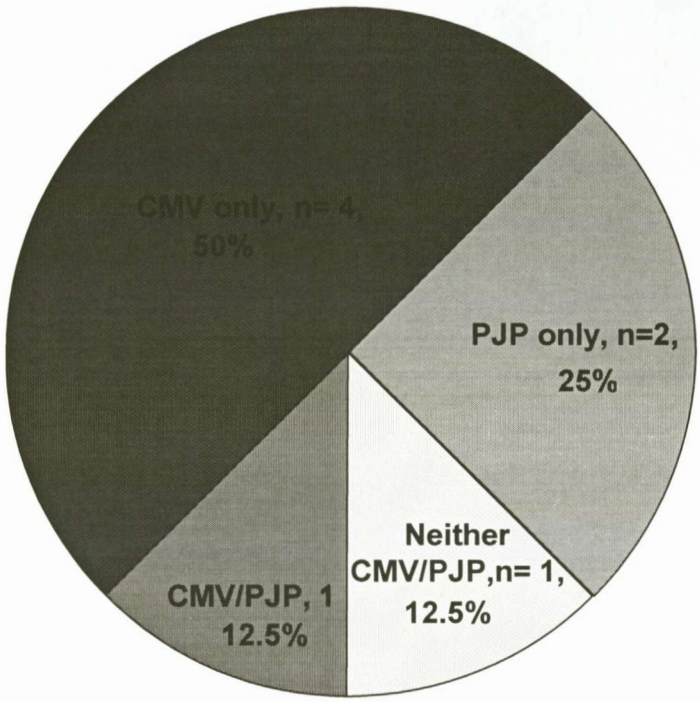
## Table 4: Summary of the entire research findings

| Variables | CMV/PJP (n=1) | CMV only (n=4) | PJP only | CMV/PJP (-ve) (n=2) |
|---|---|---|---|---|
| Average CD4% count | 2.30 | 18.80 | 4.80 | 6.30 |
| Average no. of days In hospital | 24 | 30 | 13 | 9 |
| Mechanically ventilated (n=1) | yes | No | No | No |
| Started on ART in hospital (n=3). | No | 2 (50%) | 1 (50%) | No |
| Bactrim prophylaxis (n=6) | No | 4 (100%) | 2 (100%) | No |
| Breast feeding (n=3) | 1 (25%) | 2 (75%) | 0 | 0 |
| Outcome | Died | Discharged | Discharged | Died |

Key to table 4: CMV/PJP= CMV pneumonia/PJP co-morbidity.
CMV= CMV pneumonia.
CMV/PJP (-ve) = CMV/PJP negative = patient without any of the three disease entities based on Laboratory results

**Figure 1: Pie chart illustrating prevalence of CMV pneumonia only, PJP only and CMV pneumonia/PJP co-morbidity.**

**Neither CMV/PJP= No laboratory confirmation of either CMV or PJP.**



## Chapter 4: Discussions, Conclusions and Recommendations

This study has analyzed data from a descriptive study with a limited number of subjects, with the aim to investigate the prevalence of CMV pneumonia co-infection with PJP in HIV exposed and / or infected infants admitted with suspected PJP and the effect of such co-morbidity on disease outcome. In addition to the above, the study also attempted to provide answers to other relevant questions regarding the use of ART, PJP prophylaxis with bactrim and the extent of involvement and impact of CMV pneumonia and / or PJP on the affected infants with a given CD4% count.

Our results showed that prevalence of CMV pneumonia / PJP co-infection in HIV exposed infants presenting with suspected PJP in the setting of the research (Pelonomi Hospital Paediatric wards) was 12.5% while that of PJP was 25%. These figures, when compared with the findings made available in the interim report in Cape Town study on Cytomegalovirus infection in HIV positive infants hospitalized with severe pneumonia, showed some differences. Their study found 16% incidence of PJP / CMV pneumonia co-infection and 86% PJP. We only found 12.5% prevalence of PJP / CMV pneumonia co-infection and 25% prevalence of PJP in our study. Although the Cape study used nasopharyngeal aspirates, which have a lower specificity and sensitivity (15) as opposed to induced sputum, it still found a higher incidence of CMV pneumonia / PJP co-infection and PJP. The Cape study did not indicate whether those patients studied used some modifiable factors such as co-trimoxazole prophylaxis and ART or not, which could influence the results. UNAIDS, 2009 update report (2) showed that, co-trimoxazole prophylaxis could reduce the incidence of PJP in HIV infected infants while failure to use co-trimoxazole increases the incidence. Zar et al (20) reported some reduction in the incidence of PJP with the use of ART. It is possible that the subjects studied were not on ART, while most of our subjects were started on ART in hospital. The Cape Town study, again, did not indicate whether the subjects had primary immune deficiency disorders, organ transplant or prolonged use of immune-suppressive medications. All these could render their study population vulnerable to opportunistic infection (like PJP), thereby leading to high incidence. Our study was designed to exclude these as confounding factors.

