Pneumonia in HIV-infected children admitted to hospital in Cape Town, South Africa

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Abstract

Background: There is little information on the aetiology and outcome of HIV-associated pneumonia in African children and no comprehensive data from South Africa. Studies of HIV-infected adult in Africa reported that the spectrum of pulmonary disease differs from that of developed countries with tuberculosis and pyogenic pneumonia predominating and *Pneumocystis carinii* pneumonia (PCP) occurring uncommonly. Knowledge of the aetiology and outcome of pneumonia is important for the development of paediatric management guidelines and of policies for allocation of resources especially in South Africa, where the HIV pandemic has resulted in increasing numbers of HIV-positive children requiring admission to hospital or intensive care units for pneumonia. Furthermore in countries with limited resources, development of cost effective diagnostic procedures to investigate the aetiology of pneumonia is necessary.

Objective: To investigate the aetiology, associated features and outcome of pneumonia in HIV-infected children and to determine whether sputum induction and nasopharyngeal aspirates are useful diagnostic investigations in children with pneumonia.

Design: Prospective, descriptive study

Methods: Children hospitalised for pneumonia who were known or suspected of having HIV infection or who required intensive care (ICU) support were enrolled in a 1 year study in Cape Town, South Africa. History, examination, chest radiology and blood tests (including bacterial culture and HIV testing) were performed. Induced sputum or bronchoalveolar lavage (BAL) and a nasopharyngeal aspirate (NPA) were obtained for culture and *P carinii* detection. Gastric lavages (GL) were done for *M tuberculosis* culture.

Results: Of the 250 patients [42.8% female, median age 6 (3-16) months], 151 [60.4%; 95% confidence interval (CI) = 54.2 - 66.3] were HIV-infected; 64 of these (42.4%; 95% CI = 34.7 - 50.4) were diagnosed HIV-positive on admission. Of the 76 children admitted to ICU, 36 (47%) received intermittent positive pressure ventilation. Presenting respiratory signs and chest radiology changes were similar in HIV-positive and negative children. PCP, occurring in 19 (7.6%) children (15 HIV-positive), was the AIDS-defining infection in 20.3%; 95% CI = 11.8 - 31.5. PCP was diagnosed using induced sputum (12 children) or BAL (7 children); all corresponding NPAs were negative for *P carinii*. The incidence of tuberculosis (8%) did not differ by HIV status; the yield for *M tuberculosis* from sputum induction (11%) was higher than that from gastric lavage (6%), p=0.08. HIV-positive and negative patients had a similar incidence of bacteraemia (13.5% versus 15.6%, p=0.65); *S pneumoniae* (5%) and *S aureus* (2%) were the predominant isolates. Bacteria were cultured from 53% BAL specimens, 61% induced sputa specimens and 71% nasopharyngeal aspirates. Bacterial prevalence, organism type and antimicrobial resistance were similar in HIV-positive and negative patients except for *S aureus* which was more commonly cultured from sputa and nasopharyngeal aspirates of HIV-infected children. Thirty-nine children (15.6%, 95% CI = 11.5 – 20.5) died in-hospital; the mortality rate was higher in HIV-positive [31 of 151 (20.5%), 95% CI = 14.7 – 27.5] compared with seronegative children [8 of 99 (8.1%), 95% CI = 3.8 - 14.8; RR 1.16, 95% CI = 1.05 - 1.28, p=0.008). Eight of 19 (42%, 95% CI = 20.3 - 66.5) children with PCP died compared to 31 of 231 (13.4%, 95% CI = 9.3 - 18.5) without PCP [RR=1.5 (1.02-2.2), p<0.005]. All HIV-negative children died in ICU in contrast to 6 of 31 (19.4%) HIV-positive patients (p<0.001). Using multiple logistic regression, PCP was the only significant risk factor for mortality in HIV-positive children (p=0.03).

Conclusion: A quarter of children hospitalised for pneumonia were diagnosed with HIV infection at the time of admission. Amongst these children, PCP was an important AIDS defining illness and was associated with a high mortality. In children hospitalised for acute pneumonia, bacteria were a common cause of this infection; the incidence and type of bacteria were similar in HIV-positive and seronegative children. Sputum induction could be safely and effectively performed in infants and young children and provided a satisfactory and more convenient specimen for bacteriologic confirmation of pulmonary tuberculosis than did gastric lavage. Nasopharyngeal aspirates were not useful for diagnosis of PCP but bacterial culture provided important information about the type and antimicrobial susceptibility of the bacterial pathogens prevalent in the community.

HIV-infected children with pneumonia had a higher mortality rate than HIV-negative patients which could be partly attributed to the high mortality associated with PCP. However, allocation of resources may also impact on survival as the majority of HIV-positive children who died did not have the opportunity of ICU care. Development of policy guidelines regarding the admission of HIV-infected children to health facilities including intensive care units is needed. Early identification of HIV-infected infants is important for use of chemoprophylaxis to prevent PCP. Guidelines for management of HIV-associated pneumonia in children in South Africa should include use of induced sputum as a diagnostic investigation, treatment with broad-spectrum antibiotics in patients and therapy for PCP in infants with suspected HIV.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
AFB	Acid-fast bacilli
HIV	Human immunodeficiency virus
BAL	Bronchoalveolar lavage
CMV	Cytomegalovirus
CI	Confidence interval
ELISA	Enzyme linked immunosorbent assay
FBC	Full blood count
FIO_2	Fraction of inspired oxygen
GL	Gastric lavage
HAART	Highly active anti-retroviral therapy
ICU	Intensive care unit
IF	Immunofluorescence
IPPV	Intermittent positive pressure ventilation
LIP	Lymphocytic interstitial pneumonia
LDH	Lactate dehydrogenase
LRTI	Lower respiratory tract infection
MDI	Metered dose inhaler
MIC	Minimum inhibitory concentration
NPA	Nasopharyngeal aspirate
OR	Odds ratio
$PaCO_2$	Partial pressure arterial carbon-dioxide
PaO_2	Partial pressure arterial oxygen
PCP	*Pneumocystis carinii* pneumonia

PCR	Polymerase chain reaction
PEEP	Positive end-expiratory pressure
PIP	Peak inspiratory pressure
RR	Relative risk
RSV	Respiratory syncytial virus
TB	Tuberculosis
TMP-SMX	Trimethoprim-sulphamethoxazole
WBC	White blood cell count
WHO	World Health Organisation
ZDV	Zidovudine

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study. It is my sincere hope that the results will be useful to those involved in the care of HIV-infected children and may contribute to better care.

CHAPTER 1: BACKGROUND

1.1 Pneumonia in HIV-infected children

Acute lower respiratory tract infection, particularly pneumonia is responsible for approximately 4 million deaths annually amongst children under 5 years of age (Garenne et al 1992, WHO 1993). Most of these deaths occur in infants in developing countries (WHO 1993). The magnitude of this problem has been exacerbated by the human immunodeficiency virus (HIV) epidemic in these countries as pulmonary disease, principally pneumonia is the most common cause of morbidity and mortality in children infected with HIV (Marolda et al 1991, McSherry 1996).

Approximately 90% of children with AIDS will develop respiratory problems sometime during their illness; of these, pneumonia is the major cause of hospitalisation and mortality (Bye 1995). A primary respiratory problem is frequently the first sign of HIV infection in children (Hauger 1991, Bye 1995). *Pneumocystis carinii* pneumonia (PCP) was the most common opportunistic infection in children with AIDS in developed countries prior to the introduction of chemoprophylaxis and anti-retroviral therapy, occurring in up to 65% of children diagnosed with AIDS within the first year of life (Sanders-Laufer et al 1991, Connor et al 1991).

In Africa, respiratory infections have also been reported as the dominant clinical syndromes in hospitalised HIV-infected children. A prospective study of 4480 paediatric admissions in Abidjan, Cote d"Ivoire reported that pneumonia was the most important cause of hospitalisation, accounting for 26% of admissions amongst HIV-infected patients compared to 19% in seronegative children (Vetter et al 1996). Pneumonia was also the most common reason for admission at a regional urban

hospital in South Africa during 1992 to 1997, occurring in 37% of children, 58% of whom were HIV-infected (Zwi et al 1999). A study in rural Zambia reported that 11% of children admitted for pneumonia were HIV-infected (Smyth et al 1997) while an urban Zambian study of hospital admissions in Lusaka found that 28% of children with pneumonia were HIV positive (Chintu et al 1995). Urban-rural differences in HIV prevalence probably reflect the higher rate of HIV seroprevalence in adults in urban areas of Zambia (Hira et al 1989). A Zimbabwean study found clinical or serological evidence of HIV in 31% of children with pneumonia (Nathoo et al 1993). As a result of the high incidence of respiratory infections in HIV-infected children, the World Health Organisation (WHO) has suggested that severe or recurrent pulmonary infection be recognised as a major criterion for the clinical case definition of acquired immunodeficiency syndrome (AIDS) (Weiger et al 1992, Buehler and De Cock 1993).

The propensity for HIV-positive children to develop pneumonia results from immunosuppression of the lung host defence mechanisms as a result of HIV infection. The lung is an important site of HIV infection through direct infection of CD4 lymphocytes in the parenchyma and alveolar macrophages (Lipman et al 1997, Wallace 1998). HIV within the lung may elicit a local immune response from infected lymphocytes and macrophages resulting in infection of CD8 cells (Agostini and Semenzato 1996). Impaired cytotoxic activity and reduced production of cytokines by lymphocytes and macrophages may favour the development of pulmonary infections. Furthermore, the lungs serve as the portal of entry into the body of many infectious agents and thus may be the initial site for establishment of infection (Murray and Mills 1990).

