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Clinical course and outcomes of confirmed and suspected *Pneumocystis jirovecii* pneumonia in Sowetan children living with HIV

By

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A Research Report submitted to the Faculty of Health Sciences,
University of the Witwatersrand, Johannesburg,
in partial fulfillment of the requirements for the degree of
Masters of Medicine in Paediatrics

July 2022

Declaration

I Mohiniben Darji declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of the candidate)

29 day of July 2022 in Johannesburg

Dedication

This dissertation is dedicated to my parents, Jitendra and Priti, who have been my strength and support throughout my life. I would like to thank my sisters, Komal and Shivani, for always giving me reasons not to give up whenever I thought I could not go further. To Vinay, my best friend and life partner, you inspire me everyday; thank you for always being extremely supportive and for believing in my potential. Thank you for always pushing me to be the best version of myself.

Presentations and Publications

The findings of this study were presented at the University of the Witwatersrand Paediatric Research Day in December 2021.

Abstract

Introduction

Pneumocystis jirovecii is an important opportunistic pathogen in children living with HIV (CLWH), responsible for the majority of community acquired pneumonia (CAP) admissions in infancy. There is scanty evidence on long-term survival rates in children hospitalised with *P. jirovecii* pneumonia (PCP), especially in settings with a high burden of paediatric HIV.

Methods

Admissions to general paediatric wards at Chris Hani Baragwanath Academic Hospital over the period 01 January 2011 to 31 December 2019 were assessed for a discharge diagnosis of PCP. PCP was classified as being confirmed or presumed, based on laboratory criteria. Only CLWH were included in the study.

Results

Over the 9-year study period, 390 cases of PCP were diagnosed, of which 297 (76.2%) were in CLWH. The majority of the PCP episodes were presumed (262/297, 88.2%). Sixteen children had recurrent PCP admissions. There was no difference between the median age of children at admission with confirmed PCP or presumed PCP (4.07 months vs 3.66 months; P=0.590). Children with presumed PCP were significantly underweight (weight-for-age Z-score -2.88 vs -1.74; P<0.001) and wasted (weight-for-length Z-score -1.15 vs -0.38; P=0.039) compared to those with confirmed PCP. Children with presumed PCP had a higher prevalence of confirmed pulmonary tuberculosis (7/18 (38.9%) vs 1/35 (2.9%); P=0.002). The majority (56.1%) of patients were initiated on antiretroviral therapy (ART) one week after the PCP admission episode. Of 58 PCP episodes in which data on mechanical ventilation was available, 18 (31.0%) were ventilated. Case fatality was 44.4% in ventilated patients, and the overall in-hospital case fatality was 24.8%. One-hundred, twenty-five children were followed up at the outpatient ART Clinic, of which 42 (33.6%) were still in care at the end of the study. The median duration of co-trimoxazole preventive therapy was 1278 days.

Conclusion

PCP was often the first presentation of HIV infection. Children with PCP still have poor outcomes. Despite the decline in PCP admissions, there was a high in-hospital mortality rate.

Acknowledgements

This dissertation would not have been possible without the help and support from my supervisor Prof David Moore, I sincerely thank you.

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Nomenclature

ART	Antiretroviral therapy
BDG	β-D-Glucan
CAP	Community acquired pneumonia
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	confidence interval
CLWH	children living with HIV
CMV	cytomegalovirus
CPT	co-trimoxazole preventive treatment
CRP	C-reactive protein
HIV	human immunodeficiency virus type-1
LDH	lactate dehydrogenase
NHLS	National health laboratory service
PERCH	Pneumonia Research for Child Health
PCP	*Pneumocystis jirovecii* pneumonia
PMTCT	prevention of mother-to-child transmission
PCR	polymerase chain reaction
ROC	receiver operating characteristic
WHO	World Health Organisation

1 LITERATURE REVIEW

1.1 Introduction

Pneumocystis jirovecii is an important opportunistic pathogen in children living with HIV (CLWH), responsible for the majority of community acquired pneumonia (CAP) admissions in infancy in these children.[1] *Pneumocystis jirovecii* pneumonia (PCP) classically presents as a progressive, hypoxic pneumonia with signs of severe respiratory distress and respiratory failure.[2] Mortality rates are high if not appropriately treated. Studies conducted in the late 1990s highlighted the importance of prophylactic therapy, using co-trimoxazole, to prevent PCP and all-cause mortality in CLWH.[3]

1.2 Epidemiology of PCP

With maturation of prevention of mother-to-child transmission (PMTCT) programmes, early diagnosis of HIV infection in children, and expedited access onto antiretroviral therapy (ART) in HIV-infected infants, the rates of PCP associated morbidity and mortality are declining.[4] Data from the Centers for Disease Control and Prevention Paediatric Spectrum of Disease Project (1994–2001) indicated a decline in PCP infection rates (cases per 1000 HIV-infected children) from 25 in 1994 to 18 in 1996, and 6 in 2001 in the United States.[4] Similarly, analyses of data from the Perinatal AIDS Collaborative Transmission Study revealed a 95% decline in PCP (cases per 100 child-years) from 5.8 (pre-ART) to 0.3 (in the ART era).[5] Another study demonstrated that the incidence rate for PCP (cases per 100 child-years) was 1.3 during the pre-ART era (1981–1988) and <0.5 during the ART era (2001–2004).[5]

Similar declines in the burden of PCP have not been demonstrated in South Africa, however, as there were delays in implementing the National roll-out of ART to HIV-infected persons (which commenced in 2004). In a South African study in 1998 by Fatti et al[6] which looked at paediatric pneumonia admissions across four hospitals in Cape Town, PCP accounted for 9.9% of pneumonia cases in the study population where 39.1% of the children were on co-trimoxazole preventive treatment (CPT). A prospective study done in a South African hospital between November 2006 and August 2008 enrolled 202

children with suspected PCP, of which 109 (53.9%) were confirmed cases.[7] In a cross sectional autopsy study of inpatient paediatric deaths in Zambia (2011 to 2014), PCP and cytomegalovirus were diagnosed in 7% and 5% of lung specimens, respectively.[8]

In the Pneumonia Etiology Research for Child Health (PERCH) study, *P. jirovecii* was responsible for 23.0% (95% credible interval, 12.4-31.5%) of CAP admission episodes in CLWH under-5 years of age; these cases clustered almost exclusively in the under-1 year old age group, in which 1 in 3 CAP hospitalisations were associated with *P. jirovecii*.[1] A similar incidence was found in a Cape Town study where PCP occurred in 21.3% of children of whom the majority (76.7%) were HIV-infected.[9] A study at Steve Biko Academic Hospital which looked at severe pneumonia in HIV exposed and HIV-infected children admitted to the paediatric ICU showed an incidence of 27% of PCP cases.[10] A study in a district hospital situated in southern Mozambique demonstrated a 14.3% prevalence of PCP in HIV-infected children, and 3.3% in HIV-uninfected children.[11]

With increased use of immunosuppressant therapy for conditions such as malignancies and autoimmune diseases, the incidence of PCP in the HIV-uninfected infected group has increased over time.[12,13] It is therefore essential to diagnose and treat PCP early in all immunocompromised patients.

1.3 Pathogenesis of PCP

Pneumocystis was initially discovered by Chagas in 1909. At that time, it was mistaken as a new stage in the life cycle of *Trypanosoma cruzi*.[14] However, in 1912 Carini and the Delanöes established that this organism was a new genus and species, which was named *Pneumocystis carinii*: "Pneumo" in relation with lung tropism and "cystis" because of its shape.[15] Debate regarding the fungal versus protozoal classification of the organism continued into the late 1980s, after which definite proof was provided for the new classification of *Pneumocystis* as a fungus.[15] *Pneumocystis carinii* is the species which is tropic for rodent pulmonary tissue, whereas *Pneumocystis jirovecii* is tropic for the human respiratory tract.[16] The morphologic forms involved in the *Pneumocystis* life cycle (trophic form, sporocyte and mature cyst) were described through transmission electron microscopy and have evolved through recent molecular data.[15]

Transmission of PCP is via the airborne route and the incubation period is about 4 to 8 weeks. *Pneumocystis jirovecii* predominantly affects the lungs. Once the *Pneumocystis* cysts reach the alveoli, spores are released that develop into trophic forms and adhere to type 1 alveolar cells, causing infection in the lungs of the affected individual.[14] Serological evidence shows that most healthy children have been exposed to *Pneumocystis jirovecii* by 3 to 4 years of age.[16]

HIV/AIDS had become a huge concern during the 1980s and it was during this time when PCP was found to be one of the most common clinical manifestations in persons with untreated HIV. Patients infected with PCP usually have an underlying medical condition that weakens their immune system, such as HIV infection, or they have been on medication that lowers their immune response.[16] Other risk factors for PCP include chronic lung disease, malignancy, inflammatory diseases or autoimmune diseases and haematology-oncology patients who have had solid organ or stem cell transplants. Episodic outbreaks were described in premature, malnourished infants in crowded orphanages during and after World War II, and also in immunosuppressed renal transplant patients.[14] PCP continues to be a common opportunistic pathogen in immunocompromised children.

1.4 Clinical presentation

The clinical presentation of PCP comprises signs and symptoms of a lower respiratory tract infection and includes cough, shortness of breath and low grade fever. There may also be a history of poor feeding and vomiting. Children with PCP commonly present with hypoxic pneumonia, respiratory failure and the need for mechanical ventilation in severely affected children. In a South African study of children with and without HIV diagnosed with PCP (2006 to 2008), the distinguishing features of PCP were the absence of fever, low oxygen saturations and tachypnoea.[7]

According to the National Institutes of Health, age <6 months, respiratory rate >59 breaths per minute, arterial oxygen saturation ≤ 92% in room air and the absence of vomiting are independently associated with PCP in CLWH.[17] These findings are consistent with those in a South African study in which the presence of three (interval likelihood ratio, 5.0; 95% CI 2.0-12.5) or all four clinical findings (interval likelihood ratio, 36.0;

95% CI 4.4-96.5) were associated with a diagnosis of PCP.[6] The most common presenting features in Mozambican children were cough (85.1%), vomiting (31.2%), diarrhoea (21.8%) and poor feeding (24.3%).[11] These clinical findings did not differ by HIV status of the children.[11] In multivariate analysis, PCP was independently associated with HIV infection, grunting, digital clubbing, age <12 months and infiltrates on chest radiography.[11]

In a 6 month prospective study in Malawian children aged 2 months to 5 years, PCP was diagnosed in 16 (10.7%) of 150 children, and all children with PCP were HIV infected and <6 months old.[2] Children with PCP had a lower median temperature in comparison to those with bacteraemic pneumonia (37.8 vs 39 °C; P=0.0006) and a greater prevalence of hypoxia (86% vs 60%; P=0.003).[2] A French study conducted between April 2014 and August 2020 described the characteristics of 32/279 children (11.4%) children <3 years old with PCP detected by polymerase chain reaction (PCR).[18] PCP cases presented with lower respiratory tract symptoms (78.1%) in comparison to children who tested negative for PCP (42.9%; P=0.0002).[18] Malnutrition was an important co-morbid factor in a study done on children who demised in Zambia with respiratory tract infections, where malnutrition was observed in 63% of children with CMV pneumonia and 33% of children with PCP.[8] The median age of presentation for PCP was 3.6 months in the Mozambican study, and young age has been a consistent risk factor for PCP in many other studies.[11]

Underlying conditions associated with PCP in HIV uninfected children <3 years of age include cardiopulmonary pathologies, primary immunodeficiency syndromes (especially severe combined immunodeficiency), hyaline membrane disease, asthma, acute leukaemia, other malignancies, and solid organ transplantation.[12,18]

1.5 Diagnosis of PCP

The diagnosis of PCP is made using a comprehensive approach which should include clinical examination, chest X-ray findings, blood tests and respiratory samples used to detect *P. jirovecii* DNA using molecular techniques. Currently, there is no ideal laboratory test for the diagnosis of PCP. Respiratory samples which can be used include induced sputum, bronchoalveolar lavage, endotracheal aspirates, and nasopharyngeal swabs, and lung tissue for histology.

Immunofluorescent antibody microscopy testing was previously used to diagnose PCP, but this has a low sensitivity, requires trained laboratory staff and is time consuming. PCP cysts exhibit a green fluorescence immunofluorescent antibody testing. In a prospective study done at Chris Hani Baragwanath Academic Hospital (CHBAH) in 1999, nasopharyngeal aspirates and induced sputum specimens were used to diagnose PCP in children <2 years of age presenting with severe pneumonia, and *P. jirovecii* cysts were identified in 48.6% of specimens.[19] Sensitivity and specificity of 75% and 80% using direct monoclonal antibody immunofluorescent stains were reported in that study.[19]

Blood samples for detection of *P. jirovecii* DNA are not useful in clinical practice.[20] Lung biopsy has high sensitivity and specificity, but is invasive, and rarely performed in paediatric patients with suspected PCP.[20] The Grocott-Gomori stain is used for histological evidence of *P. jirovecii*.

Bronchoalvelolar lavage fluid is considered the gold standard sample to diagnose PCP, but requires an invasive collection procedure which may not be feasible in resource-poor health care facilities.[21] Bronchoalveolar lavage is expensive, without added benefit and is not possible to obtain in all patients, however it may be a consideration in patients who are already intubated. In a retrospective analysis of PCP DNA in different samples collected from children with suspected PCP, the highest positive detection rate with PCR was found in bronchoalveolar lavage specimens, followed by bronchial secretions and endotracheal aspirates.[20] In a study which explored the cost effectiveness of various diagnostic tools for the diagnosis of PCP, PCR done on induced sputum had a sensitivity of 94% and specificity of 99% compared to a immunofluorescence study (sensitivity 81% and specificity 100%).[22] Thus, PCR on non-invasive respiratory samples may be the most cost-effective way of diagnosing PCP.

PCR for *P. jirovecii* has a sensitivity of 95% in bronchoalveolar lavage specimens. Non-invasive samples, including nasopharyngeal swabs and expectorated sputum, have lower sensitivity in HIV-infected patients.[20] PCR detects more PCP infections compared to immunofluorescence in lower respiratory tract samples.[7] Compared to a "gold standard" measure of a positive *P. jirovecii* PCR on lower respiratory tract specimens, a PCR done on a nasopharyngeal aspirate has a sensitivity of 86%, specificity of 95%, positive predictive

value of 96% and negative predictive value of 85%.[7] In another study conducted in Cape Town, *P. jirovecii* was identified through immunofluorescent staining of nasopharyngeal aspirates in 9.3% of PCP cases; 16.3% of cases were identified using induced sputum and 30.9% of cases were identified using bronchoalveolar lavage specimens.[9] With regards to post mortem biopsies in the same study, 4 of the 5 (80%) postmortem histologic examinations identified PCP.[9] Nasopharyngeal aspirate samples may be used for diagnostic purposes when PCR is utilised. PCR is the most cost effective method of establishing a diagnosis of PCP in South African patients.[22]

Laboratory tests that have been used for the diagnosis of PCP besides respiratory samples do assist in supporting a diagnosis of PCP. β-D glucan (BDG) is a cell wall component of *P. jirovecii* and other fungi, and is a nonspecific marker for PCP. Serum BDG levels have a sensitivity of 90-95% and specificity of 78-68% for PCP.[13] Serum lactate dehydrogenase (LDH) is frequently elevated in HIV-infected patients with PCP, but has limited accuracy, with sensitivity of 80-100% and specificity of 6-85%.[13] In a systematic review, serum LDH levels >450 U/L were predictive of PCP and normal LDH levels had high negative predictive value in the context of suspected PCP.[23] When used in combination, an elevated BDG and LDH with clinical features of PCP improved the diagnostic rate, with 92.8% sensitivity and 83.9% specificity.[23] C-reactive protein (CRP) levels are measured by many centres as an assisted tool to identify inflammation or infection. In a study by Lansapa et al.,[11] an abnormal CRP was found more frequently in children who did not have PCP in comparison to the children who had confirmed PCP (36.4% vs 62.4%, P=0.017).