The prognosis of the only patient with co-infection was very poor as he eventually died after a prolonged hospital stay and mechanical ventilation. This was in total agreement with the Cape study and also concurred with the conclusion made by the Institute of child health, London report, which suggested that CMV pneumonia / PJP co-infection carries a worse prognosis, requiring prolonged hospital stay and mechanical ventilation (13).

Although our clinical impression seemed to suggest that CMV pneumonia / PJP co-infection carry a less favourable prognosis, it could not be concluded that CMV pneumonia/PJP co-infection was responsible for the death since there was no statistical evidence to support this argument, and furthermore, the second patient that died in our study was HIV uninfected and did not have laboratory evidence of either CMV pneumonia or PJP, although he met our inclusion criteria. More research is needed in this area with larger sample size. However, as the results suggested, CMV/PJP co-infection, clinically, led to a less favourable outcome in the one patient that had both infections. This could also be ascribed to the severe immune-suppression with overwhelming dual opportunistic respiratory infections.

In our study, the clinical impression was that PJP (when it occurs in isolation) did not carry a poor prognosis, and occurred less commonly than CMV pneumonia. The majority of patients enrolled in the study were on bactrim prophylaxis against PJP and this might have contributed to the finding of low prevalence of PJP relative to CMV pneumonia. Furthermore, the low sensitivity of the methods used in diagnosing PJP could exert some influence on the reported outcome. PJP was diagnosed using indirect immune - fluorescence assay of induced sputum specimen which has a higher yield than nasopharyngeal aspirate specimen with low sensitivity that could be less than 50% (9) and therefore not always reliable in ruling out PJP. A negative PJP culture result on induced sputum does not absolutely exclude PJP (27). Therefore, the low prevalence of PJP found in this study could possibly be attributed to two factors: The use of bactrim prophylaxis and the low sensitivity (but highly specific) of the method used in isolating Pneumoncystis jirovici. In general, it seemed that PJP alone, as an opportunistic infection in HIV infected infants did not carry a bad prognosis. As shown in table 1, all patients diagnosed with PJP only (25%) survived and were discharged home. Although they did not require mechanical ventilation, they fell within the category of those with prolonged hospital stay (13 days only). This prevalence of PJP (25%) and the good prognosis of the condition supports the finding in the Steve Biko adacemic hospital study that "PJP is

not a bad disease" (15); but that has not always been our experience –perhaps the CMV co-infection was missed.

Out of a total of five patients with CMV infection (62.5%), four (80%) had CMV pneumonia only while one (20%) had co-infection. When the total number of cases studied (8 cases) is taken into account, this figure translates into 50% CMV only pneumonia and 12.5% CMV pneumonia / PJP co-infection. This was the highest prevalence rate amongst all the three variables studied, but none of them was treated either with Gancyclovir or ventilated and all survived, though with prolonged hospital stay. This high rate of survival could be attributed to their relatively high CD4% count (average 18.8%) compared to the average CD4% count of 2.3% and 4.8% in the groups that had co-morbidity and PJP only respectively (Table 4). It could, therefore, be inferred from this finding that high CD4% count possibly protects against some opportunistic infections, especially CMV pneumonia. Their survival/fairly good prognosis could also be ascribed to the use of ART as one half of the CMV pneumonia patients were started on ARV therapy during admission. This highlighted the need for early introduction of ART in all HIV infants who are diagnosed with CMV pneumonia.