1.2 The HIV epidemic in Africa and South Africa

The developing world now bears the major caseload of HIV-infected people (Gilks 1993). Global estimates of the magnitude of the HIV/AIDS epidemic at the end of 1999, reported that 24.5 million infected adults and children live in sub-Saharan Africa, representing over two-thirds of the 34.3 million HIV-positive people in the world (UNAIDS 2000). Moreover, almost 83% of the world's AIDS deaths have been in this region (UNAIDS 2000). Four out of five HIV-positive women and an estimated 90% of HIV-infected children live in Africa. UNAIDS estimates that 1.2 million children were HIV-infected at the end of 1999 and an additional 3.6 million had died (UNAIDS 1999). The high number of African HIV-infected children may be due to a number of factors. More women of childbearing age are HIV-infected in Africa than elsewhere and African women have more children on average than those in other continents, so one infected woman may pass the virus on to a higher than average number of children. HIV prevalence rates among women in their teens and early twenties in various urban and rural areas of Africa are extremely high (fig 1.1). In some areas of South Africa such as the town of Carletonville, almost 6 out of 10 women are HIV-positive. Furthermore, the majority of children in Africa are breastfed, which may account for a substantial proportion of all HIV transmission from mother to child (Newell 1998). Finally, anti-retroviral drugs which reduce the risk of HIV transmission from mother to child before and around childbirth are less readily available or affordable in Africa compared to developed countries (Mofenson and McIntyre 2000).

In 1999, the most severe HIV epidemics in the world were in the southern countries of Africa including South Africa (MAP 2000). Sharp rises in infection rates have been

reported in high and relatively low-prevalence areas of South Africa over the past 4 years (figure 1.2). Infection rates increased from less than 1% in the adult population at the beginning of the 1990s to approximately 12.9% in 1997 and 20% by 1999 (UNAIDS 2000, MAP 2000). With a total of 4.2 million infected people, South Africa has the largest number of people with HIV/AIDS in the world (UNAIDS 2000). South Africa is also experiencing an influx of migrant people from other parts of Africa, particularly neighbouring countries in southern Africa. Many of these countries have even higher rates of HIV infection for example in neighbouring Botswana, 35.8% of adults are HIV-positive (UNAIDS 2000). In Zimbabwe, one in four adults in 1997 were presumed infected; 32% of pregnant women were infected in Harare in 1995 (UNAIDS/WHO 1999). In one town near the South African border with a large population of migrant workers, 7 out of 10 women attending antenatal clinics tested HIV-positive in 1995.

Thus South Africa is experiencing an HIV pandemic. As the mother to child HIV transmission rate is approximately 30%, the number of HIV-infected infants in South Africa is increasing rapidly. As the government has not promoted anti-retroviral therapy for pregnant women (Makgoba 2000), this rate is unlikely to decrease in the near future. The epidemic has had a major impact on health service utilisation with approximately 20% of paediatric admissions currently HIV-related and increasing numbers of HIV-infected children requiring admission to hospital and ICU (Zwi et al 1999, Jeena et al 1999). As a result, large numbers of HIV-infected children can be expected to require primary and hospital care for pneumonia and related complications.

Figure 1.1: HIV prevalence rates among women in their teens and early twenties, various African studies, 1995-1998 *(source: UNAIDS 2000)*

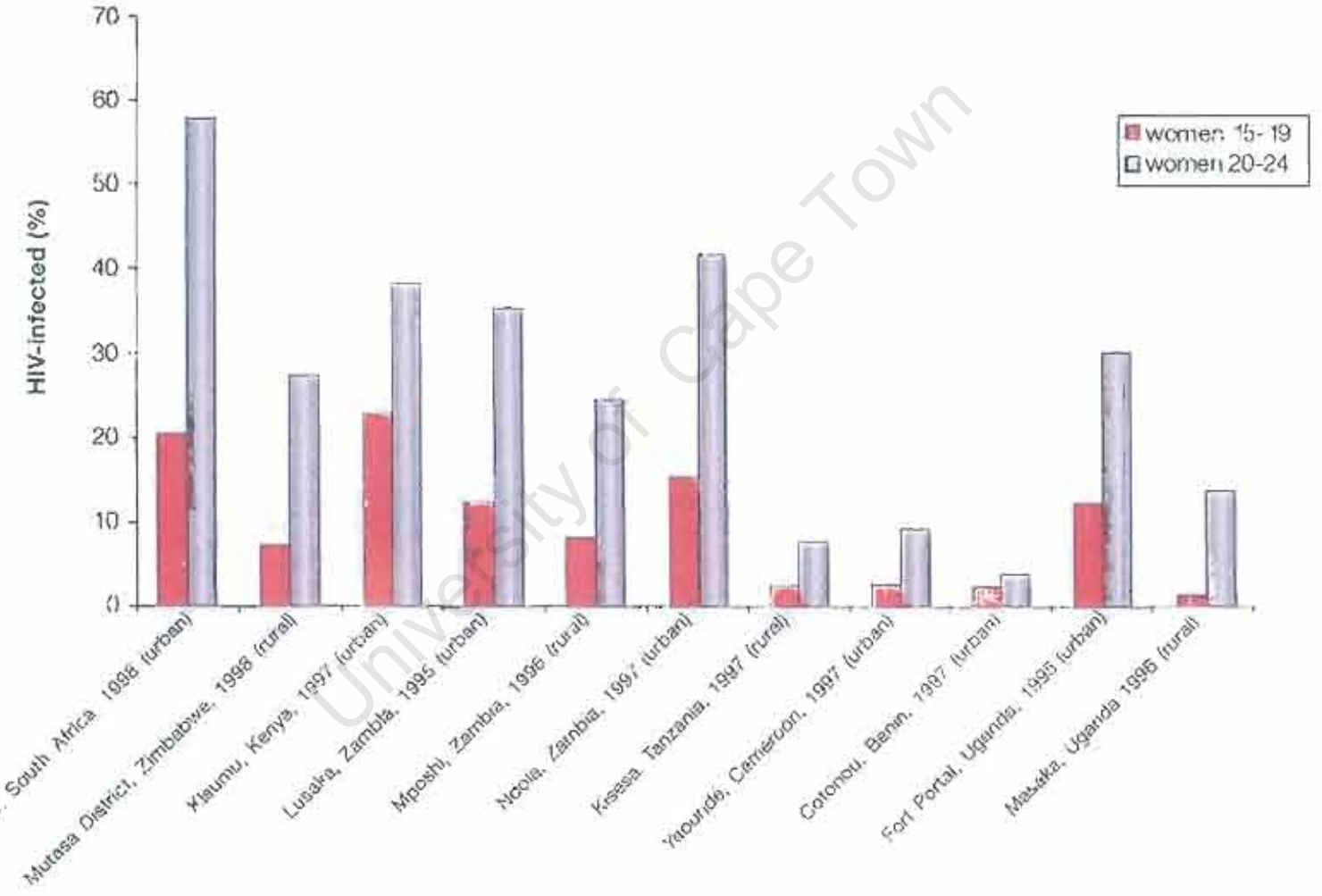
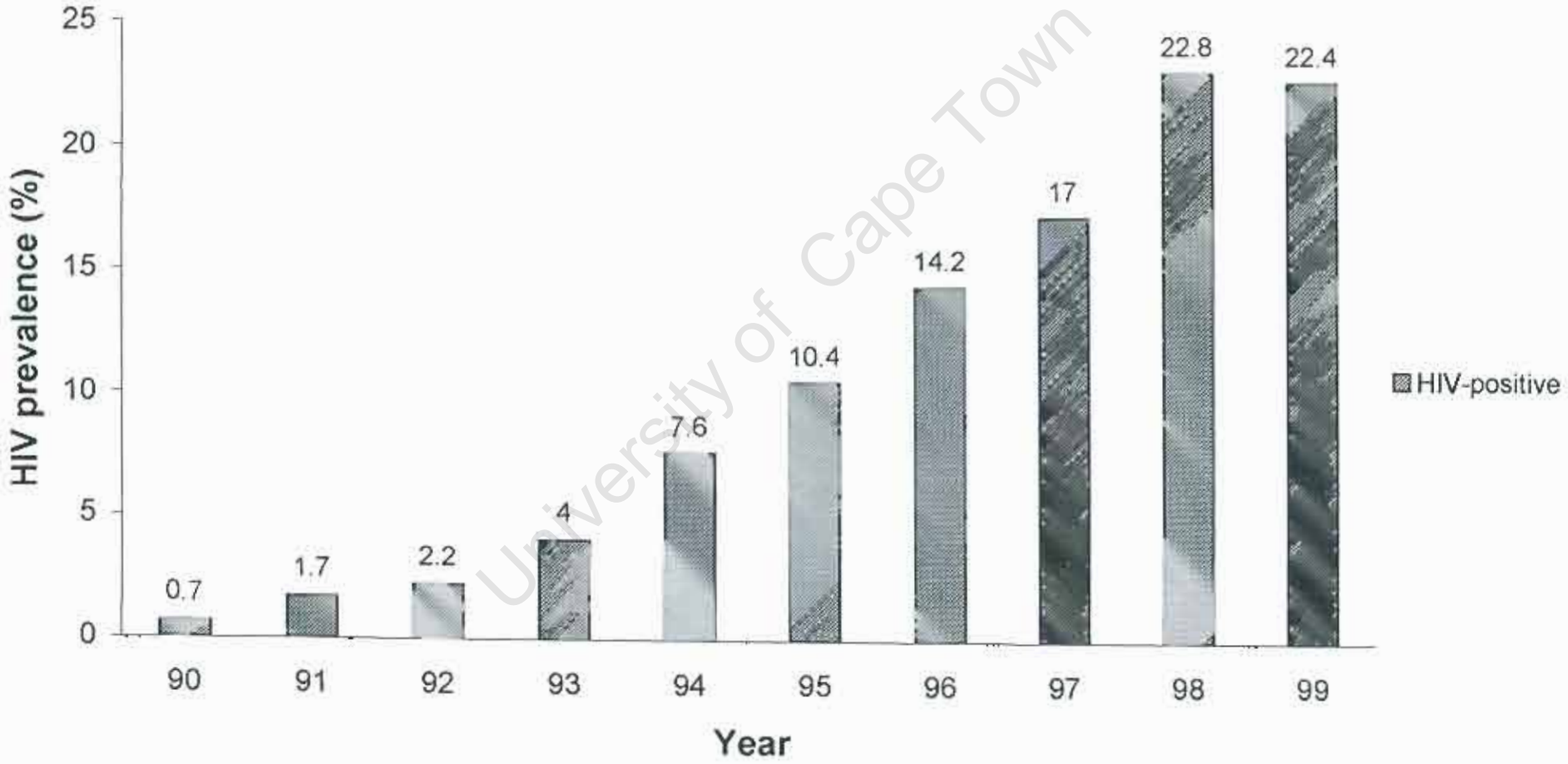