Chest radiographs of children with PCP most commonly reveal bilateral diffuse parenchymal infiltrates with a "ground-glass" or reticulo-granular appearance, but they may be normal or have only mild parenchymal infiltrates.[17] The earliest infiltrates are perihilar, progressing peripherally before reaching the apical portions of the lung. Less commonly, lobar, cavitatory, nodular, or miliary lesions may be found on chest radiograph, and pneumothorax or pneumomediastinum may be observed.[17] In a study from Malawi, the most common radiological features in children with PCP were interstitial infiltrates and hyperinflation.[2] Chest computed tomography may show a ground glass appearance of

the lungs.[13]

1.6 Co-infection with CMV

CLWH are at risk of developing cytomegalovirus (CMV) pneumonia, often as a co-infecting agent with PCP.[24] In a study by Morrow et al,[9] 83.7% of children testing positive for *P. jirovecii* also had a positive CMV PCR result, whereas the prevalence of CMV was 55.3% in children that tested negative for PCP (P=0.001). The majority of the patients with CMV and PCP were HIV infected.[9] Co-infection of CMV with PCP is associated with poorer outcomes, with mortality rates of 42% with CMV infection alone and 50% in those with CMV and PCP.[25]

1.7 Treatment of PCP

Pneumocystis jirovecii is resistant to most antifungal agents, and the antibiotic combination drug trimethoprim-sulfamethoxazole (co-trimoxazole) is the first-line treatment for PCP. PCP is treated through using a 3-week course of high-dose co-trimoxazole.[16] Co-trimoxazole has many side effects, including hypersensitivity reactions, hepatitis and myelosuppression, and long-term administration of the drug should be monitored closely. Adverse events associated with co-trimoxazole include hyponatraemia, hyperkalaemia, vomiting, and hepatitis.[12] Alternative therapies include primaquine with clindamycin, or atovaquone and intravenous pentamidine.[21] Corticosteroids are given in addition to antimicrobial therapy to reduce inflammation, and have been beneficial in patients with severe PCP and patients with HIV infection who are hypoxic.[21]

1.8 Co-trimoxazole preventive treatment

A pivotal randomised controlled trial which evaluated the impact of co-trimoxazole preventive treatment (CPT) on survival in Zambian CLWH in 2001 through 2003 established that there was a 43% (95% confidence interval (CI) 23 to 57%) lower hazard of death in children receiving CPT in the era before access to ART.[3] A recent study by Zajac-Spychala et al[20] demonstrated a significantly higher prevalence of PCP in CLWH that were not prescribed CPT compared to those who received CPT (15.7% vs 7.3%).

Current guidelines recommend that HIV-exposed infants older than 6 weeks should receive CPT to lower the risk of PCP and all-cause mortality.[26] CPT should be continued until CLWH are proven to be HIV negative 6 weeks post cessation of breast feeding. CLWH who are less than 1 year of age should be prescribed CPT. Those between 1 and 5 years who have a CD4% <25 or WHO stage 2, 3, or 4 should also be on CPT and it can be discontinued if the CD4% is >25 regardless of WHO staging. It is also recommended that CLWH aged <5 years who have had PCP should continue CPT until 5 years of age, with subsequent discontinuation of CPT only if the CD4 criteria in >5 year category are met.[26] CLWH >5 years who have CD4 counts <200 cells/μL or WHO stage 2,3 or 4 should be on co-trimoxazole until the CD4 count is >200 cells/μL.[26]

In a laboratory based surveillance study conducted in Pretoria, 32% of children <5 years of age exhibited failure of prophylaxis, and developed PCP despite accessing CPT, which may reflect difficulties in maintaining treatment adherence to CPT, or accumulating drug resistance in *P. jirovecii* to co-trimoxazole.[27]

1.9 Long term outcomes

Few studies have evaluated the long term outcomes of PCP. Most studies focus on the initial course of illness and in-hospital mortality rates associated with the condition. The case fatality rate for untreated PCP in CLWH is 100%.[6] In a prospective descriptive study of children requiring ventilation for respiratory failure at CHBAH in the pre-ART era (1992 through end-1993), PCP was detected in post-mortem lung biopsies of 42% (5/12) and CMV was detected in 8% (1/12) of HIV-infected children that died from severe pneumonia.[28] Case-fatality rates were 88% (15/17) in CLWH, and 31% (29/93) in HIV negative children in that study.[28] A study of over 200 children with suspected PCP from Cape Town demonstrated in-hospital case fatality of 32% in children in whom PCP was confirmed, compared to 17% in children without confirmation of *P. jirovecii* infection.[7] Most of the deaths (88.8%) occurred in CLWH, and all of the HIV-infected children that died had CD4 counts <15%.[7] In a two-month observational study of severe pneumonia in 121 children 2-60 months of age in Uganda, 16.5% had confirmed PCP, 90% of which were HIV-infected, and the mortality rate in those with PCP was 40%.[29] In a

study from Mozambique, CLWH diagnosed with PCP had poorer outcomes compared to HIV uninfected children, with in-hospital mortality rates of 20.8% for children diagnosed with PCP (2006 to 2007).[11] In France, mortality rates associated with PCP in a study conducted between 2014 and 2020 was 18.8% in children <3 years of age.[18]

In a Cape Town study conducted from 2006 to 2008, 30 (69.7%) of 43 children with PCP required mechanical ventilation for a median duration of 6.5 days.[9] In a study from Taiwan, conducted from 2014 to 2017, 65% of children with PCP required mechanical ventilation.[12]

A long term outcome measure of PCP recurrence in CLWH, from a study conducted in Pretoria between 2006 and 2010, was that 20% of CLWH <5 years of age had recurrent episodes of PCP and a case fatality of 27%.[27] Lanaspa et al[11] demonstrated a higher mortality rate (11.5%) up to 21 days post discharge in comparison to the group with no PCP (3.6%; P=0.044). Based on these high mortality and recurrence rates, there is a need for better surveillance and interventions to manage PCP in CLWH.

1.10 Justification for this study

PCP remains an important cause of morbidity and mortality in CLWH, especially in infancy. There are challenges in the diagnosis of HIV among the younger age groups and PCP is often the presenting illness in CLWH. There is insufficient data on the outcomes of CLWH who have been admitted with PCP in the South African setting where the burden of HIV is high. Insight into the clinical presentation, demographic characteristics and risk factors of CLWH who present to a tertiary academic South African hospital may assist in making the diagnosis of PCP in children sooner, may assist in decreasing the morbidity and mortality associated with the condition, and may improve in-hospital outcomes.

There is scanty evidence on long-term survival rates in children hospitalised with PCP, especially in settings with a high burden of paediatric HIV. In this study, we aim to evaluate the long-term outcomes of CLWH with confirmed and suspected PCP, to describe the timing of the PCP diagnosis in relation to their HIV diagnosis, and to evaluate at what stage CPT was discontinued in these children.

1.11 Hypothesis

The working hypothesis of this study is that PCP admissions to CHBAH will have declined over time, with maturation of the ART programme, including expedited access onto ART for CLWH. We further hypothesise that most of the PCP diagnoses would have been made at the child's sentinel hospital admission, prior to the diagnosis of HIV.

1.12 Aims and objectives

The aim of this study was to evaluate the burden of disease and outcomes of PCP in CLWH treated at CHBAH from 01 January 2011 to 31 December 2019.

The objectives of the study were to:

1. Describe the burden of PCP in children admitted to the general paediatric wards at CHBAH, including what proportion of children present with a recurrent admission diagnosis of PCP;
2. To describe the demographic characteristics of children with PCP;
3. To describe the timing of the PCP diagnosis in relation to the child's HIV diagnosis;
4. To describe the co-morbidities associated with confirmed or suspected PCP in CLWH, including:
    (a) CMV pneumonia
    (b) Bacteraemic pneumonia
    (c) Nosocomial sepsis
    (d) Malignancy
    (e) Auto-immune disease
5. To describe the proportion of children with PCP that were admitted to ICU for mechanical ventilation, and (where possible) to describe the duration of ICU stay and duration of hospitalisation;
6. To describe the utility of LDH and other biomarkers in establishing a diagnosis of

PCP in CLWH hospitalised with severe pneumonia;

7. To describe the outcomes (survived, died, or lost to follow up after hospital discharge) of PCP in CLWH, stratifying by certainty of PCP diagnosis, and co-morbidities;
8. To describe the mortality rates associated with PCP;
9. To describe the time at which CPT was discontinued in children with confirmed or suspected PCP.

2 MANUSCRIPT IN SUBMISSIBLE FORMAT

Clinical course and outcomes of confirmed and suspected *Pneumocystis jirovecii* pneumonia in Sowetan children living with HIV

Abstract

Introduction

Pneumocystis jirovecii is an important opportunistic pathogen in children living with HIV (CLWH), responsible for the majority of community acquired pneumonia (CAP) admissions in infancy. There is scanty evidence on long-term survival rates in children hospitalised with *P. jirovecii* pneumonia (PCP), especially in settings with a high burden of paediatric HIV.

Methods

Admissions to general paediatric wards at Chris Hani Baragwanath Academic Hospital over the period 01 January 2011 to 31 December 2019 were assessed for a discharge diagnosis of PCP. PCP was classified as being confirmed or presumed, based on laboratory criteria. Only CLWH were included in the study.

Results

Over the 9-year study period, 390 cases of PCP were diagnosed, of which 297 (76.2%) were in CLWH. The majority of the PCP episodes were presumed (262/297, 88.2%). Sixteen children had recurrent PCP admissions. There was no difference between the median age of children at admission with confirmed PCP or presumed PCP (4.07 months vs 3.66 months; P=0.590). Children with presumed PCP were significantly underweight (weight-for-age Z-score -2.88 vs -1.74; P<0.001) and wasted (weight-for-length Z-score -1.15 vs -0.38; P=0.039) compared to those with confirmed PCP. Children with presumed PCP had a higher prevalence of confirmed pulmonary tuberculosis (7/18 (38.9%) vs 1/35 (2.9%); P=0.002). The majority (56.1%) of patients were initiated on antiretroviral therapy (ART) one week after the PCP admission episode. Of 58 PCP episodes in which data on

mechanical ventilation was available, 18 (31.0%) were ventilated. Case fatality was 44.4% in ventilated patients, and the overall in-hospital case fatality was 24.8%. One-hundred, twenty-five children were followed up at the outpatient ART Clinic, of which 42 (33.6%) were still in care at the end of the study. The median duration of co-trimoxazole preventive therapy was 1278 days.

Conclusion

PCP was often the first presentation of HIV infection. Children with PCP still have poor outcomes. Despite the decline in PCP admissions, there was a high in-hospital mortality rate.

Introduction

Pneumocystis jirovecii is an important opportunistic pathogen in children living with HIV (CLWH), responsible for the majority of community acquired pneumonia (CAP) admissions in infancy in these children.[1] *Pneumocystis jirovecii* pneumonia (PCP) classically presents as a progressive, hypoxaemic pneumonia with signs of severe respiratory distress and respiratory failure.[2] Mortality rates are high if not appropriately treated.[6] Studies conducted in the late 1990s highlighted the importance of prophylactic therapy, using co-trimoxazole, to prevent PCP and all-cause mortality in CLWH.[3]

A study of over 200 children with suspected PCP from Cape Town demonstrated in-hospital case fatality of 32% in children in whom PCP was confirmed, compared to 17% in children without confirmation of *P. jirovecii* infection.[7] Most of the deaths (88.8%) occurred in CLWH, and all of the HIV-infected children that died had CD4 counts <15%.[7] In a two-month observational study of severe pneumonia in 121 children 2-60 months of age in Uganda, 20 (16.5%) had confirmed PCP, 18 (90%) of which were HIV-infected, and the mortality rate in those with PCP was 40%.[29] Fatal cases of PCP are often associated with evidence of co-infection with human cytomegalovirus (CMV) in CLWH.[3,24] Du Plessis et al[27] evaluated recurrence of PCP in CLWH <5 years of age between 2006 and 2010, and demonstrated that 20% had recurrent episodes of PCP.

There is scanty evidence on long-term survival rates in children hospitalised with PCP, especially in settings with a high burden of paediatric HIV. In this study, we aimed to describe the timing of the PCP diagnosis in relation to the HIV diagnosis in CLWH, to evaluate at what stage co-trimoxazole preventive treatment (CPT) was discontinued in these children, and evaluate the long-term outcomes of CLWH with confirmed and suspected PCP.

Methods

This was a retrospective, descriptive study of all admissions that were diagnosed with PCP in the general paediatric wards at Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa, from 1 January 2011 through 31 December 2019. CHBAH is a

secondary/tertiary public sector hospital which serves the majority of children requiring hospitalisation for severe pneumonia in Soweto. Population estimates are that 1.2 million persons reside in Soweto, 120,000 to 150,000 of which are children <5 years of age. Modelling estimates indicate that 2% of Sowetan children <5 years of age are HIV-infected.[30]

Inclusion criteria

Inclusion criteria for the study was a discharge diagnosis of PCP (ICD-10 Codes: B59 and/or B20.6), or laboratory confirmation of PCP in children treated in the general paediatric wards at CHBAH during the study time period. HIV-infected children aged birth to <14 years were eligible for inclusion in the study.

Case definitions

A confirmed case of PCP was classified as *P. jirovecii* DNA detected on PCR of respiratory specimens (nasopharyngeal swabs, induced sputum, endotracheal aspirates); or *P. jirovecii* detected using immunofluorescence microscopy on respiratory secretions; or *P. jirovecii* cysts observed on post-mortem lung biopsy in children dying from pneumonia. A presumed case of PCP was classified as no molecular, immunofluorescence microscopy or histological evidence of PCP, but a clinicianischarge or outcome diagnosis of the condition. A diagnosis of CMV co-infection was ascribed in children with CMV viraemia or CMV detected on respiratory secretions using quantitative or qualitative polymerase chain reaction (PCR). Positive blood cultures for clinically significant bacterial isolates were used to define bacteraemic pneumonia.