CMV infection is vertically transmitted (utero, perinatal and breast milk). Interestingly, in this study, all the infants that were breastfed had CMV pneumonia (Table 4). This figure sharply contrasted with the 53% transmission rate reported in literature (7). Several factors could be responsible for this: first, the viral load of the mothers of babies used in our study could be high and consequently lead to high transmission rate which culminated in the high incidence rate obtained. Second, low CD4% count and delay in initiation of ART could also be contributing factors as it had been shown that the use of ART could reduce the rate of acquisition of opportunistic infections in this group of patients (21).

The findings of this study, though not statistically significant, seemed to suggest that CMV pneumonia/PJP co-infection carries a less favourable prognosis and that bactrim prophylaxis and ART were important contributors to the improvement in the conditions of the patients based on clinical impressions. This study underscored the need to acquire a special diagnostic kit locally to prompt effective and timely diagnosis and management of these conditions.

## Limitations

One of its greatest limitations was the small size of the sample used since it might not be a true representation of the population under study. Furthermore, the study, being descriptive, only provided a snapshot. Different results may have been obtained if the study was conducted over a longer period. The lack of follow-up could also be considered, although, not part of the study.

The diagnosis of CMV pneumonia and PJP could also be a source of error in the study. No clear cut diagnosis of CMV pneumonia or PJP could have been possible without open lung biopsy. Until such time a new technology becomes available, the diagnosis of CMV pneumonia and PJP would continue to pose a diagnostic dilemma and enormous potential limitations to any study involving those fields. In other words, there are no convenient acceptable methods of confirming the diagnosis presently. However, despite this particular limitation, this study used the available technology (shell vial culture, viral load estimation on peripheral blood leucocytes) to arrive at the most acceptable diagnosis possible.

## Conclusions

It would appear from the results that:

- CMV pneumonia alone is common (50%) but under diagnosed.
- CMV pneumonia / PJP co-infection is prevalent (12.5%) in our setting and carries a poor prognosis.
- Clinically, it seems that PJP alone has excellent prognosis.

More studies need to be carried out in this field over a reasonable length of time with larger sample size.

## Recommendations

Based on the above findings, the investigator suggests the following recommendations:

1. Every HIV infected infant presenting with severe pneumonia should be investigated for possible CMV pneumonia and / or PJP.
2. Diagnostic laboratory for CMV infection be established in the Free State to ease cost and enhance the speed of diagnosis and management of affected patients.
3. All HIV infected infants with CMV pneumonia / PJP be started on ART.
4. All HIV exposed infants should be started on co-trimoxazole prophylaxis from 4-6 weeks of age.
5. More research should be carried out in this field of CMV pneumonia /PJP co-infection over a longer period of time with larger sample population.

## References:


1. Kroon M, Eley B. Burden of disease project, appendix 5: Paediatric HIV in the Western Cape 2007.

   Available from http://vula .uct.ac.za/.../CD%20volume %207%20 Appendix % 205% 20HIV.pdf [Accessed 30 October 2010].

2. UNAIDS. AIDS Epidemic Update 2009. Available from http://www.unaids.org/en/knowledgecentre/HIVData/Epiupdate/EpiupdArchive/2009/default.asp [Accessed 01 October 2010].

3. Marius BJ, Rabie H, Schaaf SH et al. Common opportunistic infections in HIV infected infants and children, part 1- respiratory infections. South African Fam Pract 2006; 48(10): 52-57.

4. Bennett NJ. Pneumocystis (carinii) jiroveci pneumonia 2008. Available from http//emedicine.medscape.com/article/225976-overview [Accessed 18 August 2009].

5. Wilfert CM, Gross PA, Kaplan E, et al. Quality standard for enumeration of CD4 lymphocytes in infants and children exposed to or infected with HIV. Clin Infect Dis 1995; 21(suppl 1): 134-5.

6. Boyton RJ. Infectious lung complications in patients with HIV/AIDS. Curr Opin Pulm Med 2005; 11(3): 203-7.

7. Zampoli M. CMV Pneumonia in HIV Infected Children (oral presentation) 2009. Available from

   http://www.scah.uct.ac.za/paediatric-refresher-course-2009/presentations/wed/Zampoli-Cytomegalovirus-pneumonia.pdf [Accessed 20 September 2009].