Fig 1.2: HIV prevalence in antenatal attendees, South Africa 1990-1999



1.3 Aetiology of pneumonia in HIV-infected patients in Africa

Bacterial and viral agents have been reported to be the predominant causes of pneumonia in children in developing countries including Africa (Shann 1986, Berman 1991). In contrast to most viral infections, which are usually self limited illnesses, bacteria are responsible for hospitalisation or death of a major proportion of children with pneumonia (Berman 1991). The most reliable aetiologic data on childhood pneumonia in developing countries is from studies that report on bacterial isolates from lung aspirates obtained from children hospitalised with pneumonia who had not received antibiotics (Abdel-Khalik et al 1938, Berman 1991, Escobar et al 1976, Diakparomre and Obi 1981, Silverman et al 1977, Shann et al 1984, Wall et al 1986). In these studies bacteria were isolated in 62% of aspirates; *S pneumoniae, H influenzae* and *S aureus* were the most frequently identified pathogens. The overall isolation rates for *S pneumoniae* and *H influenzae* were similar (27%) while *S aureus* occurred in 17%. In almost all studies *S pneumoniae* was the most frequently identified organism occurring in more than 30% of children in 60% of the studies. Viral infections have been identified in 30-40% of cases of acute respiratory infections in hospitalised children; amongst viral pathogens, respiratory syncytial virus (RSV) predominated accounting for 15-20% of such infections (Hazlett et al 1988, Sobeslavsky et al 1977). Bacteria, occurring in up to two thirds of viral pneumonias, are frequently cultured concurrently with viruses (Shann et al 1984).

There is little information on the aetiology of pneumonia in African HIV-infected children. Studies of African adult HIV positive patients suggest that the spectrum of pulmonary disease differs from that in developed nations in that bacterial pathogens

and tuberculosis predominate while PCP occurs relatively rarely (Abouya et al 1992, Atzori et al 1993, Kamanafu et al 1993, Batugwanayo et al 1994). The relative contribution of *Pneumocystis* and other pathogens such as *M. tuberculosis* or pyogenic bacteria to lower respiratory tract infection (LRTI) in HIV infected children in Africa has not been well studied. Pneumonia in HIV-infected children may be caused by a variety of pathogens (table 1.1); a detailed discussion of the evidence for these in African HIV-infected children follows.

Table 1.1: Pulmonary infections in HIV-infected children

Bacterial

- *S pneumoniae*
- *H influenzae*
- Staphylococcal species
- *M tuberculosis*
- *M avium intracellulare*
- *K pneumoniae*
- *P aeruginosa*
- Legionella species

Viral

- Respiratory syncytial virus (RSV)
- Adenovirus
- Parainfluenza
- Influenza A and B
- Measles virus
- Cytomegalovirus (CMV)
- Herpes simplex virus (HSV)
- Varicella zoster virus (VZV)
- Ebstein Barr virus (EBV)

Fungal

- *Candida* species
- Aspergillus species
- *C immitis*
- *C neoformans*
- *H capsulatum*

Other

- *P carinii*

1.3.1 PCP

Pneumocystis carinii pneumonia (PCP) was one of the major causes of hospitalisation and death in HIV-infected patients in developed countries prior to the introduction of chemoprophylaxis and anti-retroviral therapy (Murray and Mills 1990). In contrast, PCP has been considered to be rare in Africa (Lucas et al 1989, Elvin et al 1989, Carme et al 1991, Abouya et al 1992, Machiels and Urban 1992, Atzori et al 1993, Kamanfu et al 1993, Batungwanayo et al 1994). Diagnosis and treatment of PCP in African patients has therefore not been included as a priority in many empirical management algorithms. Moreover, some African studies have suggested that use of chemoprophylaxis for PCP is not cost effective in these geographic areas (Abouya et al 1992, Batungwanayo et al 1994). Such recommendations also negate the efficacy of trimethoprim-sulphamethoxazole (TMP-SMX) prophylaxis for reduction of other infections and for improving survival amongst HIV-infected people (Wiktor et al 1999). Since untreated PCP is lethal and since effective, inexpensive treatment and preventive strategies are available, accurate knowledge of the incidence of PCP in HIV-infected patients in Africa has important implications (Hughes 1991, Russian and Kovacs 1995). Furthermore, distinguishing PCP from other respiratory illnesses such as tuberculosis is desirable, as treatment of the former with steroids may worsen underlying tuberculosis.

Epidemiologic and serologic evidence for PCP in Africa

P carinii is a ubiquitous organism found throughout the world; infection is probably acquired via the airborne route. Serologic studies indicate that the majority of immunocompetent children acquire antibodies to *P carinii* early in life; 75% of children

in the United States and Europe have acquired antibodies by 4 years of age (Pifer et al 1978, Meuwissen et al 1977). Serologic studies from Africa indicate that infection with *P carinii* is also common (Wakefield et al 1990, Smulian et al 1993). A comparative study on sera from healthy Gambian and British individuals showed similar rising titers of antibody to pneumocystis in both populations from six months to a peak at ten years of age by which time 80% of subjects had sero-converted (Wakefield et al 1990). A serologic study of the frequency of anti-*P carinii* antibodies in immunocompetent and healthy HIV-infected adults from 5 different geographic regions [USA (Cincinnati), Haiti, Mexico, Korea and Africa (South Africa, Kenya and Zaire)] found an overall prevalence of anti-*P carinii* antibodies of 73% (Smulian et al 1993). Of relevance, the highest seroprevalence occurred in Africa (83%). Amongst HIV-negative subjects, the rate of seropositivity was similar in all regions. However, different patterns of antibody response were detected in the geographic regions suggesting variation in antigenic strain of *P carinii* or in host immune response.

Clinical studies of PCP in Africa

Studies of the aetiology of pneumonia in HIV-infected African adults have reported that bacterial pathogens and tuberculosis predominate while PCP occurs uncommonly (table 1.2) (Lucas et al 1989, Elvin et al 1989, Carme et al 1991, Abouya et al 1992, Machiels and Urban 1992, Atzori et al 1993, Kamanfu et al 1993, Batungwanayo et al 1994, McLeod et al 1989, Malin et al 1995). Although the majority of adult studies reported PCP in less than 10% of cases, the rate of PCP varied from 0-33% with two Zimbabwean studies reporting rates of 22% and 33% respectively (McLeod et al 1989, Malin et al 1995). These studies differ in the criteria and number of patients selected,

methodology for obtaining respiratory secretions, diagnostic tests used to identify *P carinii* and the geographic areas in which they were performed. Of note, is the high rate of tuberculosis reported in most studies occurring in up to 50% of cases (table 1.2).

Relatively few cases of PCP in HIV-infected adults have been documented in South Africa. An autopsy study of 20 patients who died with no definite clinical hepatic or pulmonary diagnosis at Baragwanath Hospital, a large tertiary hospital in Johannesburg, reported PCP in 2 (10%) (Karstaedt and Obers 1997). Isolated case reports of PCP in HIV-infected adults in Johannesburg and Pretoria have been published (Sein et al 1999, Pincus et al 1987). A chart review of an adult urban HIV population attending a major referral clinic in Cape Town from 1984-1995 reported tuberculosis to be the commonest AIDS diagnosis and the rate of PCP to be declining to 12% (Wood et al 1996). This decline in PCP incidence was noted to occur as the demographics of the clinic population shifted from a predominantly white male homosexual group to a heterosexual black population (Wood et al 1996). Data from a 5 year prospective cohort study of these HIV-infected adults attending HIV clinics in Cape Town, confirmed a significantly lower risk of developing PCP among at risk black Africans compared to Caucasians in whom the incidence was similar to that found in the United States (table 1.3) (Phair et al 1990). A retrospective chart review of HIV-positive adults hospitalised in Johannesburg also found a lower risk of PCP in black Africans compared with Caucasian patients (Mahomed et al 1999). A similar racial difference has also been found in African HIV-infected people living in London, United Kingdom, who had a lower PCP risk compared to white patients [adjusted hazard ratio 0.3 (0.1-0.7)] after initial AIDS diagnosis (Del Almo et al 1996).

Table 1.2: Summary of studies on the incidence of PCP in African HIV-infected patients with pulmonary disease

Study	n	Country	PCP	Other pathogens	Comments
Adult studies					
*Lucas SB et al	40	Uganda	0	Tuberculosis 6 (15%)	Silver & Giemsa stains for *P carinii*
McLeod DT et al	37	Zimbabwe	8 (22%)	Tuberculosis 12 (32%) Bacteria 16 (43%) Fungi (3%)	Giemsa, silver & toluidine blue stains for *P carinii* on BAL or biopsies of those smear negative for AFB
Elvin KM et al	27	Zambia	0	Tuberculosis 11/22 (50%)	
Carme B et al	45	Congo	5 (11%)	Not sought	Silver stain for *P carinii*
*Abouya YL et al	53	Cote d'Ivoire	5 (9%)	Tuberculosis 21 (40%) Bacteria 18 (34%)	In-hospital deaths Silver and H & E stains for *P carinii*
*Lucas S et al	247	Cote d'Ivoire	7 (3%)	Tuberculosis 94 (38%) Bacteraemia 46 (16%)	Autopsies on patients dying in general medical wards
Machiels, Urbani	44	Zambia	4 (9%), 2 coinfected with TB	Not sought	Patients unresponsive to antibiotics & sputum negative for AFB
Atzori C et al	83	Tanzania	3 (4%)	Tuberculosis 32 (39%)	Toluidine blue & Giemsa stains for *P carinii* on sputum
Kamanfu G et al	222	Burundi	11 (5%)	Tuberculosis 109 (49%) Community acquired pneumonia 79 (36%) Bacteraemia 23 (10%)	
Batungwanayo J et al	111	Rwanda	5 (5%)	Tuberculosis 25 (23%) Fungi 14 (13%)	Giemsa & silver stain for *P carinii*
Malin AS et al	64	Zimbabwe	21 (33%); 6 coinfected with TB	Tuberculosis 24 (39%)	Patients unresponsive to penicillin & smear negative for AFB
Mahomed et al	67	South Africa	29 (43)	Tuberculosis 9 (13)	Retrospective review of patients with sputa smear negative for AFB & *P carinii* who had bronchoscopy
Paediatric studies					
*Lucas SB et al	78	Cote d'Ivoire	11 (14%); 11/36 (31%) < 15 months	Bacteria 33 (42%) Tuberculosis 1 (1%) Measles 13 (17%)	Consecutive HIV positive children who died
*Jeena PM et al	36	South Africa	16/31 (52%)	CMV 16/31 (52%) Bacteria 8/31 (26%) Tuberculosis 1/31 (3%)	Lung biopsies on children who died in ICU
Kamiya Y et al	60	Malawi	5 (8%)	Not sought	*P carinii* from NPAs. HIV status confirmed in minority
*Ikeogu MO et al	122	Zimbabwe	19 (16%)	Bacteria 106 (87%) CMV 9 (7%) Tuberculosis 6 (5%)	Children died at home
Graham et al	93	Malawi	16 (17)	Bacteraemia 12 (13)	Children hospitalised with pneumonia. *P carinii* from NPAs