Data sources

All children with a discharge diagnosis of PCP were identified through an electronic database which is maintained by the Department of Paediatrics at CHBAH. Data from a pneumonia aetiology study, conducted in 2011 through 2013, were used to identify children that were routinely tested using a multiplex PCR assay for respiratory pathogens. The National Health Laboratory Service (NHLS) database was used to obtain ancillary investigations (immunofluorescence microscopy, PCP PCR, β-D-glucan (BDG), lactate dehydrogenase (LDH) and histology results) that were used to diagnose PCP. Outpatient

follow-up measures were obtained from the database of the paediatric HIV clinic. Timing of initiation of ART; cessation of CPT; and the time to transfer out or loss to follow-up or death were explored using this database. The last censor date was 31 December 2019 for those who were found to still be attending the outpatient HIV clinic at that time period.

Additional data

Additional data collected for analysis included patient age at presentation, sex, nutritional status (based on World Health Organization (WHO) Growth Standards) and duration of hospital stay. Blood investigations included CMV viral load, LDH, BDG and C-reactive protein (CRP), CD4 count and HIV viral load on admission. Co-morbidities such as CMV, tuberculosis (TB), malignancy and auto-immune diseases were also analysed. Data were also collected with regards to the duration of ART before admission and date of cessation of CPT. Complications during the PCP admission episode(s) were assessed, with outcomes defined as discharge, death, transfer out or lost to follow-up. CD4 and HIV viral load at or prior to CPT cessation was also evaluated. Censor dates for outcome measures included date of in-hospital death if died during the PCP hospitalisation episode, date of transfer out or loss to follow-up at the outpatient HIV clinic, or date of death during outpatient follow-up.

Analysis

All data analysis was conducted using R version 4.1.1.[31] Proportions were compared using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were assessed for normality, with means and standard deviations (SDs) reported for normally distributed variables and medians and interquartile ranges (IQRs) reported for skewed variables. The unpaired T-test was used to compare means, and the Wilcoxon test was used to compare medians. The proportion of children with confirmed or suspected PCP whose CPT was discontinued at outpatient follow-up was evaluated. Stratified analyses were conducted by certainty of the PCP diagnosis, discharge from hospital versus in-hospital death, ventilation status, and follow-up at the outpatient HIV clinic. Outcomes of children followed up at the outpatient HIV clinic were evaluated, and the clinical and laboratory characteristics of children who were known to have completed CPT were compared to

those whose CPT stopping date was unknown.

Receiver operating characteristic (ROC) curves were constructed to evaluate the performance of biomarkers in the diagnosis of PCP. Survival analysis was conducted to evaluate the duration of CPT in children who were followed up at the outpatient HIV clinic.

Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (clearance number: M210224) and the Ethics Committee of the NHLS (clearance number: PR2010473).

Results

From 09 February 2011 to 21 December 2019, there were 390 paediatric admissions to CHBAH with a discharge diagnosis of PCP. The 390 PCP admissions occurred in 363 children, indicating recurrent PCP episodes in a subset of children. Three hundred fourteen (80.5%) of the PCP episodes occurred in CLWH (Figure 2.1). All further analyses are restricted to the 297 HIV-infected children that had a discharge diagnosis of PCP.

The majority (262/314, 83.4%) of the PCP episodes in CLWH were presumed, and not microbiologically confirmed. Most of the 52 confirmed PCP cases (35, 67.3%) were diagnosed in 2011 through 2013 (Table 2.1), during conduct of the Pneumonia Etiology Research for Child Health (PERCH) Study. Confirmed PCP cases were diagnosed on the basis of *P. jirovecii* DNA detected on respiratory samples (n=40), or on visualisation of the organism on post-mortem lung biopsies (n=12).

Figure 2.2 shows the monthly number of HIV-infected PCP cases admitted to the general paediatric wards during the study time period: there was a median of 3 (IQR, 2 to 4) cases per month. The highest number of HIV respiratory admissions (n=41) were in April 2011 and August 2012. The largest burden of PCP cases (n=9) was in September 2012 (Figure 2.2). PCP constituted 14.7% (314/2136) of all respiratory admissions in CLWH during the study period, and 2.4% (390/16230) of all respiratory admissions.

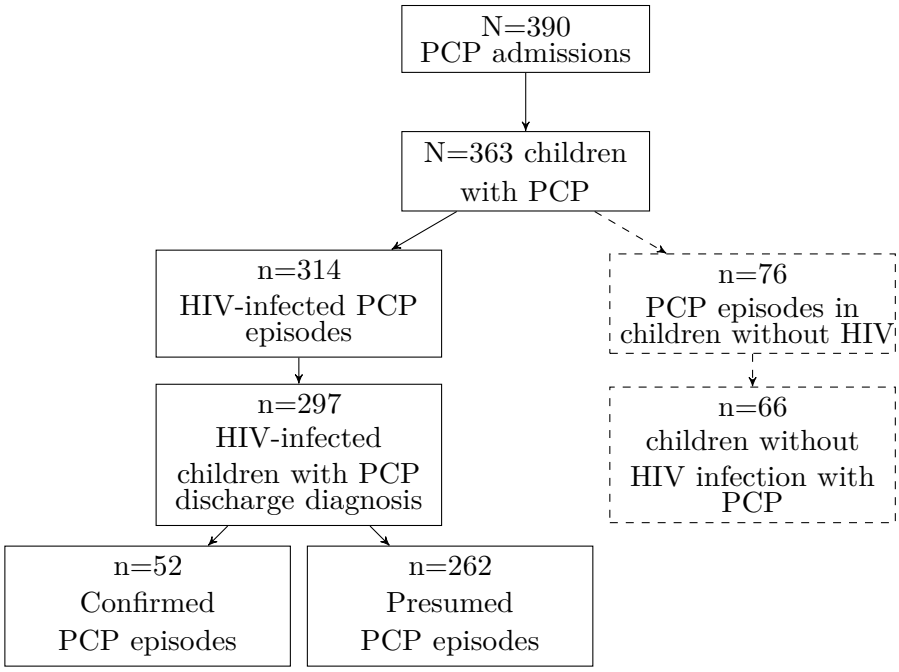

Figure 2.1: Number of PCP admissions, children and episodes included in the analysis

Table 2.1: Certainty of PCP diagnosis in HIV-infected children during the study time period

Year	Confirmed (n=52)	Presumed (n=262)	Total (n=314)
2011	17.3% (9)	7.6% (20)	9.2% (29)
2012	32.7% (17)	13.4% (35)	16.6% (52)
2013	32.7% (17)	8.4% (22)	12.4% (39)
2014	0.0% (0)	16.4% (43)	13.7% (43)
2015	0.0% (0)	14.1% (37)	11.8% (37)
2016	0.0% (0)	10.7% (28)	8.9% (28)
2017	5.8% (3)	9.2% (24)	8.6% (27)
2018	5.8% (3)	9.2% (24)	8.6% (27)
2019	5.8% (3)	11.1% (29)	10.2% (32)

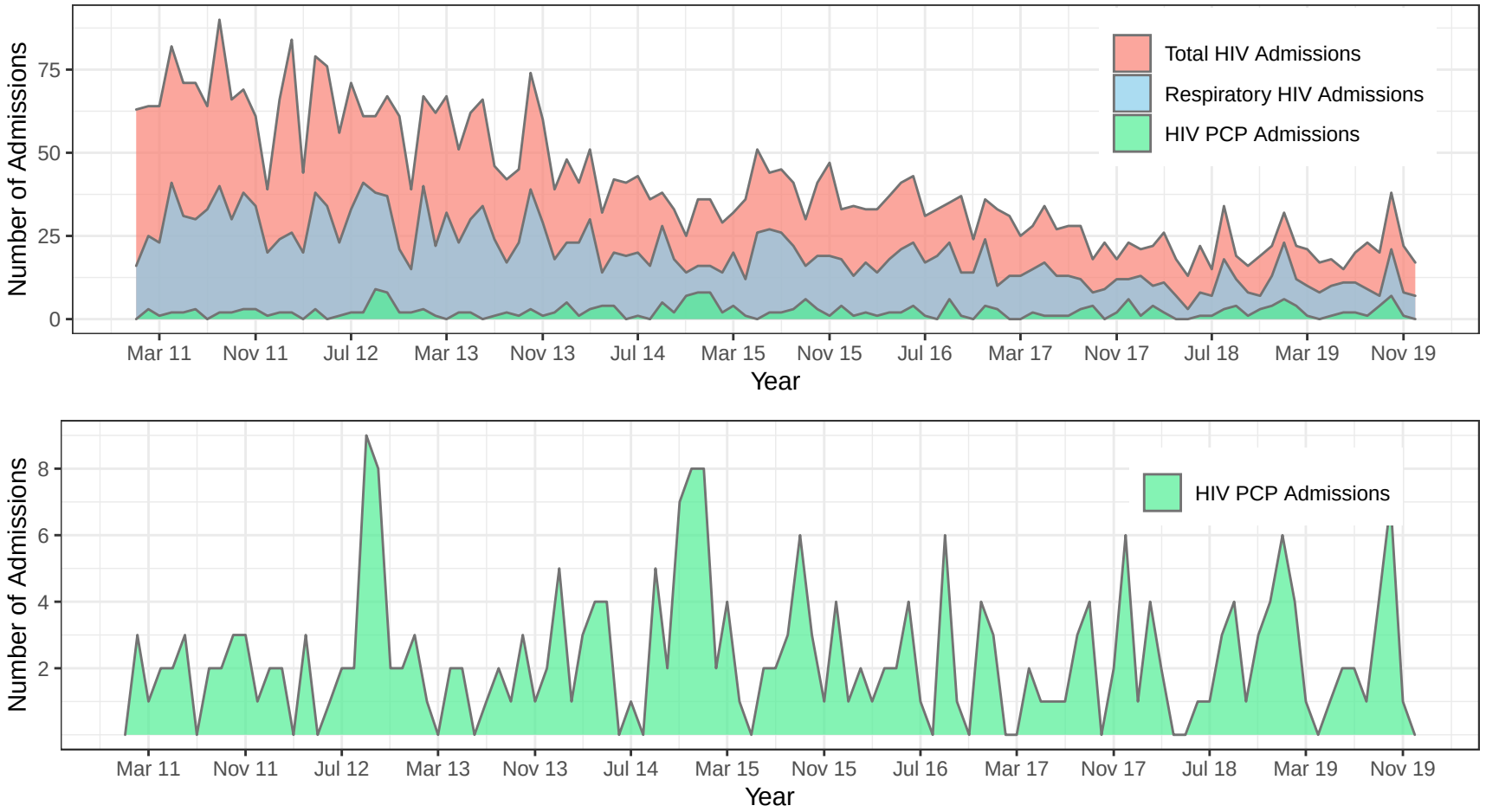

Figure 2.2: Number of PCP cases in HIV-infected children hospitalised at CHBAH during the study time period

Demographic characteristics of the patients at each of the PCP episodes are summarised in Table 2.2. The median age on admission was 3.7 months (IQR, 2.9 to 7.9 months). Seventy-seven percent of the patients in the confirmed PCP group were hypoxic as compared to 100% in the presumed PCP group (albeit information on presence of hypoxic was available in a subset of patients; n=34, 10.8%). Patients in the presumed PCP category were more underweight (median -2.88 vs -1.74; P<0.001) and wasted (median -1.15 vs -0.38; P=0.039) in comparison to patients with confirmed PCP. The median β-D-glucan levels in confirmed and presumed PCP groups were similar (355.5 vs 348.0 pg/mL; P=0.524), as were the mean LDH levels (1497.4 vs 1080.67 U/L; P=0.230). Serum CRP levels (16 vs 13 mg/L; P=0.673) and CMVVL DNAemia densities (mean 4.34 vs 3.86 $\log_{10}$ DNA copies/mL; P=0.419) were also similar in patients with confirmed and presumed PCP (Table 2.2). Of the children who had a CMVVL done, 13 (50%) had CMV disease and the majority (10/13) had presumed PCP. Patients with presumed PCP had a higher prevalence of confirmed TB (38.9%) compared to those with confirmed PCP (2.9%); P=0.002.

Significant bacteraemia was observed in 12.4% PCP episodes, with no statistically significant difference between the confirmed and presumed PCP groups (P=1.000). Twenty-five PCP episodes were complicated with hospital acquired infection (HAI), with similar rates of HAI observed in the presumed and confirmed PCP groups (P=0.446).

The majority (34/42, 81%) of the patients with available information on HIV clinical staging, were WHO stage IV at the time of their PCP hospitalisation. Median CD4 counts and percentages, and median HIV viral loads were similar, regardless of certainty of the PCP diagnosis (Table 2.2).

Timing of ART initiation was available for 114 (36.3%) of the PCP episodes, and the majority (64/114, 56.1%) were started on ART after one week of admission. The median length of hospitalisation was 15 days, significantly longer in presumed PCP episodes (Table 2.2).

Characteristics of ventilated patients

Information on requirement for ventilation was available for 58 PCP episodes, of which 18 (31.0%) received mechanical ventilation. Presumed PCP episodes were significantly more frequently ventilated (6/7, 85.7%) compared to confirmed PCP episodes (12/51, 23.5%).

Non-ventilated patients were significantly more likely to have confirmed PCP compared to ventilated patients (39/40 (97.5%) vs 12/18 (66.7%); P=0.004) and had significantly longer duration of hospitalisation (27.5 days (IQR, 10.5-30.0 days) vs 7.0 days (IQR, 10.5-30.0 days); P=0.001) (Supplementary Materials, Table 2.7). Mortality rates were similar in children that were ventilated and those that were not ventilated (44.4% and 32.5%, respectively; P=0.562) (Supplementary Materials, Table 2.7). Duration of ventilation and ICU stay was not available for the children that were ventilated.

Survival in-hospital

Mortality outcomes were available for 286 (91.1%) of the 314 PCP episodes, of which 71 (24.8%) resulted in death during the hospitalisation. Case fatality was significantly higher in confirmed (40.4%) compared to presumed PCP episodes (21.4%) (Table 2.2).

Characteristics of 71 children that died in-hospital, compared to those who survived to hospital discharge, are presented in detail in Supplementary Materials (Table 2.8). Most clinical characteristics between children that died and those survived were similar, including age on admission, anthropometry, serum LDH levels and whole blood CMVVL (Supplementary Materials, Table 2.8). The median CRP levels were significantly higher at baseline in those that died (47.5 mg/L) compared to those that survived (11 mg/L); P= 0.022. Furthermore, the median length of stay was significantly shorter in the children who died (8 days), compared to the length of stay in children that survived (16 days); P<0.001. The prevalence of confirmed PCP was significantly higher in children that died, compared to those that survived (21/71 (29.6%) vs 31/215 (14.4%); P= 0.007) (Table 2.8).