8. Cunha Ade A, Marin LJ, Aquino VH et al. Diagnosis of cytomegalovirus infections by qualitative and quantitative PCR in HIV infected patients. Rev Inst Med Trop 2002; 44(3): 127-132.

9. Arthur J. Pneumonia, viral 2010. Available from http:// emedicine.medscape.com/article/300455-overview [Accessed 10 October 2010].

10. Athter K. Cytomegalovirus Overview 2009. Available from http://emedicine.medscape.com/article/215702-overview [Accessed 10 October 2009].

11. Shelhamer JH. CMV infection as a cause of pulmonary disease in HIV infected patients 2008. Available from www.uptodate.com/patients/content/topic.do?topicKey [Accessed 09 December 2008].

12. Morrow B, Zampoli M, Hsaio NY, et al. Cytomegalovirus infection in hospitalized infants in a high HIV prevalence setting 2008: an interim research report, School of Child and Adolescent Health, University of Cape Town, South Africa.

13. William AJ, Duong T, McNally LM, et al. Pneumocystis carinii pneumonia and cytomegalovirus infection in children with vertically acquired HIV infection. AIDS 2001; 15(3): 335-339.

14. Madhi, SA. Lower respiratory tract infections in HIV-1 infected children. Southern African Journal of HIV medicine 2001; 2(3):32-39.

15. Kitchin O. Lekalala RM. Moodley T. et al. Outcome of infants admitted to Stev Biko Academic Hospital with pneumocystis carinii pneumonia (PCP) 2009. Oral Presentation at the combined congress of the critical care society of southern Africa and the South African Thoracic Society. Available from

http://www.criticare.co.za/images/copiconpress/pilanesbergB/tuesday/10h30%20Omolemo%20copicon %20Abstract.pdf [Accessed 11 December 2009].

16. Tam M. The lung in the immunocompromised patient. Int J Thoracic Med 1999; 66(3 pt 2): 199-207.

17. Slogrove AL, Cotton MF, Esser MM. Severe infections in HIV-exposed uninfected infants: Clinical Evidence of Immunodeficiency. J Trop Pediatr 2010; 56(2): 75-81.

18. Leung TF, Fok TF, Wong MCK, et al. Pneumocystis Carinii Pneumonia in immunocompetent infants with congenital CMV infection. J Clin Path 2000; 28: 184-186.

19. Thurstan S. Co-trimoxazole as a prophylaxis for HIV positive malnourished children 2005. Available from http://fex.ennonline.net/25/children.aspx [Accessed 01 November 2010].

20. Zar HJ, Dechaboon A, Hanslo D, et al. Pneumocystis Carinii Pneumonia in South African children with human immuno deficiency virus. Pediatr Infect Dis J 2000; 19(7): 603-7.

21. Zar HJ. Impact of highly Active Antiretroviral Therapy on Opportunistic Respiration Infections. Ped Pulm 2008; 43: 1-10.

22. WHO/UNICEF. Co-trimoxazole prophylaxis for HIV exposed and HIV infected infants and children 2009.

Available from www.unicef.org/aids/files/CotrimoxazoleGuide 2009.pdf [Accessed 30 October 2010].

23. Brumback BG, Wade CD. Simultaneous culture for adenovirus, cytomegalovirus, herpes simplex virus in same shell vial by using three-colour fluorescence. J Clin Microbiology 1994; 32(9): 2289-2290.

24. WHO. Clinical case definition for paediatric HIV infection in Bloemfontein. J Trop Pediatr 2005; 51(6); 387.

25. Brantsaeter AB, Holberg-Petersen M, Jeanson S, et al. CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV infection –a retrospective autopsy based study. BMC Infect Dis 2007; 7: 127-134.

26. Jiang ZF, Shen KL. CMV pneumonia in non immune children. HK J Paediatr (New series) 1996; 1(2) 119-125.

27. Hughes WT, Behrman RE, Kliegman RM. Diagnosis of Pneumocystis Carinii Pneumonia. In: Nelson Textbook of Paediatrics. 17th ed. Philadelphia: Saunders 2004; 1154.