*post mortem studies
AFB – acid fast bacilli, NPAs – nasopharyngeal aspirates, BAL- bronchoalveolar lavage, ICU- intensive care unit

There is relatively little information on the importance of *P carinii* in HIV-infected children in Africa (Lucas et al 1996, Jeena et al 1996, Kamiya et al 1997, Ikeogu et al 1997, Graham et al 2000). As primary infection with *P carinii* causes disease in children (Hughes 1991) and as the seroprevalence data demonstrate high and equivalent levels of infection, it is probable that the incidence of paediatric PCP is similar to that described in developed countries. Post mortem studies have confirmed that the infection is common, occurring in 31% of HIV-positive children younger than 15 months in Cote d'Ivoire (Lucas et al 1996) and 16% of HIV-infected Zimbabwean children who died at home (table 1.2) (Ikeogu et al 1997). Two South African studies of children dying in ICU reported that PCP was common amongst the subset of patients in whom post mortem lung biopsies were obtained, occurring in 7 of 27 (26%) and 5 of 12 (42%) in Durban and Johannesburg studies respectively (Jeena et al 1996, Mathivha et al 1998). More recently, a study of 93 HIV-infected Malawian children hospitalised with severe pneumonia reported PCP in 17% (Graham et al 2000). These studies confirm the presence of *P carinii* in the environment in Africa, provide insights into the role of *P carinii* as a pathogen in this continent and suggest that *P carinii* is an important cause of pneumonia in HIV-infected infants in Africa.

Table 1.3: PCP risk for adult patients with CD4 counts lower than 200 cells/μl

	PCP at 6 months	**PCP at 12 months**
USA (Phair et al)	8.4%	18.4%
Cape Town White	7.6%*	17.3%*
Cape Town black African	3.7%	5.9%

*$p < 0.01$ compared to the risk in black Africans in Cape Town

1.3.2 Bacterial pneumonia

HIV-infected children are at increased risk for recurrent and severe bacterial infections including pneumonia and bacteraemia (Krasinski et al 1988, Andiman et al 1994). The importance of bacterial infections has been emphasised by their inclusion as an AIDS indicator disease in the CDC definition of AIDS. The risk of bacterial infection is related to the severity of HIV infection and degree of impairment of the immune system; the risk of bacterial septicaemia increases as the CD4 count decreases (Roilides et al 1991, Vugia et al 1993). Although there does not appear to be a difference in the rates of community acquired bacterial disease between HIV-positive and seronegative infants, the incidence of invasive bacterial disease in HIV-infected children increases sharply after 12 months of age (Andiman et al 1994, Ruiz-Contreras et al 1995).

Studies from developed countries prior to the introduction of anti-retroviral therapy have reported both gram positive and negative organisms as a cause of bacteraemia. In the majority of studies *S pneumoniae* was the most frequently isolated pathogen (Lichenstein et al 1998, Andiman et al 1994, Bernstein et al 1985). A prospective study reported a relative risk of 12 for pneumococcal bacteraemia in HIV-infected compared to seronegative children (Farley et al 1994). Other gram positive organisms responsible for bacteraemia are *S aureus* and *S epidermidis* while Salmonella species have been the most frequently identified gram negative pathogens (Andiman et al 1994, Bernstein et al 1985, Ruiz-Contreras et al 1995). A study of children hospitalised in Zimbabwe reported an increased risk of bacteraemia in HIV-infected children (OR 2.68); bacteraemia occurred in 67 of 168 children (40%) and the

predominant isolates were *S epidermidis* (10%) *S pneumoniae* (9%), *S aureus* (7%) and *S enteritidis* (5%) (Nathoo et al 1996).

Precise data on the incidence of bacterial pneumonia in HIV-infected children have been more difficult to obtain principally because of the difficulty in obtaining microbiologic confirmation of infection (see section 1.4 – identification of pulmonary pathogens). Even within the context of a clinical trial to describe the characteristics of acute pneumonia HIV-infected children in the USA, only 16 of 131 (12%) of all acute episodes of pneumonia had an identified aetiology (Mofenson et al 1998). In this study, *S pneumoniae* was the predominant isolate accounting for 5 of 16 laboratory-proven bacterial pneumonias while *S aureus* and gram negative organisms accounted for 2 and 6 cases respectively. Marolda et al reviewed 132 hospital admissions for pulmonary disease in 50 HIV-positive children over a 6-year period in New York, prior to the use of anti-retroviral agents. Bacterial pneumonia was found to occur in 15 patients (30%) of which the predominant pathogens were *S pneumoniae, P aeruginosa* and *K pneumoniae* (Marolda et al 1991).

There are few studies of the importance of bacterial pathogens in the aetiology of pneumonia in HIV-infected children in Africa (table 1.4). Nathoo et al reported bacteraemia in 99 of 443 (22%) of children admitted to hospital in Zimbabwe for ALRI; the predominant bacterial isolates included *S pneumoniae* in 25, coliforms in 23, and *S aureus* and Klebsiella species in 10 each (Nathoo et al, 1993). Of the 99 children with bacteraemia, 32 were clinically suspected of having HIV; the predominant bacterial isolates in these children were *S pneumoniae* in 10, *S aureus* in 5, coliforms and other streptococcal species in 4 each. Lung aspirates of Zimbabwean

children who died outside hospital found bacterial isolates in 106/122 (86%) HIV positive compared to 46/62 (74%) seronegative children (Ikeogu et al 1997). However, bacterial growth was strongly correlated with malnutrition (OR 5.19) and no association was found between HIV serostatus and bacterial lung infection that could not be attributed to nutritional status. Pyogenic pneumonia was reported on post mortem examination in 33 of 78 (42%) HIV-positive and 24 of 77 (31%) seronegative children who died in hospital in Cote d'Ivoire (Lucas et al 1996). Lung biopsies of South African children who died in ICU from pneumonia reported histological evidence of bacterial pneumonia in 8 of 31 HIV-infected (26%) and 11 of 34 (32%) seronegative children (Jeena et al 1996). A study in Malawi reported bacterial pneumonia (defined as abnormal chest Xray and positive blood culture) in 12 of 93 (13%) HIV-infected children (Graham et al 2000).

Table 1.4: Major bacterial causes of pneumonia in HIV-infected children in Africa

Study, place	n	Patient population	Bacteria	Culture rate	Specimen
Nathoo et al 1993 Zimbabwe	219	Hospitalised, ALRI	*S pneumoniae* 12 *S aureus* 5 Coliforms 4	32*	Blood culture
Lucas et al 1996 Cote d'Ivoire	78	Died in hospital	Pyogenic pneumonia 33	33 (42%)	Necropsy
Ikeogu et al 1997 Zimbabwe	122	Children < 5yrs who died at home	Klebsiella spp 28 *S pneumoniae* 10 *S aureus* 8 Coliforms 8 Pseudomonas spp 6	106 (86%)	Lung aspirates
Graham et al 2000 Malawi	93	Hospitalised, ALRI	*S pneumoniae* 4 Salmonella spp 3 *H influenzae* 2	12 (13%)	Blood culture

*number of HIV-positive children in whom blood cultures were obtained not stated

1.3.3 Pulmonary Tuberculosis

In contrast to the data in HIV-infected adults, in whom an increased incidence of TB has been found, studies in children have reported conflicting results. Accurate information on HIV and TB coinfection in African children is scanty because of the difficulties in diagnosing TB (Osborne 1995). Obtaining microbiologic confirmation of TB in HIV-infected children is difficult, the tuberculin skin test has a reduced sensitivity due to the development of anergy and chest Xray changes are not specific for TB (Harries 1990). The majority of studies have relied on a suggestive history and clinical presentation for diagnosing TB. Published studies differ in their methodology, patient selection and method of diagnosis. Most cross sectional studies of children diagnosed with TB have reported high levels of association with HIV seropositivity (table 1.5). Similarly, cross sectional studies of HIV-infected children have reported high rates of TB (Vetter et al 1996, Wilkinson 1994). A South African study in Kwazulu-Natal reported TB in 29 of 76 (38%) HIV-infected children (Wilkinson 1994). A study of 4480 children hospitalised in Cote d"Ivoire, reported TB in 5.6% of HIV positive compared to 1.3% of seronegative children older than 5 years; however, the overall rate of TB was not significantly higher in HIV-infected patients (1.9% versus 0.7%) (Vetter et al 1996).

Longitudinal studies of cohorts of perinatally HIV-infected infants have not shown an increased risk of TB compared to seronegative children. No cases of TB were found in an 18-month follow-up study of 106 Zairean children born to HIV-infected mothers (Ryder et al 1991). Similarly no cases of TB were detected in a 2-year follow up study of 218 children of HIV-positive women in Rwanda (Lepage et al 1993). Another 2-

year follow-up study of 118 infants in the Congo also did not find any TB cases (Lallemant et al 1994). In a South African cohort study (48 HIV-infected and 93 seronegative infants) followed up for a mean of 26 months, only 2 cases of TB were detected (Bobat et al 1998). However, a small Rwandan study of older HIV-infected children aged 5 to 12 years reported that TB occurred in 4 of 16 (25%) (Lepage et al 1991), suggesting that long term follow-up is needed in order to assess TB risk in HIV-infected children.