Table 2.2: Characteristics of children with a discharge diagnosis of PCP, stratified by certainty of PCP diagnosis

	Overall	Confirmed	Presumed	p	test
n	314	52	262		
Median age [months, IQR]	3.70 [2.86, 7.84]	4.07 [2.82, 6.85]	3.66 [2.87, 8.00]	0.590	nonnorm
Hypoxic (%)	34 (81.0)	27 (77.1)	7 (100.0)	0.380	
Median Wt-for-age Z-score [IQR]	-2.68 [-3.71, -1.52]	-1.74 [-2.52, -0.85]	-2.88 [-3.95, -1.70]	<0.001	nonnorm
Median Lt-for-age Z-score [IQR]	-2.22 [-3.62, -0.88]	-2.11 [-3.03, -0.35]	-2.22 [-3.70, -1.14]	0.064	nonnorm
Median Wt-for-Lt Z-score [IQR]	-0.98 [-2.29, 0.25]	-0.38 [-1.87, 0.76]	-1.15 [-2.43, 0.00]	0.039	nonnorm
Median Beta-D-glucan [IQR]	348.00 [237.00, 523.00]	355.50 [261.50, 407.50]	348.00 [232.50, 523.00]	0.524	nonnorm
Mean LDH (SD)	1229.50 (604.76)	1497.40 (672.41)	1080.67 (546.33)	0.230	
Median CRP [IQR]	15.00 [4.00, 54.00]	16.00 [15.00, 38.00]	13.00 [4.00, 54.00]	0.673	nonnorm
Mean CMVVL (log10 DNA copies/mL, SD)	3.93 (1.06)	4.34 (0.69)	3.86 (1.11)	0.419	
CMV disease = Yes (%)	13 (50.0)	3 (75.0)	10 (45.5)	0.587	
Significant BC = Yes (%)	39 (12.4)	6 (11.5)	33 (12.6)	1.000	
HAI = Yes (%)	25 (8.0)	6 (11.5)	19 (7.3)	0.446	
Definite TB (%)	8 (15.1)	1 (2.9)	7 (38.9)	0.002	
Clinical TB (%)	18 (34.0)	12 (34.3)	6 (33.3)	1.000	
WHO Clinical Stage (%)				0.577	
I	2 (4.8)	2 (5.7)	0 (0.0)		
II	1 (2.4)	1 (2.9)	0 (0.0)		
III	5 (11.9)	5 (14.3)	0 (0.0)		
IV	34 (81.0)	27 (77.1)	7 (100.0)		
Median CD4% [IQR]	13.01 [8.46, 23.02]	13.07 [8.68, 22.74]	11.40 [6.71, 25.87]	0.678	nonnorm
Median CD4# [IQR]	837.00 [442.00, 1435.00]	846.50 [527.25, 1417.25]	403.00 [76.50, 1871.00]	0.436	nonnorm
Median HIVVL [log10 RNA copies/mL, IQR]	6.52 [6.23, 6.87]	6.48 [6.24, 6.88]	6.62 [6.22, 6.74]	0.697	nonnorm
Ventilated = Yes (%)	18 (31.0)	12 (23.5)	6 (85.7)	0.004	
Median length of hospitalization [days, IQR]	15.00 [8.00, 24.00]	9.50 [4.00, 15.25]	16.00 [9.00, 26.00]	<0.001	nonnorm
Median time on ART at PCP diagnosis [days, IQR]	-10.00 [-19.00, 1.75]	-15.00 [-34.50, -3.00]	-9.00 [-18.00, 2.00]	0.226	nonnorm
Timing of ART initiation category (%)				0.570	
>1 week prior to admission	23 (20.2)	3 (20.0)	20 (20.2)		
Within 1 week of admission	27 (23.7)	2 (13.3)	25 (25.3)		
>1 week post admission	64 (56.1)	10 (66.7)	54 (54.5)		
Died in Hospital (%)	71 (24.8)	21 (40.4)	50 (21.4)	0.007	

Note:
ART = antiretroviral therapy; BC = blood culture; CRP = C-reactive protein; CMVVL = cytomegalovirus viral load in whole blood; HAI = healthcare-asccociated infection; HIVVL = HIV viral load in whole blood; IQR = interquartile range; LDH = lactate dehydrogenase; Lt-for-age = length-for-age; PCP = Pneumocystis jirovecii pneumonia; SD = standard deviation; TB = tuberculosis; WHO = World Health Organization; Wt-for-age = weight-for-age; Wt-for-Lt = weight-for-length. P-values are derived from tests that compare confirmed to presumed PCP cases according to type of variable: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Recurrent PCP episodes

Recurrent PCP episodes occurred in 16 (5.4%) of the 297 CLWH during the study period (Table 2.3). The median age at admission during the second PCP admission was 7.5 months, and the second PCP episode occurred at a median of 39.5 days (IQR, 24.5 to 81.5 days) after the first PCP episode. The median length of hospitalisation during the first PCP admission was 18.5 days, longer than the length of hospitalisation during the second PCP admission episode (median 9.0 days). Seven (87.5%) of eight children with available information of timing of ART in relation to the first PCP episode, commenced ART subsequent to the first PCP admission. In contrast, at the time of the second PCP episode, only one (12.5%) of the eight children with available data on time of ART commencement was not yet on ART at the time of admission: she was commenced onto ART seven days into her second PCP admission episode (Patient 7, Table 2.3). One patient had three PCP admission episodes, the first at 3.1 months of age, the second 18 days later, and the third which occurred 79 days after the second admission.

Blood results were inconsistently available for the children that experienced recurrent PCP admission episodes (Table 2.4). Patient 1, ART naïve at the initial PCP episode but ART experienced at the second PCP episode, had increased CD4 T-helper cell count and percentage between the two PCP episodes (from 856 to 1586 cells/mm^3; 10.59% to 21.88%). She was the only patient with blood results available for comparison between the PCP episodes (Table 2.4).

Utility of biomarkers in the diagnosis of PCP

Biomarkers typically used to evaluate PCP showed poor ability to distinguish between confirmed and presumed PCP cases (best AUC in the ROC analysis was 0.69, for serum LDH), and performed equally poorly in the comparison between children that died in-hospital and those that survived (best AUC in the ROC analysis was 0.67, for CRP; Table 2.5). ROC curves for those for β-D-glucan, CRP and CMV viral load are presented in Supplementary Materials (Figure 2.4).

Table 2.3: Characteristics of children with recurrent PCP admission episodes

Patient	Sex	First PCP Episode			Second PCP Episode					Third PCP Episode			
		Age (months)	Length stay (days)	Time on ART (days)	Age (months)	Time difference (days)	Length stay (days)	Time on ART (days)	Died	Age (months)	Time difference (days)	Length stay (days)	Died
Patient 1	F	2.6	19	-95	11.3	252	4	157	No				
Patient 2	M	3.2	12	-13	15.5	377	12	364	No				
Patient 3	F	3.1	27		3.7	18	9		No	6.2	79	0	No
Patient 4	F	6.6	26		7.9	39	6		No				
Patient 5	F	5.6	12		7.6	61	16		No				
Patient 6	F	7.8	21	-21	11.2	102	14	81	No				
Patient 7	F	1.7	24	-47	3.0	40	5	-7	No				
Patient 8	M	3.0	18		14.8	361	1		Yes				
Patient 9	F	1.9	42		2.9	28	14		No				
Patient 10	F	6.8	7		7.4	21	9		No				
Patient 11	F	8.6	16	-16	9.2	18	4	2					
Patient 12	M	4.1	17	-7	4.8	21	2	14	No				
Patient 13	F	2.4	19	-1	3.6	37	20	36	No				
Patient 14	M	37.6	5	184	38.7	35	8	219	No				
Patient 15	M	2.4	8		4.0	50	9		No				
Patient 16	U	3.6	23		5.5	58							

Note:
ART = antiretroviral therapy; F = female; M = male; PCP = Pneumocystis jirovecii pneumonia; U = sex unrecorded.

Table 2.4: Key laboratory results of children with recurrent PCP admission episodes

Patient	PCP Episode	BDG	LDH	CRP	CD4 count	CD4%	HIVVL (log10 copies/mL)	CMVVL (log10 DNA copies/mL)
Patient 1	1				856	10.59		
Patient 1	2				1586	21.88	5.68	
Patient 3	3				72	6.81	3.66	
Patient 4	1	158	2093	44				7715
Patient 5	1				2224	43.76	7.00	
Patient 9	2				2152	8.64	6.42	
Patient 10	2			180				
Patient 13	2	523		4				

Note:
BDG = beta-D-glucan; CD4 = CD4 T-helper cell; CMVVL = cytomegalovirus viral load; CRP = C-reactive protein; HIVVL = HIV viral load; LDH = lactate dehydrogenase; PCP = Pneumocystis jirovecii pneumonia.

Table 2.5: ROC analysis of biomarkers commonly used in the work-up of PCP cases

Predictor	Optimal cutpoint	Youden	Sensitivity	Specificity	Accuracy	AUC
Confirmed vs Presumed PCP						
LDH	1442	0.38	0.60	0.78	0.71	0.69
BDG	326	0.15	0.67	0.48	0.52	0.42
CRP	11	0.32	0.89	0.43	0.49	0.54
CMVVL	3433	0.50	1.00	0.50	0.58	0.68
Died vs Survived						
LDH	1442	0.25	0.50	0.75	0.64	0.56
BDG	301	0.35	0.71	0.64	0.69	0.62
CRP	54	0.35	0.50	0.85	0.73	0.67
CMVVL	12985	0.48	0.78	0.71	0.73	0.63

Note:
AUC = area under the curve; BDG = beta-D-glucan; CRP = C-reactive protein; CMVVL = human cytomegalovirus viral load; LDH = lactate dehydrogenase; PCP = Pneumocystis jirovecii pneumonia; ROC = receiver operating characteristic.

Outcomes

One hundred twenty-five (42.1%) of the 297 patients were followed up at the outpatient HIV clinic, 42 (33.6%) of which were still following up at the clinic at the end of the study period. Eight (6.4%) of the children followed up at the outpatient HIV clinic were known to have died during follow-up, 45 (36.0%) were transferred out to continue ART at down-referral clinics, and 30 (24.0%) were lost to follow-up. The median duration of CPT for those following up at the outpatient clinic was 617.0 days (IQR 297.5 to 1136.0).

Children that were followed up at the outpatient HIV clinic were similar in almost all respects, compared to those that were not followed up at the clinic (Supplementary Materials, Table 2.9). The only characteristic that differed between the children that were and were not followed up at the outpatient HIV clinic, was the timing of ART initiation at presentation with PCP. Timing of ART initiation was known in a subset of the children, and 16.0% (16/100) of those treated at the outpatient HIV clinic had accessed ART more than one week prior to the PCP admission episode, whereas 50.0% (7/14) of those that were not followed up at the outpatient clinic had accessed ART >1 week prior; P=0.012 (Supplementary Materials, Table 2.9). As outpatient HIV clinic attendance usually commences following a sentinel admission episode, it is likely that this discrepancy in timing of ART reflects PCP as being a sentinel admission episode in CLWH.

When stratifying the outpatient HIV clinic patients by whether they were still in care or not at the end of the study period, similar proportions of those still in care and those that were censored had discontinued CPT (18/42 (42.9%) vs 41/83 (49.4%); P=0.616) (Supplementary Materials, Table 2.10). Median age at CPT cessation was significantly younger (21.6 months (IQR, 9.4-38.0 months) vs 47.9 months (IQR, 34.2-58.2 months); P=0.001) in those that were not in care at the end of the study period, and they were younger at last follow-up (19.0 months (IQR, 9.1 to 37.5) vs 48.3 (IQR, 21.3 to 80.6); P<0.001) (Supplementary Materials, Table 2.10).

Table 2.6 shows the characteristics of the 125 children who were followed up at the outpatient HIV clinic, stratified by whether CPT was known to have been stopped or not. Fifty-nine children (48.8%) had stopped CPT by the end of the study period. The

median age at discontinuation of CPT was 27.6 months. Children who had stopped CPT were significantly older at the last follow-up visit, compared to children that were still on CPT (Table 2.6). Of those that had stopped CPT, 18 (30.5%) were still in care at the outpatient HIV clinic, 36 (61%) had been transferred out or were lost to follow-up, and 5 (8.5%) had died (Table 2.6). The median duration of CPT in children whose CPT was known to have been stopped was 1278 days (Figure 2.3).

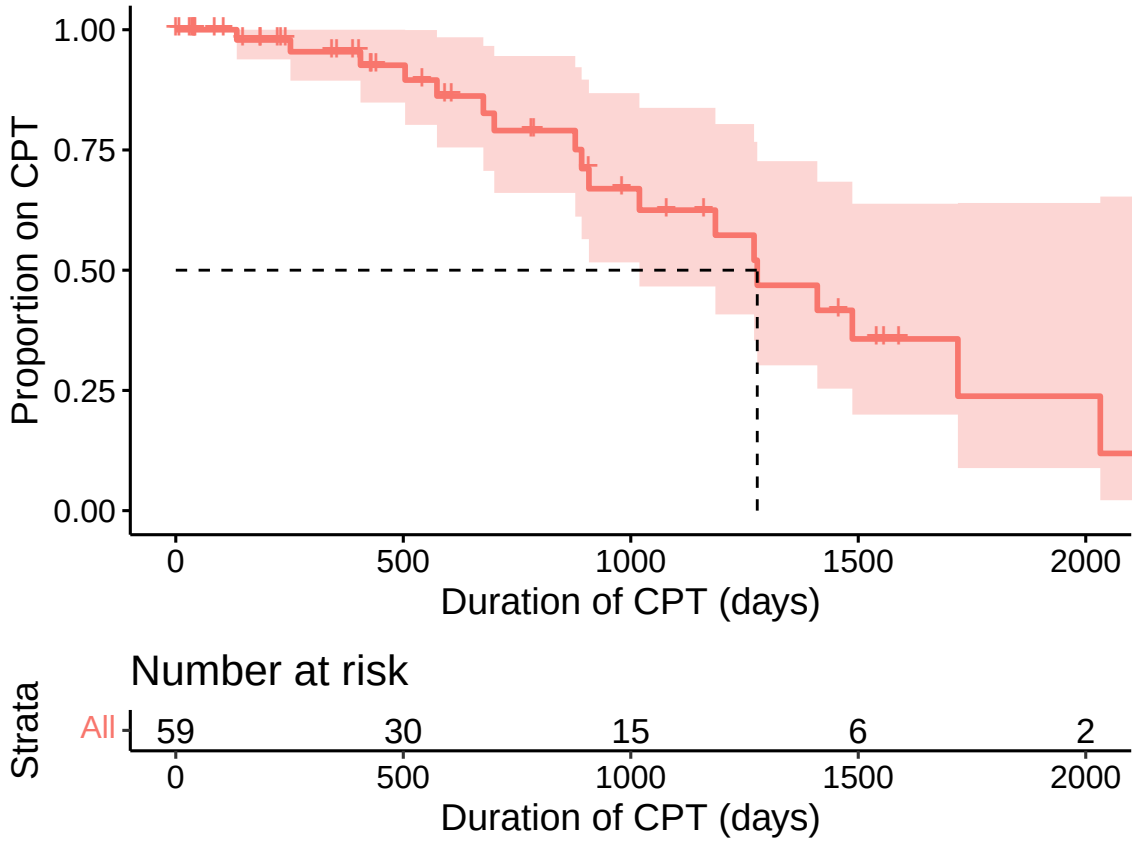

Figure 2.3: Kaplan-Meier plot of duration of CPT in children that were followed up at the outpatient HIV clinic, and whose CPT cessation dates were known

Table 2.6: Outcomes of children that were followed up at the outpatient HIV clinic, stratified by CPT stop category