## Appendix 1: Data Sheet.

# Cytomegalovirus pneumonia co-infection in HIV exposed infants

## Data Collection Sheet

Subject nr: _____

PM nr: _____

### Demographic information

Date of admission: _____/_____/2009
*dd* *mm*

Date of birth: _____/_____/_____
*dd* *mm* *yy*

Age: _____months, _____weeks

Sex: male/female

### Treatment before admission

Prevent mother to child transmission (PMCT): Yes/No
Bactrim Prophylaxis: Yes/No
Feeding after birth: _____
Current: _____
Previous admission: _____
_____
_____

### Management in hospital

_____

_____

_____

_____

_____

_____

_____

_____

_____

## Special investigations

**HIV PCR** _____

**CD4 Count%** _____

**CMV PCR (blood)** _____

**CMV SVC (Induced sputum)** _____

**LDH** _____

**PJP Culture (Induced sputum)** _____

## Outcome

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

## Appendix 2: Informed Consent.

# Informed Consent (ENGLISH VERSION)

CONSENT TO PARTICIPATE IN RESEARCH

Patient's initials & Surname:
.....
Patient's date of
birth:.....

**STUDY: Cytomegalovirus pneumonia co-infection in HIV exposed infants admitted with clinical PJP pneumonia to Pelonomi Regional Hospital**

You have been asked to participate in a research study.
You have been informed about the study by ..... .
You have been informed about any available compensation or medical treatment if injury occurs as a result of study-related procedures;
Please note that by completing this form you are voluntarily agreeing to participate in this research study. You and your infant will remain anonymous and your data will be treated confidentially at all times. You may withdraw your infant from this study at any given moment during the completion of the form/procedure.
You will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.
If you agree to participate, you will be given a signed copy of this document as well as the participant information sheet, which is a written summary of the research.
The research study, including the above information has been verbally described to me. I understand what my involvement in the study means and I voluntarily agree to participate.
There are no risks involved to your infant with regards to the procedures of drawing blood and collecting stomach aspirates except for minor bleeding and perhaps a little local irritation of the nasal passages.
You may contact the department of Paediatrics at 051 405 3151 any time if you have questions about the research or if you are injured as a result of the research.

You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, UFS at telephone number (051) 4052812 if you have questions about your rights as a research subject.

_____ _____
Signature of Participant Date

_____ _____
Signature of Witness Date
*(Where applicable)*

_____ _____
Signature of Translator Date

## Appendix 3: Information Guide.

Study title: The prevalence of Cytomegalovirus pneumonia co-infection in HIV exposed infants admitted with clinical Pneumocystis Jiroveci pneumonia.

Dear intended participant,

I am doing research on the above subject area. Research is just the process to learn the answer to a question. In this study I want to learn how common a specific lung infection which is caused by a virus (called cytomegalovirus pneumonia) is in HIV exposed babies. These babies must be admitted in Pelonomi Hospital with a severe lung infection {pneumonia} called Pneumocystis jiiroveci pneumonia. This is a study involving research and not routine care. The study will assist us in treating small babies who may develop severe disease of the chest {lower respiratory tract}.

**Invitation to participate:** I am asking for your permission to include your child in this research.

**What is involved in the study**: When you decide to include you child in this study we will take blood and some fluid from his/her throat. This will be sent to a laboratory to determine whether a specific virus is present or not. These samples will just be taken once and this will be the end of your child's participation in this study. A total of at least 20 subjects are expected to participate in the study and every race/nationality can participate.

**Risks**: No major risks or side effects are expected in the study. However, in the event of any unforeseen negative effect during procedures, our emergency trolley and personnel are on standby to handle such cases.

**Benefits**: None attached

**Participation is voluntary,** and refusal to participate will involve no penalty or loss of benefits to which your child is otherwise entitled; he/she may discontinue participation at any time without penalty or loss of benefits to which he/she is otherwise entitled.

**Confidentiality:** Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy the research records for quality assurance and data analysis include groups such as the Ethics Committee for Medical Research etc.

**The results of this research** will be published without names and copies made available at the Department of Paediatrics, Universitas Hospital, Bloemfontein.

**Contact details of researcher** – for further information/reporting of study-related adverse events, contact Dr.A.O Ogugua. Dept of Paediatrics, Universitas hospital Bloemfontein. Phone: 0514053181.

Signed.