Mortality studies in HIV-infected children have rarely reported TB (Lucas et al 1996, Jeena et al 1996, Ikeogu et al 1997). A post mortem study of 78 HIV-positive and 77 seronegative children in Cote d"Ivoire found TB in 2% and 4% respectively (Lucas et al 1996). Similarly, a Zimbabwean study of 184 children (122 HIV-infected) found TB in 8 cases (4%), 6 of whom (5%) were seropositive (Ikeogu et al 1997). A report of post mortem liver and lung biopsies obtained in 72 children (36 HIV-infected) who died in an ICU in South Africa with pneumonia, detected TB in only 1 HIV-infected patient (Jeena et al 1996).

Overall, it appears that HIV infection does increase the risk of TB in children and the discrepancies in reported association between TB and HIV co-infection may be a result of the differences in study methodology (Coovadia et al 1998). In general, longitudinal studies are limited by their small sizes and relatively short duration of follow up. Moreover, TB appears to become increasingly important as a pulmonary pathogen in children older than 18 months; however, mortality data reported is predominantly for infants or young children. TB is unlikely to account for a large proportion of deaths in HIV-infected infants who succumb to bacterial or opportunistic infections.

Table 1.5: Cross sectional African studies of HIV prevalence in children with tuberculosis

Study	Country	n	HIV prevalence in TB cases	HIV prevalence in controls
Sassan Morokro et al	Cote d'Ivoire	289	11%	0.5%
Luo et al	Zambia	270	38%	13%
Jeena et al	South Africa	180	11%	-----
Mukadi Y et al	Cote d'Ivoire	161	19%	0%
Harries et al	Malawi	4691	64%	-----

1.3.4 Viral pneumonia

The most common virus isolated from lower respiratory tract secretions in HIV-infected children is cytomegalovirus (CMV) but its role in the aetiology of pneumonia is uncertain. In HIV-infected patients, the presence of CMV in BAL fluid is usually not predictive of CMV pneumonia as definitive diagnosis requires pathologic evidence (Drew 1988). As evidence of this, over half of BAL samples from adult HIV patients with pulmonary symptoms contain CMV, but this is not associated with a more severe course or worse outcome than patients with other causes of pneumonia (Miles et al 1990, Millar et al 1990). Moreover, pneumonia with CMV as the sole pathogen rarely occurs in HIV-infected patients (Millar et al 1990, Rodriguez-Barradas et al 1996).

Asymptomatic shedding of CMV is common amongst children with low CD4 counts, but it is rarely a cause of clinically recognisable pneumonia (Chadwick 1997). Furthermore, when children with PCP and CMV (identified from a lung biopsy or BAL fluid) were compared to those with PCP alone there was no difference in the severity of disease, need for mechanical ventilation or outcome (Glaser et al 1992). Although CMV lung infection has been frequently identified in autopsy studies of HIV-infected children, the specific contribution of CMV to mortality has been difficult to determine because of other co-existing pathological processes (Lucas et al 1996, Jeena et al 1996). Nevertheless, biopsy-proven CMV pneumonia, described in relatively few HIV-infected patients, has been associated with severe immunosuppression and a high mortality (Salomon et al 1997).

Other viruses rarely reported to cause pneumonia in HIV-infected children include respiratory syncytial virus (RSV), measles, adeno and influenza virus (Chandwani et al 1990, King et al 1993, Park et al 1993, Lucas et al 1996, Safrin et al 1990).

1.4 Identification of pulmonary pathogens

Pulmonary pathogens are infrequently isolated in HIV-infected children with pneumonia due to the difficulty in obtaining lower respiratory tract secretions. Even in the setting of a controlled clinical trial in the USA in which HIV-infected children were followed for years, pulmonary pathogens were identified in only 12% of pneumonic episodes (Mofenson et al 1998). Although lower respiratory tract secretions can be successfully obtained using BAL, this procedure requires resources, specialised equipment and a skilled bronchoscopist (Bye et al 1987, Boccon-Gibod et al 1997) all

of which are generally lacking in developing countries. Moreover, bronchoscopy and BAL may be associated with morbidity and clinical worsening of hypoxic lung disease in children (Bye et al 1987, Abadco et al 1992). Most of the data on the aetiology of pneumonia in African HIV-infected children is therefore derived from blood culture results or from post mortem studies (tables 1.2 and 1.4).

Two diagnostic procedures, sputum induction and nasopharyngeal aspiration may provide specimens that are useful for determination of the aetiology of pneumonia. However, neither of these procedures has been well studied in infants or young children as discussed below.

1.4.1 Sputum induction

Sputum induction has been successfully used to diagnose PCP, tuberculosis and other pulmonary pathogens in HIV-infected adults (Leigh et al 1989, Kirsch et al 1990). The sensitivity of sputum induction compared to BAL for diagnosing PCP in adults ranges from 55 to 95% depending on the methodology used to obtain sputum and identify *P carinii* while the specificity is 99 to 100% (Leigh et al 1989, Kovacs et al 1988, Kirsch et al 1990). As a result this procedure has become a standard first line practice for the investigation of HIV-infected adults with pneumonia (Van der Els and Stover 1996). However, this technique has not been used in infants or young children and has rarely been studied in older patients. A study of 18 immunosuppressed paediatric patients (mean age 7.3 years) detected 9 episodes of PCP in 8 children using sputum induction and immunofluoresence for *P carinii* detection (Ognibene et al 1989). Sputum induction done in 14 immunocompromised children with respiratory

symptoms, identified CMV in one and *H Influenzae* in two (Foot et al 1992). In a Malawian study of the efficacy of sputum induction in children aged 3 to 15 years, sputum was successfully obtained in 29 of 30 patients undergoing investigation for pulmonary tuberculosis and the diagnosis was confirmed in 8 (28%) (Shata et al 1996). However, gastric aspiration was not performed so the yield from induced sputum could not be compared with that from the standard diagnostic procedure of GL.

1.4.2 Nasopharyngeal aspiration

Upper respiratory tract secretions in the form of a nasopharyngeal aspirate (NPA) may provide a possible means of identifying pulmonary pathogens. This technique is attractive as it is relatively non-invasive, inexpensive, requires minimal skill and is well tolerated by children. The successful identification of *P carinii* on NPA was first described in a single HIV-infected infant using immunofluorescence (Hague et al 1990). Subsequently PCP was diagnosed using NPAs in 5 of 60 Malawian children hospitalised for acute lower respiratory tract infection (Kamiya et al 1997). More recently PCP was found in 16 of 150 Malawian children using the same diagnostic methodology (Graham et al 2000). However, *P carinii* is not usually found in upper airway secretions as the organism inhabits the lungs exclusively. It is possible that in overwhelming infection, *P carinii* may be detected from the upper airways using methods that are sufficiently sensitive to detect small numbers of organisms. As evidence of this *P carinii* has been detected using PCR in nasopharyngeal aspirates from 3 leukaemic infants with pneumonia (Richards et al 1994) and in oropharygeal washes from an HIV-infected child (Martino et al 1999). Nevertheless, no study has

compared NPAs to lower respiratory tract secretions for the diagnosis of PCP and the diagnostic sensitivity of this method remains to be determined.

1.4.3 Identification of *P carinii*

Identification of *P carinii* has relied on identification of *P carinii* in lower respiratory tract secretions using specialised stains in particular silver methenamine or toluidine blue for the cyst forms and Giemsa for the trophozoites. The development of immunofluorescence techniques using monoclonal antibodies has improved the detection rate for P carinii (Orholm et al 1990, Midgley et al 1991). A study comparing four stains (silver, modified Giemsa, direct and indirect immunofluorescence) in 100 specimens (50 induced sputa and 50 BAL fluid) found that the sensitivities for detection of *P carinii* in sputum were 92% with silver stain or Giemsa and 97% with immunofluorescence. The sensitivities for detection in BAL fluid were 86% with silver stain, 81% with Giemsa, 86% for indirect immunofluorescence and 90% for indirect immunofluorescernce (Cregan et al 1990). More recently the use of polymerase chain reaction (PCR) techniques has been reported to be more sensitive than standard cytological techniques for detection of *P carinii* (Wakefield et al 1990, Leibovitz et al 1995, Kahn et al 1999), particularly in samples with a relatively low parasitic load (Graves et al 1997).

1.5 Outcome of HIV-positive children with pneumonia.

Studies in both developed and developing countries have found that HIV infection increases infant and child mortality (Blanche et al 1989, Lallemant et al 1989, European Collaborative Study 1991, Taha et al 1995, Vetter et al 1996). The mortality

in African children was higher and occurred earlier (Lallemant et al 1989, Vetter et al 1996) than in developed countries (Blanche et al 1989, European Collaborative Study 1991). Disease progression in HIV-infected children in developing countries is more rapid compared with developed countries with the probability of death in sub-Saharan Africa 0.23-0.35 by 12 months and 0.57-0.68 by 5 years (Tudor-Williams 2000). In contrast, in Europe prior to the use of highly active anti-retroviral therapy (HAART) these probabilities were 0.1 and 0.2 respectively. The impact of the HIV epidemic on infant and child mortality is large; it has been estimated that 50-75% of all HIV-infected children in Africa will die before their fifth birthday (Nicoll et al 1994). Currently in South Africa, approximately 45% of all deaths among children younger than 5 years are AIDS-related, in neighbouring Zimbabwe that percentage is 70% (MAP 2000).