	CPT stopping data after censor date or unknown	CPT known to have been stopped	p	test
n	66	59		
Median age at PCP admission episode [months, IQR]	4.12 [3.00, 8.48]	3.81 [2.79, 10.12]	0.701	nonnorm
Median age at CPT cessation [months, IQR]	-	27.62 [15.25, 52.60]	-	nonnorm
Median age at last follow-up [months, IQR]	19.03 [9.13, 35.58]	42.03 [15.85, 68.08]	0.005	nonnorm
Male (%)	28 (42.4)	29 (49.2)	0.566	
Hypoxic (%)	6 (60.0)	7 (87.5)	0.444	
PCP confirmed (%)	11 (16.7)	5 (8.5)	0.271	
Definite TB (%)	1 (10.0)	1 (10.0)	1.000	
Clinical TB (%)	2 (20.0)	4 (40.0)	0.626	
WHO Clinical Stage (%)			0.417	
I	0 (0.0)	1 (12.5)		
II	1 (10.0)	0 (0.0)		
III	1 (10.0)	2 (25.0)		
IV	8 (80.0)	5 (62.5)		
Median CD4% at CPT stop or at last follow-up if censored [IQR]	40.37 [26.10, 41.78]	31.10 [26.53, 35.37]	0.332	nonnorm
Median CD4# at CPT stop or at last follow-up if censored [IQR]	1204.00 [1084.00, 1905.00]	1550.50 [1153.50, 2141.75]	0.614	nonnorm
Median HIVVL at CPT stop or at last follow-up if censored [log10 RNA copies/mL, IQR]	2.33 [2.17, 4.17]	1.63 [1.40, 3.01]	0.135	nonnorm
Shezi outcome (%)			0.001	
Died	3 (4.5)	5 (8.5)		
In care	24 (36.4)	18 (30.5)		
LTFU	7 (10.6)	23 (39.0)		
TF out	32 (48.5)	13 (22.0)		

Note:
CPT = Co-trimoxazole preventive therapy; HIVVL = HIV viral load in whole blood; IQR = interquartile range; LTFU = Lost to follow-up; PCP = Pneumocystis jirovecii pneumonia; TF = transfer; TB = tuberculosis; WHO = World Health Organization. P-values are derived from tests that compare CPT known to have been discontinued to cases in which CPT was not known to have been discontinued, according to type of variable: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Discussion

Pneumocystis jirovecii is a common opportunistic pathogen in CLWH, and PCP is often the sentinel infection with which these children reach medical attention. In this study, we aimed to assess the outcomes CLWH that were diagnosed with PCP during a 9 year period at an academic hospital in South Africa. The total HIV admissions during the study period decreased over time. This could be attributed to optimisation of PMTCT and ART programmes. There was a steady decline in the total respiratory admissions in CLWH over the 9 year study period, possibly associated with incorporation of pneumococcal conjugate vaccine (PCV) into the immunisation programme,[32] as well as increasing ART coverage of CLWH.

Only 52 (16.6%) of all PCP episodes were confirmed in the current study. The majority of the confirmed PCP cases (43/52, 82.7%) were diagnosed in 2011 to 2013, during conduct of the PERCH Study. In PERCH, *P. jirovecii* was associated with 23% (95% credible interval 12.4%-31.5%) of community-acquired pneumonia (CAP) admissions in South African CLWH.[1] In the current study, the burden of PCP was 14.7% (314/2136) among respiratory admissions in CLWH, and 2.4% (390/16230) of all respiratory admissions over a 9 year period. Similarly, PCP was attributed in 15/151 (9.9%) of respiratory admissions in CLWH in Cape Town in the era before access to ART.[6]

In our study, the highest number of PCP admissions occurred in 2012 (52 admissions) and the least admissions in 2017 and 2018 (27 admissions in each year). A presumptive diagnosis of PCP was made by treating clinicians in the majority of children included in this study, which likely reflects clinician reliance on the use of ancillary tests (e.g. β-D-glucan) to support a diagnosis of the condition. PCR for *P. jirovecii* is infrequently used by clinicians working in the study hospital, despite the substantially higher sensitivity of this diagnostic modality over immunoflourescence, even on upper respiratory tract specimens.[7,33] .

Three hundred fourteen PCP episodes occurred in 297 CLWH who were included in this study. Sixteen (5.4%) children had recurrent PCP episodes, lower than the recurrent PCP incidence (19.9%) observed by Du Plessis in Pretoria in 2006 to 2010.[27] The lower

rates of recurrent PCP observed in the current study may be due to better ART coverage rates in the study time period (2011 through 2019).

PCP is commonly seen between the age group of 6 weeks to 6 months, and the median age of the children on admission in this study was 3.7 months (IQR, 2.85 to 7.85 months), similar to other studies.[1,7,11] Children with presumed PCP had significantly lower anthropometric indices compared to those with confirmed disease. This may indicate that clinicians are prone to consider a diagnosis of PCP in CLWH that are malnourished. This suggests that CLWH with confirmed PCP may in fact have better nutritional status that what clinicians typically assume to be the case in infants suffering from this condition.

This study had limited ability to definitively evaluate the role of biomarkers for the diagnosis of PCP in CLWH. This is likely as a result of the study design which did not include a group of "non-PCP" patients, and the fact that clinicians likely based a clinical diagnosis of PCP on the results of commonly used biomarkers, such as β-D-glucan and LDH. There was no significant difference between the confirmed and presumed PCP groups in terms of serum LDH, β-D-glucan, CRP and CMVVL. A control group of CLWH hospitalised with LRTI but without a discharge diagnosis of PCP may have assisted evaluation of test performance of these biomarkers in children in whom clinicians are considering a diagnosis of PCP. The sensitivity and specificity of LDH as a biomarker for the diagnosis of PCP was 67% and 56% respectively in our study, which was lower than that observed through a meta-analysis by White et al[13] where the sensitivity and specificity were of 80-100% and 6-85%, respectively. BDG had a sensitivity of 67% and 48% specificity in our study, which was also lower in comparison to the study by White et al[13] in which the sensitivity and specificity of BDG were 90-95% and 78-68%, respectively.

In our study, CMVVL at a cut-off density of 3433 copies/mL had a sensitivity of 100% and specificity of 50% for distinguishing between confirmed and presumed PCP cases, however there was no statistically significant difference between both groups with regards to CMV disease. Dual infection with *P. jirovecii* and CMV is associated with more severe disease, and worse outcomes in children presenting with hypoxaemic pneumonia.[12,25]

In this study, children with presumed PCP had a significantly higher proportion of

definite TB compared to those with confirmed PCP (38.9% vs 2.9%; P=0.002), but there was no significant difference between the groups with regards clinical TB (34.2% vs 33.3%; P=1.000). Furthermore, 12.4% of children had positive blood cultures that were considered significant with no difference between the two groups, which was similar to the proportion of blood culture positivity among PCP cases (14.6%) in a Mozambican study.[11] Eight percent (25/314) of the PCP episodes were complicated by hospital-acquired infections, which could may have contributed to overall morbidity, increased hospital stay and overall mortality. We were unable to assess other underlying co-morbidities such malignancy and autoimmune disease in this study. Immunological and virological status of the CLWH in the presumed and confirmed PCP groups was similar in our study, and most children were not on ART on admission. This indicates that PCP was frequently the sentinel admission episode in children newly diagnosed with HIV in our study.

Of the 58 children in whom ventilation status was known, 18 (31%) were ventilated and eight (44.4%) of those that were ventilated died. The case fatality rate was similar (32.5%) in those that were not ventilated; P=0.562. In a study done at CHBAH in 1992 to 1993, case fatality was 88% in CLWH that were ventilated in ICU, and 42% (5/12) in those with PCP.[28] The similar case fatality rates in our study and that which was conducted in the pre-ART era is concerning, but speaks to the clinical severity and complexity of management of infants with PCP requiring mechanical ventilation, and likely similarity in patient characteristics (i.e. ART naive at presentation with PCP) in both studies. In a study from Taiwan, which included adults and children with confirmed PCP, 13/20 (65%) children were ventilated and survival among those that were ventilated was 39% (5/13).[12] Four (6.9%) of the 58 ventilated children in our study had information on CMV co-infection, three (75%) of whom had had evidence of CMV disease.

Mortality rates are high in children with untreated PCP. The baseline characteristics in the groups of children who died and survived were similar in most aspects, although the median CRP was significantly higher in the children who died (47.5 vs 11.0 mg/L; P=0.022) which may have been an indicator of a more severe inflammatory or infective process leading to a poorer outcome. In this study there were 71 deaths in hospital and 8 deaths out of hospital post discharge. There was a statistically significant difference

between the confirmed and presumed PCP groups with regards to in-hospital mortality rates (40.4% in the confirmed PCP group vs 21.4% in the presumed PCP group; P=0.007). The overall in-hospital mortality rate during the study period was 24.8%. This is similar to mortality rates (18.8% to 40%) described in other studies conducted in hospitals over the years.[7,11,18,29] This is a concerning factor as despite an increase in the provision of CPT and earlier treatment of PCP with better diagnostic methods available for the diagnosis of PCP, the overall mortality rates remained high. These high mortality rates indicate that PCP is still a life threatening illness in CLWH. There is a need for better screening and and high index of suspicion for PCP. Early infant diagnosis of HIV, expedited access onto ART and CPT are essential to prevent PCP and its associated morbidity and high mortality rates.

Long term outcomes of children previously diagnosed with PCP were evaluated in our study. One hundred twenty-five (42.1%) of the 297 CLWH that were diagnosed with PCP during the study period were followed up at the outpatient HIV clinic. Forty-two (33.6%) of those in follow-up were still in care at the end of the study and 8 children died (6.4%). The median age at last follow-up was 48.3 months. Fifty-nine (59/125, 47.2%) children were known to have discontinued CPT, and the median duration of CPT for these children was 1278 days. The median CD4% at discontinuation of CPT was 31.1%, which is in keeping with the current South African guidelines. A limitation in reviewing total duration of CPT for all children was that children that were lost to follow-up or transferred out to continue ART at other facilities were not known to have stopped CPT.

Limitations

Use of a PCR test for the diagnosis of PCP was infrequently used by clinicians working at the study hospital, which accounted for a much smaller study population of confirmed PJP cases. The definition of presumed PCP relied on clinical suspicion by the treating clinician, and may result in bias as the use of biomarkers such as LDH and β-D-glucan may have been more widely used to make a diagnosis of PCP. Blood results were also inconsistently available to assess the utility of biomarkers for the diagnosis of PCP in the study. Information regarding ventilation status was available in very few cases (58/297,

19.5%) of which only 18 (31.0%) were ventilated. This limits the conclusions regarding characteristics and outcomes of ventilated and non-ventilated children. Furthermore, information regarding duration of ICU stay was not available, and we were unable to assess underlying co-morbidities (e.g. malignancy, autoimmune conditions) in this study.

Conclusions

This study highlights that PCP continues to be an important opportunistic infection in CLWH, and is frequently a primary admission episode for the diagnosis of HIV in previously undiagnosed children, with a median CD4% <15% upon diagnosis. In the South African setting, PCP is still associated with poor outcomes although there has been a decline in total admissions over the years during this study, attributed to better PMTCT and provision of CPT. Children with PCP who are ventilated still have high mortality rates and the overall in-hospital mortality rates are unchanged compared to observational studies which were conducted in the pre- and early-ART eras. Early intervention and treatment of PCP is important to improve the overall outcomes of children admitted with PCP. Further studies investigating long term outcomes of children that were previously diagnosed with PCP, with longer follow up times, could be a possible scope for future studies.

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Supplementary Materials

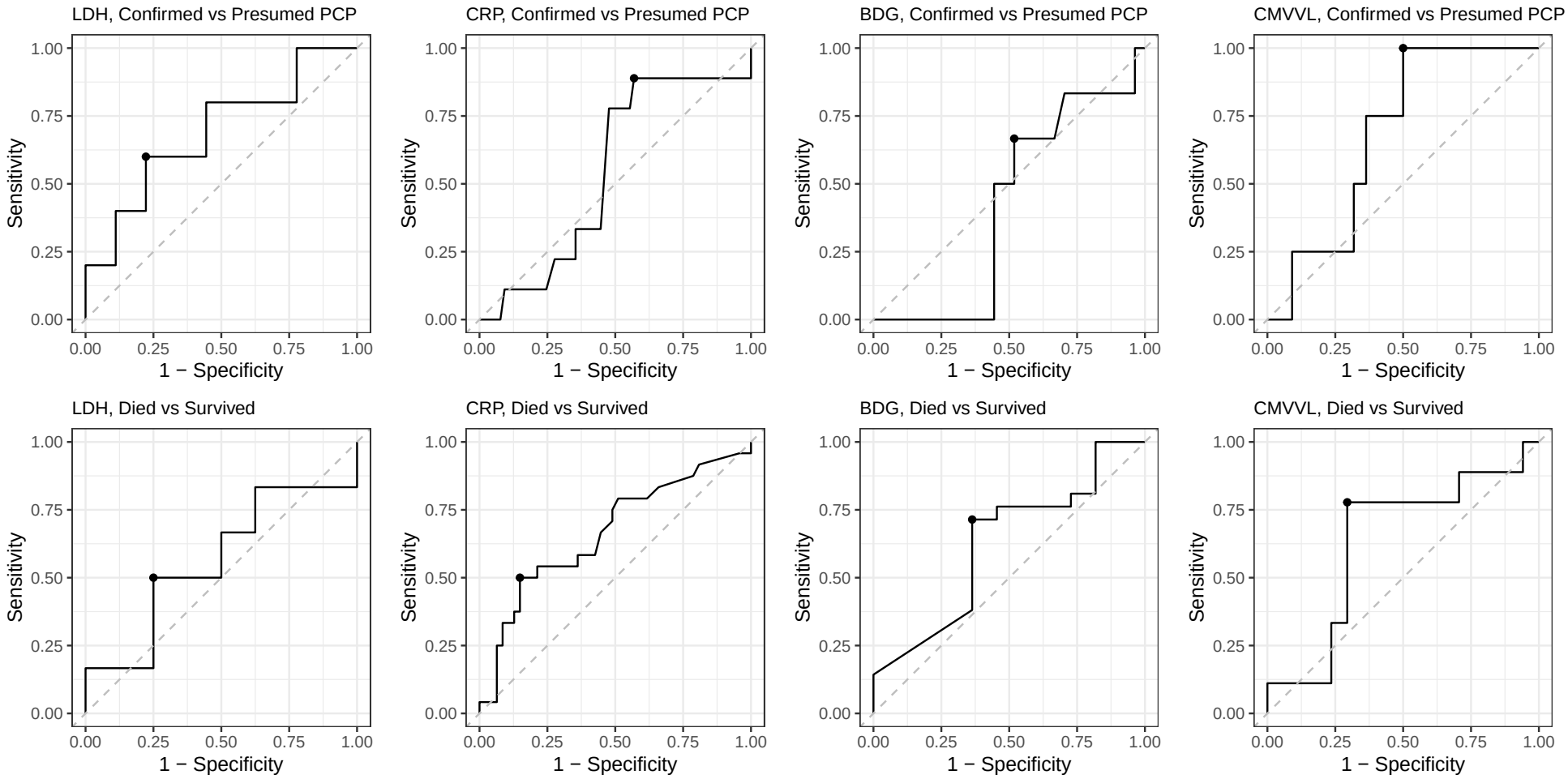

Figure 2.4: ROC analysis of commonly used biomarkers in children with PCP, stratified by certainty of PCP diagnosis and in-hospital mortality

Table 2.7: Characteristics of children with a discharge diagnosis of PCP, stratified by ventilation status

	Overall	Not ventilated	Ventilated	p	test
n	58	40	18		
Median age [months, IQR]	3.40 [2.73, 6.62]	3.73 [2.74, 6.73]	3.21 [2.71, 5.00]	0.405	nonnorm
Hypoxic (%)	34 (81.0)	24 (75.0)	10 (100.0)	0.195	
Median Wt-for-age Z-score [IQR]	-1.71 [-2.48, -0.89]	-1.69 [-2.67, -0.70]	-1.77 [-2.35, -1.16]	0.729	nonnorm
Median Lt-for-age Z-score [IQR]	-2.00 [-2.99, -0.34]	-1.61 [-3.05, -0.30]	-2.45 [-2.95, -0.68]	0.572	nonnorm
Median Wt-for-Lt Z-score [IQR]	-0.36 [-1.65, 0.82]	-0.26 [-2.12, 1.05]	-0.47 [-1.29, 0.45]	0.702	nonnorm
PCP Category confirmed (%)	51 (87.9)	39 (97.5)	12 (66.7)	0.004	
Median Beta-D-glucan [IQR]	355.50 [261.50, 407.50]	-	355.50 [261.50, 407.50]	-	nonnorm
Mean LDH (SD)	1497.40 (672.41)	1952.00 (721.25)	1194.33 (547.48)	0.267	
Median CRP [IQR]	15.50 [12.00, 32.50]	15.50 [14.00, 25.50]	15.50 [5.25, 32.50]	0.830	nonnorm
Mean CMVVL (log10 DNA copies/mL, SD)	4.34 (0.69)	3.54 (n=1)	4.60 (0.53)	-	
CMV disease = Yes (%)	3 (75.0)	0 (0.0)	3 (100.0)	0.505	
Significant BC = Yes (%)	7 (12.1)	3 (7.5)	4 (22.2)	0.247	
HAI = Yes (%)	7 (12.1)	3 (7.5)	4 (22.2)	0.247	
Definite TB (%)	1 (2.4)	1 (3.1)	0 (0.0)	1.000	
Clinical TB (%)	14 (33.3)	11 (34.4)	3 (30.0)	1.000	
WHO Clinical Stage (%)				0.378	
I	2 (4.8)	2 (6.2)	0 (0.0)		
II	1 (2.4)	1 (3.1)	0 (0.0)		
III	5 (11.9)	5 (15.6)	0 (0.0)		
IV	34 (81.0)	24 (75.0)	10 (100.0)		
Median CD4% [IQR]	13.01 [8.46, 23.02]	13.14 [8.71, 23.56]	11.00 [6.30, 21.07]	0.331	nonnorm
Median CD4# [IQR]	837.00 [442.00, 1435.00]	837.00 [531.50, 1462.50]	629.50 [145.25, 1206.00]	0.379	nonnorm
Median HIVVL [log10 RNA copies/mL, IQR]	6.52 [6.23, 6.87]	6.47 [6.03, 6.87]	6.68 [6.52, 6.81]	0.170	nonnorm
Median length of hospitalization [days, IQR]	11.00 [4.00, 18.50]	7.00 [3.75, 14.25]	27.50 [10.50, 30.00]	0.001	nonnorm
Median time on ART at PCP diagnosis [days, IQR]	-16.50 [-34.25, -6.00]	-17.50 [-34.75, -1.00]	-15.50 [-22.50, -10.00]	0.967	nonnorm
Timing of ART initiation category (%)				0.468	
>1 week prior to admission	3 (15.0)	3 (21.4)	0 (0.0)		
Within 1 week of admission	3 (15.0)	2 (14.3)	1 (16.7)		
>1 week post admission	14 (70.0)	9 (64.3)	5 (83.3)		
Died in Hospital (%)	21 (36.2)	13 (32.5)	8 (44.4)	0.562	

Note:
ART = antiretroviral therapy; BC = blood culture; CRP = C-reactive protein; CMVVL = cytomegalovirus viral load in whole blood; HAI = hospital-acquired infection; HIVVL = HIV viral load in whole blood; IQR = interquartile range; LDH = lactate dehydrogenase; Lt-for-age = length-for-age; PCP = Pneumocystis jirovecii pneumonia; SD = standard deviation; TB = tuberculosis; WHO = World Health Organization; Wt-for-age = weight-for-age; Wt-for-Lt = weight-for-length. P-values are derived from tests that compare ventilated to unventilated PCP cases, according to type of variable: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.8: Characteristics of children with a discharge diagnosis of PCP, stratified by in-hospital outcome (survived vs died)

	Survived	Died in-hospital	p	test
n	215	71		
Median age [months, IQR]	3.81 [2.88, 7.75]	3.53 [2.85, 7.57]	0.724	nonnorm
Hypoxic (%)	24 (75.0)	10 (100.0)	0.195	
Median Wt-for-age Z-score [IQR]	-2.48 [-3.58, -1.31]	-2.89 [-3.66, -1.80]	0.187	nonnorm
Median Lt-for-age Z-score [IQR]	-2.15 [-3.39, -0.97]	-2.38 [-3.81, -0.97]	0.641	nonnorm
Median Wt-for-Lt Z-score [IQR]	-0.91 [-2.29, 0.35]	-0.60 [-2.56, 0.22]	0.783	nonnorm
Median Beta-D-glucan [IQR]	385.00 [286.00, 523.00]	240.00 [226.00, 523.00]	0.253	nonnorm
Mean LDH (SD)	1161.88 (564.97)	1319.67 (697.82)	0.648	
Median CRP [IQR]	11.00 [4.00, 35.50]	47.50 [11.75, 86.75]	0.022	nonnorm
Mean CMVVL (log10 DNA copies/mL, SD)	3.75 (1.02)	4.28 (1.11)	0.228	
Definite TB (%)	4 (11.4)	4 (23.5)	0.469	
Clinical TB (%)	11 (31.4)	6 (35.3)	1.000	
WHO Clinical Stage (%)			0.378	
I	2 (6.2)	0 (0.0)		
II	1 (3.1)	0 (0.0)		
III	5 (15.6)	0 (0.0)		
IV	24 (75.0)	10 (100.0)		
Median CD4% [IQR]	13.07 [8.60, 24.13]	11.40 [7.23, 18.61]	0.361	nonnorm
Median CD4# [IQR]	846.50 [535.75, 1472.75]	442.00 [323.00, 1093.00]	0.306	nonnorm
Median HIVVL [log10 RNA copies/mL, IQR]	6.47 [6.07, 6.75]	6.62 [6.50, 6.94]	0.192	nonnorm
Ventilated = Yes (%)	10 (27.0)	8 (38.1)	0.562	
Median length of hospitalization [days, IQR]	16.00 [10.00, 26.00]	8.00 [3.50, 22.00]	<0.001	nonnorm
Median time on ART at PCP diagnosis [days, IQR]	-10.00 [-19.00, 0.00]	129.00 [61.50, 148.00]	0.036	nonnorm
Timing of ART initiation category (%)			0.065	
>1 week prior to admission	18 (17.8)	2 (66.7)		
Within 1 week of admission	24 (23.8)	1 (33.3)		
>1 week post admission	59 (58.4)	0 (0.0)		
PCP confirmed (%)	31 (14.4)	21 (29.6)	0.007	

Note:
ART = antiretroviral therapy; CRP = C-reactive protein; CMVVL = cytomegalovirus viral load in whole blood; HIVVL = HIV viral load in whole blood; IQR = interquartile range; LDH = lactate dehydrogenase; Lt-for-age = length-for-age; PCP = Pneumocystis jirovecii pneumonia; SD = standard deviation; TB = tuberculosis; WHO = World Health Organization; Wt-for-age = weight-for-age; Wt-for-Lt = weight-for-length. P-values are derived from tests that compare the characteristics of children who died to those of PCP cases who survived, according to type of variable: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.9: Characteristics of children with a discharge diagnosis of PCP, stratified by whether or not they were followed up at the outpatient HIV clinic

	Not followed-up at Outpatiet HIV Clinic	Followed up at Outpatiet HIV Clinic	p	test
n	189	125		
Median age [months, IQR]	3.53 [2.82, 7.16]	3.97 [3.00, 8.65]	0.266	nonnorm
Hypoxic (%)	21 (87.5)	13 (72.2)	0.395	
Median Wt-for-age Z-score [IQR]	-2.66 [-3.65, -1.51]	-2.82 [-3.75, -1.53]	0.805	nonnorm
Median Lt-for-age Z-score [IQR]	-2.37 [-3.66, -0.88]	-2.08 [-3.48, -1.01]	0.902	nonnorm
Median Wt-for-Lt Z-score [IQR]	-0.89 [-2.06, 0.34]	-1.22 [-2.69, 0.08]	0.347	nonnorm
Median Beta-D-glucan [IQR]	326.00 [237.00, 523.00]	400.00 [271.50, 560.00]	0.316	nonnorm
Mean LDH (SD)	1254.60 (680.44)	1166.75 (434.16)	0.817	
Median CRP [IQR]	17.00 [6.00, 59.00]	11.00 [4.00, 34.50]	0.148	nonnorm
Mean CMVVL (log10 DNA copies/mL, SD)	4.15 (1.16)	3.64 (0.87)	0.228	
Definite TB (%)	6 (18.2)	2 (10.0)	0.681	
Clinical TB (%)	12 (36.4)	6 (30.0)	0.861	
WHO Clinical Stage (%)			0.518	
I	1 (4.2)	1 (5.6)		
II	0 (0.0)	1 (5.6)		
III	2 (8.3)	3 (16.7)		
IV	21 (87.5)	13 (72.2)		
Median CD4% [IQR]	11.47 [8.55, 22.91]	15.65 [7.81, 22.74]	0.875	nonnorm
Median CD4# [IQR]	808.00 [463.00, 1282.00]	923.00 [454.25, 1548.25]	0.646	nonnorm
Median HIVVL [log10 RNA copies/mL, IQR]	6.49 [6.13, 6.94]	6.54 [6.30, 6.75]	0.927	nonnorm
Ventilated = Yes (%)	12 (32.4)	6 (28.6)	0.992	
Median length of hospitalization [days, IQR]	14.50 [7.00, 23.00]	16.00 [10.00, 27.00]	0.035	nonnorm
Median time on ART at PCP diagnosis [days, IQR]	7.00 [-14.50, 57.00]	-10.00 [-19.25, -1.00]	0.111	nonnorm
Timing of ART initiation category (%)			0.012	
>1 week prior to admission	7 (50.0)	16 (16.0)		
Within 1 week of admission	2 (14.3)	25 (25.0)		
>1 week post admission	5 (35.7)	59 (59.0)		
PCP confirmed (%)	36 (19.0)	16 (12.8)	0.193	

Note:
ART = antiretroviral therapy; CRP = C-reactive protein; CMVVL = cytomegalovirus viral load in whole blood; HIVVL = HIV viral load in whole blood; IQR = interquartile range; LDH = lactate dehydrogenase; Lt-for-age = length-for-age; PCP = Pneumocystis jirovecii pneumonia; SD = standard deviation; TB = tuberculosis; WHO = World Health Organization; Wt-for-age = weight-for-age; Wt-for-Lt = weight-for-length. P-values are derived from tests that compare children that were followed up at the outpatient ART clinic to those that were not followed up at the outpatient ART clinic, according to type of variable: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.10: Outcomes of children that were followed up at the outpatient HIV clinic

	In care	Censored	p	test
n	42	83		
Median age at PCP admission episode [months, IQR]	4.28 [3.08, 11.09]	3.81 [2.92, 8.59]	0.364	nonnorm
CPT stopped (%)	18 (42.9)	41 (49.4)	0.616	
Median age at CPT cessation [months, IQR]	47.87 [34.17, 58.16]	21.55 [9.35, 38.00]	0.001	nonnorm
Median age at last follow-up [months, IQR]	48.29 [21.27, 80.55]	19.32 [9.13, 37.52]	<0.001	nonnorm
Median time of follow-up [months, IQR]	26.11 [11.55, 55.53]	10.85 [3.02, 22.85]	<0.001	nonnorm
Hypoxic (%)	1 (100.0)	12 (70.6)	1.000	
Median Wt-for-age Z-score [IQR]	-2.36 [-3.85, -1.41]	-2.83 [-3.70, -1.68]	0.611	nonnorm
Median Lt-for-age Z-score [IQR]	-2.16 [-3.38, -1.05]	-2.04 [-3.62, -0.97]	0.886	nonnorm
Median Wt-for-Lt Z-score [IQR]	-0.76 [-2.43, 0.05]	-1.48 [-2.69, 0.18]	0.632	nonnorm
Median Beta-D-glucan [IQR]	597.00 [442.00, 800.75]	1713 (n=1)	-	nonnorm
Mean LDH (SD)	984.67 (289.52)	1713.00 (NA)	NA	
Median CRP [IQR]	5.00 [2.00, 14.00]	16.00 [4.00, 57.00]	0.098	nonnorm
Mean CMVVL (log10 DNA copies/mL, SD)	3.40 (0.76)	4.05 (1.01)	0.256	
Definite TB (%)	1 (50.0)	1 (5.6)	0.456	
Clinical TB (%)	0 (0.0)	6 (33.3)	0.871	
WHO Clinical Stage (%)			0.151	
I	0 (0.0)	1 (5.9)		
II	0 (0.0)	1 (5.9)		
III	1 (100.0)	2 (11.8)		
IV	0 (0.0)	13 (76.5)		
Median CD4% [IQR]	11.85 [11.85, 11.85]	17.50 [7.49, 23.02]	0.772	nonnorm
Median CD4# [IQR]	1435.00 [1435.00, 1435.00]	837.00 [442.00, 1586.00]	0.500	nonnorm
Median HIVVL [log10 RNA copies/mL, IQR]	6.56 [6.56, 6.56]	6.52 [6.26, 6.75]	0.923	nonnorm
Ventilated = Yes (%)	1 (50.0)	5 (26.3)	1.000	
Median length of hospitalization [days, IQR]	17.50 [9.25, 31.75]	15.00 [10.50, 26.50]	0.428	nonnorm
Median time on ART at PCP diagnosis [days, IQR]	-10.50 [-19.00, -1.50]	-10.00 [-20.25, -1.00]	0.985	nonnorm
Timing of ART initiation category (%)			0.860	
>1 week prior to admission	5 (17.9)	11 (15.3)		
Within 1 week of admission	6 (21.4)	19 (26.4)		
>1 week post admission	17 (60.7)	42 (58.3)		
PCP confirmed (%)	2 (4.8)	14 (16.9)	0.103	

Note:
ART = antiretroviral therapy; CPT = co-trimoxazole preventive therapy; CRP = C-reactive protein; CMVVL = cytomegalovirus viral load in whole blood; HIVVL = HIV viral load in whole blood; IQR = interquartile range; LDH = lactate dehydrogenase; Lt-for-age = length-for-age; PCP = Pneumocystis jirovecii pneumonia; SD = standard deviation; TB = tuberculosis; WHO = World Health Organization; Wt-for-age = weight-for-age; Wt-for-Lt = weight-for-length. P-values are derived from tests that compare CPT known to have been discontinued to cases in which CPT was not known to have been discontinued, according to type of variable: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

3 RECOMMENDATIONS

This study has shown that *Pneumocystis jirovecii* pneumonia (PCP) is an important opportunistic infection presenting as the first presentation to hospital in previously undiagnosed children living with HIV (CLWH) in the South African context. This is concerning, as these children presenting often have advanced immunosuppression, co-infection with cytomegalovirus (CMV)N and may have a poorer prognosis. There has been a dramatic improvement in mortality associated with HIV infection in infants and children, through provision of early ART and prevention of vertical transmission of HIV through PMTCT programmes in South Africa. However, there are still major gaps in recognising and diagnosing HIV infection in children. This could potentially be due to failure to comply to PMTCT programmes, infrequent screening for HIV at health care facilities or poor health seeking behaviour.