A number of African studies have reported increased mortality rates for HIV-positive compared to seronegative children; these studies have also highlighted the importance of pneumonia as a cause of death. A prospective study of 1385 children in Malawi found that the independent risk of mortality was 5 times higher for infants born to HIV seropositive mothers than for those born to seronegative women (Tana et al 1995). Infant mortality rates for children born to HIV-positive and negative mothers were 223 and 68 per 1000 respectively while rates at 24 months were 317 and 106 per 1000 (Taha et al 1995). Amongst children born to seropositive women, the commonest causes of death were pneumonia, prematurity, diarrhoea and failure to thrive. A prospective study of 4480 children hospitalised in Cote d'Ivoire reported the mortality rate in HIV positive children to be 20.8% compared to 8.7% in seronegative patients [RR 2.4 (1.9-3.1)]; the highest death rate (28%) occurred in those under 15 months of

age (Vetter et al 1996). Acute respiratory infection accounted for 24% of deaths in HIV-positive compared to 18% in seronegative children ($p<0.005$). A longitudinal cohort study of infants with vertically transmitted HIV infection in Durban, South Africa found that 17 of 48 (35%) HIV-infected compared to 8 of 93 (8.6%) HIV-uninfected children died; 83% of HIV-related deaths occurred before 10 months of age (Bobat et al 1999). A retrospective study of paediatric hospital admissions at an urban South African hospital Chris Hani Baragwanath from 1992 to 1997, reported a mortality of 13.2% in HIV-infected children compared to 5.1% in uninfected patients (Zwi et al 1999). Although an overall decline in mortality rates in HIV-negative children occurred over the period of the study, HIV infection was responsible for an increase in overall in-hospital mortality of 42%. Pneumonia was the commonest cause of mortality accounting for 24.6% of deaths.

The risk of dying from acute lower respiratory tract infection amongst hospitalised HIV-infected children in Zimbabwe was 3 times that for seronegative patients (mortality rate of 28% compared to 9%) even after controlling for nutritional status, age and birthweight (Nathoo et al 1993). Although the age-specific mortality rate in the seronegative group declined with age, this trend was not evident amongst those who were HIV-positive. A study of 132 Zambian children admitted to hospital for ARI found a similarly increased risk of death (RR 2.6) amongst HIV-positive patients (Smyth et al 1997). A study of 150 Malawian children (93 HIV-infected) hospitalised with pneumonia, reported the case fatality rate for pneumonia to be 22%; death was independently and significantly associated with HIV infection [RR 2.98 (1.1-7.9)] (Graham et al 2000).

1.6 Issues for developing countries

The importance of pneumonia as a cause of morbidity and mortality in HIV-infected children in developing countries, the paucity of available data on the aetiology and outcome of pneumonia and the magnitude of the HIV epidemic in these areas have prompted international organisations to prioritise research on the aetiology of pulmonary infections in HIV-infected children in developing countries (Lepage et al 1998).

1.6.1 Development of diagnostic and management guidelines

Early diagnosis and treatment of pulmonary disease has been shown to improve survival in children with AIDS (Marolda et al 1991, Bye 1995). Thus it is important to develop diagnostic and management plans to identify pulmonary problems and to recommend appropriate treatment and chemoprophylaxis. Knowledge of the spectrum of pneumonia in HIV-infected children is important for the development of such plans for all levels of care. Since untreated PCP is lethal and since effective, inexpensive treatment and preventive strategies are available, determining the prevalence of PCP has important implications (Hughes 1991). Cost-effective, appropriate guidelines for management of HIV-infected children with pneumonia in developing countries need to be developed. Current guidelines for pneumonia produced by the World Health Organisation for use in developing countries (WHO 1990) do not contain information on management of HIV-infected children. Diagnostic and management guidelines for HIV-infected children with pneumonia in South African and developing countries do not exist.

1.6.2. Development of appropriate diagnostic procedures

Difficulties in diagnosing pulmonary infections are particularly relevant to HIV-infected infants in developing countries in whom invasive, costly and technically difficult diagnostic procedures are not feasible. In particular, obtaining an ante-mortem diagnosis of PCP has been hampered by the difficulties in obtaining lower respiratory secretions in infants and the lack of laboratory facilities or skill for identifying the organism in developing countries. As there are no clinical signs specific for PCP and as the peak incidence of paediatric PCP occurs between 3 to 6 months of age (Hughes 1991), bronchoalveolar lavage (BAL) has been the standard diagnostic procedure used in developed countries (Bye et al 1987).

Similarly, pulmonary tuberculosis has been difficult to diagnose in HIV-infected children as there is no specific clinical presentation and the development of anergy renders the skin test unreliable. Microbiologic confirmation of pulmonary TB has relied on repeated gastric aspiration which is a time-consuming and unpleasant procedure that usually necessitates hospitalisation and from which the yield is relatively low (Lloyd 1968, Starke and Yalor-Watts 1989). For these reasons, diagnosis of childhood TB in particular has been identified as a priority for research (Tudor-Williams 2000).

Sputum induction may be a relatively easy and inexpensive method for obtaining lower respiratory tract secretions for diagnosis of pulmonary infections if the procedure could be safely and effectively performed in young children. The study will investigate the efficacy of sputum induction in such children. The study will also investigate the

efficacy of nasopharyngeal aspiration for the diagnosis of PCP in HIV-infected children as this is a relatively simple procedure that may be potentially useful particularly in developing countries.

1.6.3. Development of policies for resource allocation

The sharp rises in HIV infection rates in South Africa over the past 4 years have created an increased demand for hospital and ICU beds for HIV positive infants and children (Zwi et al 1999, Jeena et al 1999). However, the number of children who can be accommodated in these facilities is limited. The resultant increased pressure on costly and precious ICU resources, necessitates clear criteria for utilisation of such facilities. Furthermore, competition for paediatric ICU beds has been exacerbated by cutbacks in tertiary health expenditure and the increased emphasis on primary care. As a result, some hospitals such as Chris Hani Baragwanath in Johannesburg, have implemented a policy that excludes children known to be infected with HIV from ICU (Zwi et al 1999).

The HIV epidemic has therefore resulted in additional financial and ethical concerns regarding the allocation and use of scarce and costly resources such as ICU facilities. Knowledge of the aetiology and outcome of children admitted to hospital and ICU with pneumonia in developing countries is vital in order to develop admission and management policies and to establish effective treatment algorithms.

1.7 Summary

Given the sharp rise in HIV prevalence rates in South Africa increasing numbers of

children will require health care for pneumonia as this infection is a major cause of morbidity and mortality in HIV positive children. However, there is a paucity of data on the aetiology, clinical course and outcome of HIV-infected children with pneumonia in developing countries including South Africa. Current, limited data suggest that bacterial and mycobacterial pathogens predominate and that PCP is uncommon. Knowledge of the spectrum and outcome of pneumonia in HIV-infected children is essential in order to develop effective diagnostic and management algorithms and to develop policies for resource allocation. Development and evaluation of improved and cost effective methods for identifying aetiological pulmonary pathogens particularly sputum induction and nasopharyngeal aspirates are also desirable.

CHAPTER 2: METHODS

2.1 Purpose

To develop an effective strategy for healthcare workers to diagnose and manage HIV-infected children with acute pneumonia.

2.2 Aim

To investigate the aetiology, associated features and outcome of pneumonia in HIV-infected children

2.3 Objectives

To investigate HIV-infected children hospitalised with pneumonia with respect to:

2.3.1 clinical, radiological and laboratory features and to compare these with those in HIV-negative patients

2.3.2 aetiological pathogens and antimicrobial susceptibility patterns compared with those in HIV-negative patients

2.3.3 clinical, radiological or laboratory features associated with specific pulmonary pathogens

2.3.4 proportion with severe pneumonia who are admitted to intensive care unit (ICU)

2.3.5 need for mechanical ventilation and outcome of pneumonia as compared to that in HIV-negative patients

2.3.6 efficacy and safety of sputum induction and nasopharyngeal aspirates as diagnostic procedures for recovery of pulmonary pathogens

2.4 Study design

A prospective descriptive study

2.5 Participants

The study population comprised children consecutively admitted to 4 hospitals linked to the University of Cape Town in South Africa (Red Cross War Memorial Children's, Somerset, Conradie and Groote Schuur) between January and December 1998. Children were prospectively enrolled if they

- had a primary diagnosis of pneumonia according to WHO criteria i.e. presence of tachypnoea or lower chest indrawing (World Health Organisation 1995)

and

- were known to be HIV infected *or* were suspected of having HIV *or* were admitted to an ICU. A suspicion of HIV infection was based on the presence of 2 or more of the following: generalised lymphadenopathy, weight below the 3rd percentile for age, hepatomegaly, splenomegaly, oral candidiasis, enlarged parotid glands or chronic diarrhoea.

Children were enrolled during working hours from Monday to Friday. Exclusion criteria were admission to hospital within a month prior to the study to preclude enrolment of children with nosocomially acquired pathogens, antibiotics for more than 48 hours prior to enrolment, cystic fibrosis and known immunodeficiency, cardiac or neurological disease except if this was HIV-associated.

2.6 Clinical evaluation and management

A history and physical examination were performed on every child enrolled and socio-demographic, clinical and outcome data collected (appendix 1). Information on the birth history and immunisation status was obtained from the Road to Health Chart (immunisation record) of each child. Children were evaluated and treated by the admitting medical officer. Use of TMP-SMX prophylaxis in children known to be HIV-infected was recorded. Current guidelines for use of prophylaxis at these hospitals are oral TMP-SMX (5mls if less than 10kg or 10mls if greater or equal to 10kg) given twice daily three times per week on alternate days (Monday, Wednesday and Friday) in all HIV-positive children under a year of age; a similar regime is used for those older than a year who are moderately or severely symptomatic (clinical category B or C) as it is too costly to perform CD4 counts.

A daily record sheet of clinical signs and treatment given was kept for a maximum of 14 consecutive days for each patient (appendix 2). When patients were hospitalised for longer than 14 days, a daily record exceeding 14 days was not kept but the child was followed till discharge from hospital or death. When PCP was suspected, a standard treatment regime of intravenous TMP-SMX (10mg/kg loading dose followed by 5mg/kg/dose 6hrly) and corticosteroids (prednisone 2mg/kg for 5 days then 1 mg/kg for 5 day, then 0.5mg/kg for 5 days) was started on the day of admission (Bye et al 1994). Children were switched to the same dose of oral TMP-SMX following clinical improvement for a total of 21 days of therapy. In addition, a broad spectrum antibiotic (cefuroxime 20mg/kg/dose given eight hourly) was recommended for all children. Use of oxygen, additional antibiotics or other therapy was at the discretion of

the ward registrar and consultant paediatrician. The decision to admit to ICU or institute intermittent positive pressure ventilation (IPPV) was made solely by the ward or ICU doctors. IPPV modes included conventional pressure limited, time-cycled ventilation and high frequency, high mean airway pressure, low tidal volume ventilation for severe hypoxaemic respiratory failure. Primary outcome measures were the duration of hospital and ICU stay and in-hospital mortality.