PCP presents as a lower respiratory tract infection with a 100% mortality rate if not appropriately treated.[6] It is important to have a high index of suspicion for the diagnosis of PCP in children who may be at risk of acquiring PCP such as immunocompromised, younger children, HIV exposed children and those who are HIV infected. Children who present with tachypnoea and hypoxia, particularly in the first 6 months of life, should be investigated for PCP.[1,7,17] It is important to have screening measures in place for such children at a hospital level, and clinics should have protocols to refer infants and children at risk of acquiring PCP.

There are various testing modalities available for the diagnosis of PCP such as immunoflourescence and PCR, which can be done on induced sputum, bronchoalveolar lavage, endotracheal aspirates, nasopharyngeal swabs, and lung tissue; however, there is no ideal test for the diagnosis of PCP. PCR testing for PCP is currently the most cost effective test for the diagnosis of PCP, and is available at most healthcare facilities who have access to a laboratory in the South African setting. Immunofluorescent antibody microscopy testing is not recommended as it is time consuming and has low sensitivity.[33] Once children at risk are identified, sputum or nasopharyngeal swabs may be sent for PCR testing, however bronchoalveloar lavage specimens have a greater sensitivity and is the gold standard for

the diagnosis of PCP but it is not feasible to do in every patient with suspected PCP.[21] In this study, the majority of confirmed PCP cases were diagnosed using PCR on respiratory samples (40/52, 76.9%) with the remaining confirmed cases diagnosed on histology of post-mortem lung biopsies. The majority of the confirmed PCP cases were diagnosed between 2011 and 2013 during conduct of the PERCH study.[1] The likely reason of the high proportion of presumed PCP cases in this study could be clinician dependence on ancillary diagnostic modalities, e.g. immunoflourescence, β-D-glucan, and serum LDH, rather than use of PCR for PCP on respiratory specimens.

We also evaluated the use of chemical and virologic biomarkers for the diagnosis of PCP with/without CMV co-infection using LDH, β-D-glucan, CRP and CMV viral load. These modalities did not confer a higher yield when stratifying by certainty of the diagnosis of PCP in our analyses. Our retrospective review of routinely collected data was incomplete, which compromised our ability to fully evaluate these biomarkers in the diagnostic work-up for PCP with/without CMV co-infection. Other studies have shown that LDH and β-D-glucan are useful for the diagnosis of PCP.[13,23]

This study highlighted the burden of CMV and tuberculosis (TB) co-infection in CLWH who have been diagnosed with PCP. It is crucial to investigate and consider treatment for CMV and TB, to optimise management in these children. Children with PCP and CMV co-infection have poorer outcomes.[25]

There is great emphasis on the provision of co-trimoxazole preventive therapy (CPT) for HIV exposed infants and CLWH, as CPT assists in decreasing the risk of acquiring PCP and is associated with lower all-cause mortality.[3] Upon every healthcare visit, children who qualify for CPT should be recognised and placed on CPT to optimise their health and prevent such opportunistic infections. Current guidance incorporates routine testing for HIV infection at post-natal follow up visits and routine vaccination visits throughout the first year of life for South African mothers who tested HIV negative in the antenatal period, and in labour.[34] Children placed on CPT should be followed up regularly to monitor and screen for symptoms associated with PCP. CPT should be discontinued once CD4 percentages are >25% regardless of WHO stage in children 1 to 5 years and for CLWH >5 years who have CD4 counts <200 cells/μL.[26] For children >5 years with WHO stage 2,3

or 4 should be on CPT until immune recovery on ART has been demonstrated.[26]

Children with confirmed and presumed PCP who were ventilated had poorer survival outcomes in this study (mortality rate 44.4%). Data regarding duration of stay in the intensive care unit (ICU), and complications in ICU were not evaluated. A study done at Chris Hani Baragwanath Academic Hospital (CHBAH) in the pre-ART era (1992 to 1993) showed a similar mortality rate (42%).[28] This shows that emphasis must be placed on preventative measures, including PMTCT to decrease the burden of vertically-acquired HIV infection, and CPT, to prevent morbidity and mortality associated with PCP.

It is important to screen for HIV in all children at healthcare visits and to institute prompt initiation onto ART for CLWH, to prevent opportunistic infections such as PCP, TB and CMV. Providing CLWH with ART and CPT are two important measures in preventing PCP and reducing overall burden of disease and mortality.

This study was done over a 9-year period, however there is scope for longer follow up studies to assess the long term outcomes of children with PCP in the South African setting. Further studies could also include comparisons of children with HIV admitted to hospital with pneumonia who do not have PCP and those who have PCP, to add insight into the differential demographic, clinical, laboratory and outcomes measures between CLWH with pneumonia, and those with presumed and confirmed PCP.

APPENDIX 1: Study Protocol

Clinical course and outcomes of confirmed and suspected *Pneumocystis jirovecii* pneumonia in Sowetan children living with HIV

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Supervisor: David Paul Moore

Degree for which study is being conducted: MMed (Paediatrics)

Abstract

Pneumocystis jirovecii pneumonia (PJP) is the most important community-acquired pneumonia in children living with HIV (CLWH) in settings with a high burden of HIV, and often presents as the sentinel diagnosis in infants with HIV. Little is known about the long-term outcomes of children with PJP from high-burdened settings of childhood HIV. In this study, we will describe the long-term outcomes of children diagnosed with confirmed or suspected PJP at Chris Hani Baragwanath Academic Hospital (CHBAH) over a 9-year period (1 January 2011 through 31 December 2019), as well as the diagnostic methodologies that were used to establish a diagnosis of the condition, survival outcomes, and timing of the illness in terms of their HIV diagnosis. As human cytomegalovirus (CMV) is also associated with PJP, we will describe the co-infection rates and outcomes of children with PJP-CMV co-infection. We will

also describe the timing of cessation of co-trimoxazole preventive therapy (CPT) in children with a previous diagnosis of PJP.

Nomenclature

ART	antiretroviral therapy
BDG	beta-D-glucan
CAP	community-acquired pneumonia
CHBAH	Chris Hani Baragwanath Academic Hospital
CLWH	children living with HIV
CMV	cytomegalovirus
CPT	co-trimoxazole preventive treatment
CRP	C-reactive protein
LDH	lactate dehydrogenase
PJP	*Pneumocystis jirovecii* pneumonia
PMTCT	prevention of mother-to-child transmission
PCR	polymerase chain reaction
WHO	World Health Organization

Introduction

Pneumocystis jirovecii is an important opportunistic pathogen in children living with HIV (CLWH), responsible for the majority of community acquired pneumonia (CAP) admissions in infancy in these children.(1) *Pneumocystis jirovecii* pneumonia (PJP) classically presents as a progressive, hypoxic pneumonia with signs of severe respiratory distress and respiratory failure.(2) Mortality rates are high if not appropriately treated. Studies conducted in the late

1990s highlighted the importance of prophylactic therapy, using co-trimoxazole, to prevent PJP and all-cause mortality in CLWH.(3)

With maturation of prevention of mother-to-child transmission (PMTCT) programmes, early diagnosis of HIV infection in children, and expedited access onto antiretroviral therapy (ART) in HIV-infected infants, the rates of PJP associated morbidity and mortality are declining.(4) Data from the Centers for Disease Control and Prevention Paediatric Spectrum of Disease Project (1994–2001) indicated a decline in PJP infection rates (cases per 1000 HIV-infected children) from 25 in 1994 to 18 in 1996, and 6 in 2001 in the United States.(4) Similarly, analyses of data from the Perinatal AIDS Collaborative Transmission Study revealed a 95% decline in PJP (cases per 100 child-years) from 5.8 (pre-ART) to 0.3 (in the ART era).(5) Another study demonstrated that the incidence rate for PJP (cases per 100 child-years) was 1.3 during the pre-ART era (1981–1988) and <0.5 during the ART era (2001–2004).(5) Similar declines in the burden of PJP have not been demonstrated in South Africa, however, as there were delays in implementing the National roll-out of ART to HIV-infected persons (which commenced in 2004). In the Pneumonic Etiology Research for Child Health (PERCH) study, *P. jirovecii* was responsible for 23.0% (95% credible interval, 12.4-31.5%) of CAP admission episodes in CLWH under-5 years of age; these cases clustered almost exclusively in the under-1 year old age group, in which 1 in 3 CAP hospitalisations were associated with *P. jirovecii*.(1)

The diagnosis of PJP is made using a comprehensive approach which should include clinical examination, chest X-ray findings, blood tests and respiratory samples used to detect *P. jirovecii* DNA. There is no current ideal laboratory test for the diagnosis of PJP. The respiratory samples which can be used are induced sputum, bronchoalveolar lavage, endotracheal aspirates, and nasopharyngeal swabs for immunofluorescence antibody testing or

polymerase chain reaction (PCR), and lung tissue for histology. Immunofluorescent antibody microscopy testing was previously used to diagnose PJP, but this has a low sensitivity, requires trained laboratory staff and is time consuming. Blood samples for detection of *P. jirovecii* DNA are not valuable. Lung biopsy has high sensitivity and specificity, but is invasive and is rarely performed in paediatric patients with suspected PJP.(6) Laboratory tests that have been used for the diagnosis of PJP besides respiratory samples do assist in supporting a diagnosis of PJP. ß-D glucan (BDG) is a cell wall component of *P. jirovecii* and other fungi, and is a nonspecific marker for PJP. In a review done by White et al. (7) serum BDG levels had a sensitivity of 90-95% and a specificity of 78-68% for PJP. Serum lactate dehydrogenase (LDH) is elevated in HIV-infected patients with PJP. LDH accuracy for the diagnosis of PJP had a sensitivity of 80-100% and specificity of 6-85%.(7) In a systematic review by Yuan-Ti Lee et al, (8) serum LDH levels >450 U/L were predictive of PJP and normal LDH levels had high negative predictive value in the context of suspected PJP. The average sensitivity and specificity of BDG were 95%-96% and 84%-86%, respectively and, when used in combination, an elevated BDG and LDH with clinical features of PJP improved the diagnostic rate with 92.8% sensitivity and 83.9% specificity.(8)

PCR for *Pneumocystis Jirovecii* has a sensitivity of 95 % in bronchoalveolar lavage specimens. Non-invasive samples, including nasopharyngeal swabs and expectorated sputum, have lower sensitivity in HIV-infected patients.(6) PCR detects more PJP infections compared to immunofluorescence in lower respiratory tract samples.(9) Compared to a “gold standard” measure of a positive *P. jirovecii* PCR on lower respiratory tract specimens, a PCR done on a nasopharyngeal aspirate has a sensitivity of 86%, specificity of 95%, positive predictive value of 96% and negative predictive value of 85%. (9) Nasopharyngeal aspirate samples may be

used for diagnostic purposes when PCR is utilised. PCR is the most cost effective method of establishing a diagnosis of PJP in South African patients.(10)

CLWH are also at risk of developing cytomegalovirus (CMV) pneumonia, often as a co-infecting agent with PJP.(11) Co-infection of CMV with PJP is associated with poorer outcomes, with mortality rates of 42% with CMV infection alone and 50% in those with CMV and PJP.(12)

Current guidelines recommend that HIV-exposed infants older than 6 weeks should receive co-trimoxazole prophylaxis to lower the risk of PJP.(13) This should be continued until they are proven to be HIV negative 6 weeks post cessation of breast feeding. CLWH who are less than 1 year of age should be on co-trimoxazole preventive treatment (CPT). Those between 1 and 5 years who have a CD4% <25 or WHO stage 2,3, or 4 should also be on CPT and it can be discontinued if the CD4% is >25 regardless of WHO staging. It is also recommended that CLWH aged <5 years who have had PJP should continue CPT until 5 years of age and should be discontinued thereafter only if the CD4 criteria in >5 year category are met. CLWH >5 years who have CD4 counts <200 cells/μL or WHO stage 2,3 or 4 should be on co-trimoxazole until the CD4 count is >200 cells/μL. (13)

There have not been many studies that have evaluated the long term outcomes of PJP. Most have described in-hospital mortality rates. A study from Cape Town evaluated case-fatality rates in children hospitalised with pneumonia, demonstrating in-hospital case fatality of 32% in children with PJP compared to 17% in children without PJP.(9) Most of the deaths occurred in CLWH (88.8%), and all of the HIV-infected children that died had CD4 counts <15%. Another study done in Uganda had 16.5% of confirmed PJP cases in children, of which 80% were classified as WHO HIV stage 4, and mortality rates in those with PJP was 40%.(14) The case

fatality rate for untreated PJP in CLWH is 100%.(15) A long term outcome measure by du Plessis et al (16) evaluated recurrence of PJP in HIV-infected children between 2006 and 2010, and demonstrated that 20% of HIV-infected children <5 years of age had recurrent episodes of PJP. Based on these high mortality rates, it is important to appreciate the need for better surveillance and interventions to manage PJP in CLWH.

There is scanty evidence on long-term survival rates in children hospitalised with PJP, especially in settings with a high burden of paediatric HIV. In this study, we aim to evaluate the long-term outcomes of CLWH with confirmed and suspected PJP, to describe the timing of the PJP diagnosis in relation to their HIV diagnosis, and to evaluate at what stage CPT was discontinued in these children.

Aims and objectives

The aim of this study is to evaluate the burden of disease and term outcomes of PJP in CLWH treated at Chris Hani Baragwanath Academic Hospital (CHBAH) from 01 January 2011 to 31 December 2019.

Objectives of the study are to describe:

1. The burden of PJP in children admitted to the general paediatric wards at CHBAH, including what proportion of children present with a recurrent admission diagnosis of PJP;
2. Demographic characteristics of children with PJP;
3. The timing of the PJP diagnosis in relation to the child's HIV diagnosis;
4. Co-morbidities in CLWH with confirmed or suspected PJP, including:
    a. CMV pneumonia
    b. Bacteraemic pneumonia

c. Nosocomial sepsis
d. Malignancy
e. Auto-immune disease

5. The proportion of children with PJP that were admitted to ICU for mechanical ventilation, duration of ICU stay and duration of hospitalisation;
6. The utility of LDH in establishing a diagnosis of PJP in young children hospitalised with severe pneumonia.
7. Outcomes (survived, died, or lost to follow up after hospital discharge) for PJP in CLWH, stratifying by certainty of PJP diagnosis, and co-morbidities;
8. Mortality rates associated with PJP;
9. The time at which CPT was discontinued in children with confirmed or suspected PJP.