2.7 Investigations

2.7.1 Blood tests

Blood tests including HIV testing, bacterial culture, a complete blood count (CBC) and white cell differential, lymphocyte phenotyping and chemistry were done. HIV infection was diagnosed by 2 consecutive positive ELISA tests (Vironostika HIV Uniform II, Organon Teknika, Holland) in children older than 18 months or by a positive ELISA and polymerase chain reaction (PCR, Amplicor HIV-1, Roche Diagnostic Systems) in those younger. Baseline arterial oxygen saturation in air was recorded using a pulse oximeter (Ohmeda, Biox 3760, BOC Health Care, Louisville, USA) but arterial blood gas measurements were only taken when a ward doctor considered this necessary.

2.7.2 Skin tests

A Mantoux test (2 TU PPD RT23, Staten Serum Institut, Denmark, Copenhagen) was placed on the left forearm of each child using subcutaneous injection. The Mantoux test was read within 48 to 72 hours of placement. A positive test was regarded as greater than or equal to 10mm transverse induration in HIV-negative children or 5mm

in HIV-positive children.

2.7.3 Chest radiography

Antero-posterior and lateral chest Xrays were performed on entry into the study. The chest Xray was interpreted according to a standardised format (appendix 3) by a single paediatric radiologist who was blinded as to the HIV status of the child or aetiology of the pneumonia. If a follow-up Xray was taken prior to discharge this was also reported according to the same format.

2.7.4 Sputum induction

Sputum induction was undertaken on the day of enrolment after a 2 to 3 hour fast. A physiotherapist or research nurse trained in the use of this technique performed the procedure. Children were pre-treated with 200μg salbutamol given via metered dose inhaler (MDI) with attached spacer (Babyhaler, GlaxoWellcome) to prevent the occurrence of bronchoconstriction (Belcher et al 1989). A jet nebuliser (GRS, Intersurgical, UK) attached to oxygen at a flow rate of 5 l/min delivered 5mls of 5% sterile saline for 15 minutes. Thereafter physiotherapy techniques including chest percussion, vibration, shaking and active cycle breathing techniques were applied. Sputum was obtained either by expectoration (in children able to cooperate) or by suctioning through the nasopharynx or oropharynx using a sterile, mucus extractor of catheter size 6. Specimens were transported directly to the laboratory for processing.

2.7.5 Non-directed bronchoalveolar lavage

Non-bronchoscopic bronchoalveolar lavage (BAL) was performed on intubated children according to a standardised procedure (Koumbourlis and Kurland 1993). A sterile suction catheter was passed blindly down the endotracheal tube and wedged as distally as possible. Three lavages of 1ml/kg of 0.9% sterile saline instilled through the catheter were performed.

As there is currently no dedicated bronchoscopy suite at Red Cross Children's Hospital, bronchoscopy and BAL would be done under general anaesthetic in the operating room in non-intubated children. Due to limitations in resources both for doing the BAL and for supporting the child after the procedure, this procedure could therefore not be undertaken in non intubated children who were sputum negative for pathogens.

2.7.6 Gastric lavage

Early morning gastric lavage (GL) was performed in non-intubated children younger than 5 years old after an overnight fast of at least 4 hours. A nasogastric tube was passed before the child arose and the gastric contents aspirated. Twenty millilitres of normal saline was inserted down the tube, left for 2-3 minutes and then aspirated. Additional 5-10mls normal saline aliquots were inserted and aspirated until a minimum of 20mls of aspirate was obtained. Gastric aspirates were placed in a sterile, sodium carbonate containing tube and taken to the laboratory. GL specimens were pooled, stained for acid fast bacilli and cultured for *M tuberculosis*. GL was ideally performed on 2 to 3 consecutive mornings; however repeated lavages were not possible in many children due to other factors such as discharge from hospital or subsequent intubation.

2.7.7 Nasopharyngeal aspirate

A nasopharyngeal aspirate (NPA) for IF for *P carinii* was obtained by instilling 4 drops of sterile saline into each nostril and suctioning the nasopharynx using a sterile suction catheter attached to a mucus trap at one end and wall suction at the other.

2.8 Culture procedures

(i) Bacterial

Before culture, the sputum and BAL samples were digested with Sputasol (Oxoid SR 089A, Oxoid Ltd, UK). All respiratory samples were cultured on chocolate and blood agar plates incubated in CO_2 and on McConkey agar plates incubated in air. Plates were incubated for 48 hours at 37°C. Blood for culture was inoculated into BACTEC PedPlus/F bottles and monitored for growth on a BACTEC 9240 machine. Potential pathogens were subcultured and identified using standard techniques (Balows et al 1991, Baron and Finegold 1990). Susceptibility testing was performed by disc diffusion using National Committee for Clinical Laboratory Standards criteria (NCCLS 1993). In addition, isolates of *S. pneumoniae* from blood culture had minimal inhibitory concentrations (MICs) for penicillin determined by the E-test method (AB BIODISK, Sweden). Pneumococcal strains with MICs less than 0.06 mg/L were regarded as susceptible, those with MICs 0.12 - 1.0 mg/L were of intermediate resistance, and those with MICS greater or equal to 2mg/L were resistant.

(ii) Mycobacterial

GL specimens collected on consecutive days were pooled before culturing, while induced sputum or BAL samples were cultured singly. Specimens were decontaminated with sodium hydroxide, and after neutralisation with buffered saline were concentrated by centrifugation. The resuspended deposit was used to make a smear for microscopy. Prepared smears from the concentrated samples were stained with Auramine-O and examined by fluorescent microscopy for the presence of acid fast bacilli. A Bactec® 12B bottle (Becton Dickinson, USA) containing supplemented Middlebrook medium was inoculated with 0.5 ml of sample, incubated at 37 °C and monitored for growth twice weekly for the first two weeks, then weekly for a total incubation period of 6 weeks. Positive mycobacterial growth was confirmed by a stain for acid fast bacilli as well as by PCR using primers prepared in-house.

(iii) Viral

For viral culture, specimens were placed in viral transport medium. After vortexing and centrifugation, 0.1ml of the supernatant was inoculated into paired primary monkey kidney and human fibroblast tube cell cultures (prepared in-house). Cultures were incubated at both 33°C and 37°C in roller drums. Monkey kidney and fibroblast cell cultures were observed for cytopathic effects for 10 and 21 days respectively. Viruses were identified by characteristic features on haemotoxylin and eosin staining of the tube coverslips. Other distinguishing tests were haemadsorption with guinea pig red cells and and immunofluorescence using guinea pig immune sera (Flow Laboratories, Irvine, Scotland) for typing of the parainfluenza isolates.

2.9 *P carinii* identification

Sputum or BAL specimens were liquefied with Sputasol (SR 089A, Oxiod Ltd, Basingstoke UK) washed thrice with sterile water, then concentrated by centrifugation. The resuspended deposit was used to make 2 smears and fixed with acetone. One smear was stained by a silver methenamine technique, the other was stained with monoclonal antibody (IF, Detect IF *Pneumocystis carinii*, Shield Diagnostics, UK) according to the manufacturer's instructions and scanned using a fluorescent microscope. For nasopharyngeal aspirates only IF was performed as this is a more sensitive technique than silver stain for identifying *P carinii*. A positive result on IF was regarded as visualisation of 5 or more cysts.

2.10 Definition of specific pulmonary diagnoses

Pulmonary tuberculosis was diagnosed when cultures of induced sputum, BAL or GL grew *M tuberculosis*. PCP was diagnosed when BAL or sputum demonstrated *P carinii* either on IF or silver stain.

2.11 Statistical analysis

Results were analysed using EpiInfo 6.4 (CDC, Atlanta, USA). Children were categorised as being HIV positive or negative. Differences between these 2 groups were assessed using the chi-square (for categorical variables) and Kruskal – Wallis tests (for continuous variables). Values were expressed as median (25^{th} – 75^{th} percentiles). Confidence intervals for proportions were calculated using the exact binomial method provided in the EpiTable calculator of EpiInfo. The yield of *M tuberculosis* from induced sputum and gastric lavage were compared using the McNemar test. No adjustment for multiple comparisons were made for univariate

analysis. Multiple logistic regression was performed using Stata 5.0 (Stata Corporation, Texas, USA).

2.12 Ethical issues

Written informed consent for enrolment in the study and for HIV testing was obtained from a parent (appendix 4). Consent forms were translated from English into Afrikaans and Xhosa as these are also widely spoken in the communities that the hospitals serve. Pre and post-test counselling for HIV testing was provided by a research nurse with the assistance either of a hospital social worker or an interpreter from the National Language Programme who had been trained as an AIDS counsellor. Patients were not enrolled in the study if informed consent for HIV testing was refused or unobtainable. The study was approved by the Research and Ethics Committee of the University of Cape Town, South Africa.