Hypothesis

The working hypothesis of this study is that PJP admissions to CHBAH will have declined over time, with maturation of the ART programme, including expedited access onto ART for CLWH. We further hypothesise that most of the PJP diagnoses would have been made at the child's sentinel hospital admission, prior to the diagnosis of HIV.

Methods

This will be a retrospective, descriptive study of all paediatric admissions hospitalised in the general paediatric wards (Wards 17, 18, 19, 33 and 36) at CHBAH from 1 January 2011 through 31 December 2019.

Inclusion Criteria

Criteria for inclusion in the study will be:

1. Discharge diagnosis of PJP (ICD-10 Codes: Pneumocystosis B59 and HIV disease resulting in *P. jirovecii* pneumonia B20.6), or laboratory confirmation of PJP in children treated in wards 17, 18, 19, 33 and 36 at CHBAH during the study time period;
2. Age range birth to <14 years;
3. HIV-infected children.

Case definitions

Certainty of PJP diagnosis will be classified as follows:

1. Confirmed case of PJP: *P. jirovecii* DNA detected on respiratory specimens (nasopharyngeal-oropharyngeal swabs, induced sputum specimens, tracheal aspirates, broncho-alveolar lavage fluid) in children hospitalised with severe pneumonia; or *P. jirovecii* detected using immunofluorescence microscopy on respiratory secretions; or *P. jirovecii* cysts observed on post-mortem lung biopsy in children dying from pneumonia;
2. Presumed cases of PJP: no molecular, immunofluorescence microscopy or histologic evidence of PJP, but discharge/outcome diagnosis of the condition.

A diagnosis of CMV co-infection will be ascribed in children with CMV viraemia detected on blood using quantitative or qualitative PCR, during the PJP admission episode.

A diagnosis of bacteraemic pneumonia will be assigned in children who have blood cultures positive for clinically significant isolates during the course of their PJP admission episode. Contaminant organisms, including coagulase negative staphylococcus, Micrococcus spp, Corynebacterium spp and Bacillus spp, will not be included as a cause of bacteraemia in this study.

Data sources

The Paediatric Admissions Database for all paediatric admissions to the general wards (wards 17, 18, 19, 33 and 36) at CHBAH will be used to identify all PJP cases, with permission from the Head of Department (Professor Sithembiso Velaphi) and the Director of the Vaccine and Infectious Diseases Analytics (VIDA) Unit (Professor Shabir Madhi). The PERCH database will be used, with permission, to identify children that had a confirmed diagnosis of PJP during the course of the PERCH Study (which enrolled children from 17 August 2011 through 30 September 2013). The NHLS database will be used to identify children with confirmed PJP (through immunofluorescence microscopy, PJP PCR, BDG, LDH testing and histology), through application to the NHLS Ethics Committee. In the subset of children that were discharged and followed up at Harriet Shezi Children's ART Clinic, a large paediatric ART Clinic that caters for the outpatient care of children with a diagnosis of HIV, the following long-term outcomes will be explored:

1. Timing of initiation of ART (by comparison of date of PJP admission to date of ART initiation);
2. Timing of cessation of CPT (by comparison of date of PJP admission to date of discontinuation of CPT);
3. Time to transfer out/ loss to follow-up/ death (by comparison of date of PJP admission to date of outcome).

Harriet Shezi Clinic data will be extracted, with permission, from the electronic database that is maintained by the clinic.

Data collection

The Data Collection Tool is shown in Appendix 1. Variables to be collected will include:

1. Age at presentation;
2. Sex;
3. Nutritional status (calculated from weight (kilograms), height (centimetres) and mid upper arm circumference (millimetres)) based on the World Health Organization (WHO) Growth Standards;
4. Duration of hospital stay (date of outcome minus date of admission);
5. Basis of PJP diagnosis:
    a. Microbiology: PJP PCR positive, PJP immunofluorescence positive;
    b. Histology: *P. jirovecii* cysts seen on post-mortem lung biopsy;
    c. Biochemistry: BDG suggestive of fungal infection, serum LDH level.
6. Co-morbidities;
7. Blood investigations: CMV viral load, LDH, BDG and C-reactive protein (CRP);
8. CD4 count and HIV viral load on admission;
9. Duration of ART before admission;
10. Complications during admission secondary to PJP;
11. Outcome (discharge/ death/ transfer out/ lost to follow-up);
12. Date of cessation of CPT, with CD4 and HIV viral load results at that visit, or in the outpatient visit preceding the date of CPT cessation;
13. Date of CD4 and HIV viral load at or prior to the date of CPT cessation;
14. Censor dates for outcome measures:
    a. Date of in-hospital death if died during the sentinel PJP episode;
    b. Date of loss-to-follow up at Harriet Shezi Children's Clinic;
    c. Date of death during outpatient follow-up;

d. Last censor date will be 31 December 2019 (children found to still be attending at Harriet Shezi Children's Clinic at that time period will be censored at that time point).

Analysis plan

All data analysis will be conducted using R version 4.0.4 (17) The analysis will be directed at exploring each of the study objectives, as follows:

1. The burden of PJP in children admitted to the general paediatric wards at CHBAH:
    a. The number of children with a diagnosis of confirmed or suspected PJP will be analysed, using the denominator of all paediatric admissions to the hospital. The admission rates for PJP will be calculated per month, and then pooled by year for 2011 through 2019.
    b. Hospitalisation rates will be expressed as "proportion of PJP per hospital admission" over the study period, as well as in age-bands (<6 weeks; 6 weeks to 6 months; 6 months to 12 months; 12 to 59 months; 60 to 168 months).
2. Demographic characteristics of children with PJP:
    a. The median age, sex and nutritional status of the children will be analysed pooled by study year.
3. The timing of the PJP diagnosis in relation to the child's HIV diagnosis:
    a. The proportion of children diagnosed with PJP as a sentinel presentation of HIV will be evaluated.
4. Co-infection rates with CMV and bacteraemic pneumonia in children with confirmed or suspected PJP:

a. The proportion of children with a discharge diagnosis of PJP that had a CMV test done will be evaluated. This will be evaluated in time bands over the study period to see if the proportion of children with PJP that were tested for CMV declined, stayed the same, or increased over time.
b. The proportion of children diagnosed with PJP that had bacteraemic pneumonia will also be established.

5. The proportion of children with PJP that were admitted to ICU for mechanical ventilation, duration of ICU stay and duration of hospitalisation:
    a. The proportion of children with a discharge diagnosis of PJP that were admitted to ICU or ventilated during the hospital admission will be evaluated. This will be evaluated in time bands over the study period to see if the proportion of children with PJP that were managed in intensive care settings decreased, stayed the same, or increased over time.
6. The utility of LDH as a non-specific biomarker for PJP will be evaluated by comparison of the median LDH levels between children with confirmed PJP and those with presumed PJP.
7. Outcomes for PJP in CLWH, stratifying by certainty of PJP diagnosis, and co-morbidities:
    a. Cox proportional hazards models will be constructed to evaluate the outcomes of PJP over time, based on the certainty of PJP diagnosis and the absence or presence of co-morbidities.
8. Mortality rates associated with PJP:
    a. The case fatality rates of PJP will be assessed over time during the study period. This will be evaluated in time bands over the study period to see if the

proportion of children with PJP that died decreased, stayed the same, or increased over time.

9. Outcomes of children diagnosed with PJP:
    a. Harriet Shezi outpatient follow-up data will be used to assess the outcomes of children that survived to hospital discharge. Cox proportional hazards models will be constructed to evaluate differences in outcome, based on the certainty of PJP diagnosis and the presence of co-morbidities.
10. The time at which CPT was discontinued in children with confirmed or suspected PJP:
    a. The proportion of children with confirmed or suspected PJP whose CPT was discontinued at outpatient follow-up will be evaluated through analysis of the Harriet Shezi Clinic database. The timing of cessation of CPT will be assessed, as will be the CD4 counts and HIV viral loads at the point of CPT discontinuation.

Linkage between data sources

The starting point for the study will be to identify which children fulfil the eligibility criteria in the CHBAH Paediatric Electronic Database. Once the identifying criteria of these children have been obtained, they will be linked to the NHLS database in order to determine the laboratory data on record for the child's admission episode: PJP PCR or PJP immunofluorescence, CD4 and HIV viral load, CMV viral load and LDH (if done). Blood culture results and serum CRP levels will also be obtained for the PJP admission episode, to determine whether bacterial co-infection was important in children that were diagnosed with PJP.

The PERCH database includes approximately 40 children with confirmed PJP, with the diagnosis based on positive PCR of upper respiratory tract and lower respiratory tract specimens.

Sample Size

The study is designed to describe as accurately as possible all children hospitalised with PJP at CHBAH during the study period, and thus relies on a convenience sample of children based on their discharge diagnoses. It is anticipated that as access to ART improved from 2013 through 2019, fewer HIV-positive infants would have developed PJP. The estimated number of children with PJP each year during the study period is summarised in Table 1.

Year	Estimated number of PJP cases
2011	20
2012	20
2013	15
2014	15
2015	10
2016	10
2017	10
2018	10
2019	10
Total	120

Collaborator from the NHLS

Dr Jeannette Wadula, the Head of Clinical Microbiology at the CHBAH NHLS, has been identified as the NHLS representative to collaborate on this study.

Collaborator from Harriet Shezi

Dr Nosisa Sipambo has been identified as the representative from Harriet Shezi and will be co-author.

Ethics

This is a retrospective study and the data that will be used from established electronic databases, through existing channels of permission seeking. We request a waiver of informed consent in view of the retrospective nature of the study. Permission for the study will be obtained from the Head of Department of Paediatrics (Professor Velaphi), VIDA (Prof Madhi), Harriet Shezi (Dr Sipambo) and the Hospital CEO (through application to the CHBAH Medical Advisory Committee). Permissions to access laboratory results will be made through the NHLS Ethics Committee.

Anticipated outcomes

From the literature review, the mortality rates from PJP in CLWH in developing countries such as South Africa is still high. It is anticipated that this study will show a declining proportion of PJP cases among children hospitalised at CHBAH over time, which may be attributed to the change in strategy whereby a diagnosis of HIV was made programmatically through use of a birth HIV PCR from 2015 onwards. Despite an anticipated decline in the proportion of hospitalisations with PJP, we anticipate that there will be similar rates of mortality associated with PJP over time.

Anticipated limitations to the study

This is a retrospective study, thus the data that is being used to make comparisons will be obtained from electronic databases maintained at the Facility. Retrieval of clinical notes is very difficult, and it is anticipated that information relating to the details of management of each cases will not be obtainable. Dependence on retrospective data may impact on the analysis, as there may be an element of recall bias or misclassification bias in terms of severity

of presentation. Patients admitted to CHBAH are managed by different clinicians in their respective units, thus patient management may differ. There may be patients lost to follow up which may not allow for complete data analysis.

Study Timelines

	2020						2021										
Milestones	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
Literature review																	
Protocol preparation																	
Protocol assessment																	
Ethics approval																	
Post graduate approval																	
Data collection																	
Data analysis																	
Writing up research report																	
Supervisor review																	
Writing up publication and examination																	

Funding Statement

Costs to access the databases, printing and photocopying for the study will be funded by the candidate. The estimated cost will be R2500.

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Appendix

Data collection tool

Patient demographics at PJP admission episode				
Age (in months) at PJP admission episode				
Age	<6 weeks	6 weeks – 6 months	6 months – 59 months	5 years to 14 years
Gender	Male		Female	
Date of ART initiation				
Date of PJP admission				
Date of discharge				

Nutritional Status at PJP admission episode			
Weight for age (SD used to classify nutritional status as per WHO)	+ 2 to -2 SD	-2 to -3 SD	-less than -3 SD
Height for age	+ 2 to -2 SD	-2 to -3 SD	-less than -3 SD
Weight for height	+ 2 to -2 SD	-2 to -3 SD	-less than -3 SD
Mid upper arm circumference	>13.5cm	13.5cm to 11.5cm	<11.5cm
Presence of oedema	Yes	No	

Comorbidities		
CMV	Yes	No
TB	Yes	No
Malignancy	Yes	No
Autoimmune disease	Yes	No
Other: _____	Yes	

Laboratory investigations during PJP admission episode		
LDH	Date of test: _____	
BDG	Date of test: _____	
CRP	Date of test: _____	
Blood culture 1	Date of test: _____	
Blood culture 2	Date of test: _____	
Blood culture 3	Date of test: _____	
Blood culture 4	Date of test: _____	
Blood culture 5	Date of test: _____	

CD4 count	Date of test: _____	
CD4 %	Date of test: _____	
HIV VL	Date of test: _____	
CMV VL	Date of test: _____	

PJP diagnosis		
	PCR	Immunofluorescence
Sputum		
Nasopharyngeal swab		
Tracheal aspirates		
Other respiratory specimen: _____		
Histology from lung biopsy		
PJP Classification	Definite	Presumed

Outcomes		
Required ventilation	Yes	If yes, duration of ventilation:_____ (days)
Complications during treatment	Yes	No
If yes, describe the complication:		

Date of outcome		
Discharged	Yes	No
Death	Yes	No
Lost to follow up	Yes	No
Date of cessation of CPT		
CD4% prior to cessation of CPT		Date of test:_____
CD4 count prior to cessation of CPT		Date of test:_____
Censoring date	a. Date of in-hospital death if died during the sentinel PJP episode; b. Date of loss-to-follow up at Harriet Shezi Children's Clinic; c. Date of death during outpatient follow-up; d. Last censor date will be 31 December 2019 (children found to still be attending at Harriet Shezi Children's Clinic at that time period will be censored at that time point).	Date:_____

APPENDIX 2: Ethics Approval Certificates

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr M Darji

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210224

NAME: (Principal Investigator)	Dr M Darji
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Medical School University
PROJECT TITLE:	*Clinical course and outcomes of confirmed and suspected Pneumocystis jirovecii pneumonia in Sowetan children living with Human Immunodeficiency Virus*
DATE CONSIDERED:	2021/02/26
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor D Moore
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/04/09

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and therefore reports and re-certification will be due in the month of **February** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

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21 June 2021

Applicant: Mohiniben Darji
Institution: University of the Witwatersrand
Department: Paediatric
Email: A00029812@wits.ac.za
Tel: 011 854 6598 **Cell:** 083 230 3453

CC: David Moore – Paediatric Infectious Diseases

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"Clinical course and outcomes of confirmed and suspected Pneumocystis jirovecii pneumonia in Sowetan children living with HIV, Ref No: PR2010473"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

APPENDIX 3: Turnitin Report

Clinical course and outcomes of confirmed and suspected Pneumocystis jirovecii pneumonia in Sowetan children living with HIV

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