CHAPTER 3: RESULTS I

Aetiology and outcome of pneumonia in HIV-positive compared to seronegative children

3.1 Patients enrolled

Two hundred and fifty children (42.8% female) were enrolled in the study. The median age of children was 6 (3-16) months. One hundred and fifty one (60.4%) were HIV positive; 64 (42.4%) of these were diagnosed with HIV infection at the time of admission (newly diagnosed). Therefore 64 of 250 (25.6%) children hospitalised with pneumonia were diagnosed as HIV-positive at the time of admission. HIV-positive children were older and had received tuberculosis treatment more frequently (table 3.1). However, children newly diagnosed as HIV-infected were of a similar age [3 (3-8) months] to HIV-negative patients. Almost 60% of HIV-infected children had had a prior hospital admission (table 3.1); 60% for a respiratory illness. The majority of HIV-positive and negative children had been born at full term (77% and 70% respectively; p=0.08)

Most children came from poor socio-economic circumstances with 59.6% living in a shack (self constructed, flimsy structure with no indoor running water). HIV-positive children were less likely to have been exposed to maternal or household cigarette smoke but breast feeding rates were similar (table 3.1). As significantly more HIV-infected than HIV-negative children were of African descent (table 3.1), such differences in passive smoke exposure are probably a result of a lower smoking prevalence among African compared to mixed race or caucasian South African women (Reddy 1996). Trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis was used by 58 of 87 (67%, 95% CI = 55.7 - 76.4) children previously diagnosed as HIV-positive. The median age of HIV diagnosis in those on prophylaxis was 7 (3-10) months. HIV-infected children on prophylaxis were older than those not taking prophylaxis [16 (9-

36) versus 4 (3-11) months, p<0.005]. Children previously diagnosed as HIV-positive who were using prophylaxis [16 (9-36) months] were of a similar age to those known to be HIV-infected but not on prophylaxis [11.5 (6-23) months], p= 0.21.

3.2 Clinical signs

Signs of HIV infection (generalised lymphadenopathy, hepatomegaly, splenomegaly) were more common in HIV-infected children (table 3.1). In addition, clubbing was present in 30 of 150 HIV-infected children (20%) compared to 1 of 99 HIV negative patients [1%; OR 24.5 (3.9-1009.5), p<0.001]. HIV-positive children with clubbing were older than those without clubbing with a median age of 38 (25-60) compared to 6 (3-12) months, p<0.001. Amongst HIV-positive children, enlarged parotid glands occurred in 26 children (17.2%); this sign occurred more commonly in those with clubbing [11 of 30 (37%)] compared to 15 of 121 (12%) without clubbing, p=0.003.

The severity of pulmonary disease, as reflected by respiratory signs and chest radiology, was similar in HIV positive and negative children (table 3.1). Median baseline arterial oxygen saturation in air of 94 % (92-97) was similar for both groups. A bacille Calmette-Guerin (BCG) vaccination scar, present in 66 (66.6%) HIV-negative children, occurred in only 59 (39.1%) HIV-positive patients, p<0.001.

3.3 Blood studies

Haemoglobin, platelet count, CD4 count and CD4:CD8 ratio were significantly lower in HIV-positive children (table 3.1). HIV-infected patients had lower serum albumin but higher serum protein, reflecting an HIV-associated hypergammaglobulinemia.

3.4 Radiology

There were no significant differences in the frequency and type of chest Xray changes between HIV-positive and negative children (table 3.1). Hilar or mediastinal adenopathy occurred more commonly in children with tuberculosis (43% compared to 12.2% of those without TB, p=0.006). Chest Xray findings associated with clubbing in HIV-infected children included a diffuse nodular pattern [occurring in 5 of 24 (21%) with clubbing compared to 4 of 108 (4%) without clubbing, p=0.003] and hilar lymphadenopathy [reported in 8 of 24 (33%) with clubbing compared to 13 of 108 (12%) without clubbing, p=0.01]. A radiological diagnosis of lymphocytic interstitial pneumonia (LIP) based on the presence of a diffuse nodular pattern was made in 9 of 132 HIV-infected children (6.8%).

Table 3.1: Presenting features of children by HIV status

	HIV positive (n=151)	HIV negative (n=99)	p
Age (months)	9 (3-23)	3 (2-7)	<0.001
Male:female	80:71	63:36	0.096
African race, n (%)	144 (95.3)	71 (71.7)	<0.001
First admission, n (%)	59 (39)	80 (81)	<0.001
History, n (%)			
TB contact	30 (19.9)	16 (16.3)	0.06
Prior TB treatment	17 (11.2)	0 (0)	<0.001
Maternal smoking	12 (8.9)	21 (22.1)	0.005
Household smoking	77 (56.6)	66 (69.5)	0.05
Breastfed	104 (76.5)	70 (71.4)	0.385
Vital signs			
Heart rate	140 (120-158)	150 (137-160)	0.003
Respiratory rate	50 (40-60)	52 (44-68)	0.312
Temp (° C)	37 (36.5-37.5)	37 (36.5-37.5)	0.793
Weight for age (%)	72 (59-85)	82 (65-94)	0.006
Height for age (%)*	89 (82-94)	92 (83-97)	0.331
Weight for height	92 (81-103)	96 (89-106)	0.02
Physical exam, n (%)			
Clubbing	30 (20)	1 (1)	<0.001
Generalised adenopathy	134 (89.3)	57 (57.5)	<0.001
Hepatomegaly	145 (96)	72 (72.7)	<0.001
Splenomegaly	98 (64.9)	25 (25.2)	<0.001
Lower chest indrawing	82 (54.3)	53 (53.5)	0.905
Lung findings**	140 (92.7)	92 (92.9)	0.949
Blood tests			
Haemoglobin (g/dl)	8.9 (8.1-10.2)	10.0 (9.2-11.6)	<0.001
Platelets (10^9/l)	337 (219-444)	438 (327-618)	<0.001
WBC (10^9/l)	12.7 (9.6-17.9)	14.1 (9.8-20.8)	0.152
CD4 (% lymphocytes)	15.0 (10.8-21.9)	39.7 (31.3-45.6)	<0.001
CD4/CD8	0.3 (0.2-0.5)	2.0 (1.4-2.9)	<0.001
Protein (g/l)	76 (62-87)	60 (52-67)	<0.001
Albumin(g/l)	29 (25-33)	33 (27.5-38)	<0.001
Radiology(n=223),n (%)			
Normal Xray	4 (3%)	0 (0)	0.151
Hyperinflation	84 (63.2)	58 (64.4)	0.902
Alveolar infiltrate	121 (91)	82 (91.1)	0.681
Consolidation	113 (85)	84 (93.3)	0.988
Adenopathy	23 (17.3)	13 (14.4)	0.231
Cavitation	3 (2.3)	6 (6.6)	0.103
Pleural effusion	15 (11.3)	7 (7.8)	0.334

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)

: * height available for 170 children

: ** presence of adventitious sounds including wheezes, bronchial breathing or crackles

3.5 Microbiology and antimicrobial susceptibility

3.5.1 Microbiology

P carinii was identified in 19 (7.6%, 95% CI = 4.6 - 11.6) children. Fifteen of these (9.9%, 95% CI = 5.7 - 15.8) were in HIV-infected patients (table 3.2). PCP occurred predominantly in those newly diagnosed as HIV-positive with 13 of 64 (20.3%, 95% CI = 11.3 – 32.2) having PCP compared to 4 of 99 (4%, 95% CI = 1.1 - 10) HIV-negative children (OR (95% CI)= 6.04 (1.7 - 26.5); p<0.005). One HIV-infected child developed PCP while on TMP-SMX prophylaxis but adherence to medication could not be established. The 4 HIV-negative children with PCP were malnourished (median weight for age 69.9%). The median age of children with PCP [3(2-4) months] was lower than those without PCP [6 (3-17.5) months, p=0.01]. A detailed analysis of children with PCP is contained in chapter 5.

Bacteraemia (excluding Aeromonas species, Flavobacterium, Agrobacterium tumefa, Diptheroids, *S epidermidis* and Bacillus organisms which were regarded as possible contaminants) occurred in 35 of 244 (14.3%, 95% CI = 10.2 - 19.4) blood cultures and was equally prevalent in HIV-positive and negative children (13.5% and 15.6% respectively, p=0.65, table 3.2). *S pneumoniae* [12 children (4.9%)] and *S aureus* [5 cases (2%)] were the predominant isolates. *P aeruginosa, E coli, and H influenzae* occurred in 3 cultures (1.2%) each, *S typhi, C jejuni* and *E faecalis* in 2 children each (0.8%) and *K oxytoca, K pneumoniae* and *S marcescens* in 1 child each (0.4%). Organism type and antimicrobial resistance of bacteria did not differ by HIV status. Of the *S. pneumoniae* isolates, 6 (50%) were susceptible, 3 intermediate and 3 resistant to penicillin; the pattern of resistance did not differ by HIV status or by use of TMP-SMX

prophylaxis. All *S. aureus* isolates from blood were resistant to penicillin but susceptible to cloxacillin.

Bacteria were cultured from 17 of 32 (53%) BAL, 128 of 210 (61%) induced sputa and 166 of 231 (71%) nasopharyngeal specimens (Table 3.2). The type and number of bacteria cultured from respiratory secretions was similar in HIV positive and seronegative children except for *S. aureus*, which was more common in specimens from HIV-positive children. Nineteen of 25 (76%) children with *S. aureus* cultured from sputum or BAL had a prior hospital admission compared to 92 of 225 (41%) without *S. aureus* [RR 1.86 (1.42-2.44), p<0.001]. Similarly, 33 of 50 (66%) children with *S. aureus* in NPAs had previously been hospitalised compared to 67 of 114 (37%) without this organism, p<0.001. HIV-infected children with *S. aureus* in lower respiratory secretions were older than those without [13(9-36) versus 5 (3-13.5) months, p<0.001] and most (19 of 22) had been previously diagnosed as HIV positive. The prevalence of *S aureus* in lower respiratory secretions did not differ by HIV status when those previously diagnosed with HIV infection were excluded. Using multiple logistic regression (including the variables age, prior admission, use of TMP-SMX prophylaxis, previous diagnosis of HIV infection and HIV status), only a previous diagnosis of HIV-infection was associated with a positive *S aureus* nasopharyngeal (p=0.004) or sputum culture (p=0.006). For the majority of respiratory isolates, resistance to selected antimicrobials was higher in HIV-positive children but the only statistically significant difference was an increased TMP-SMX resistance in *M. catarrhalis* isolates from seropositive children (table 3.3).

The incidence of culture proven tuberculosis [in 20 children (8%)] was similar in HIV-positive and negative children (7.9% and 8% respectively, p=0.97). All *M tuberculosis* isolates were sensitive to isoniazid and rifampicin. Of the children culture positive for *M tuberculosis*, 4 of 12 (33.3%) HIV-positive and 5 of 7 (71.4%) negative children had a positive tuberculin skin reaction, p=0.12. In one HIV-negative child no record of the tuberculin skin reaction was kept.

Viruses, cultured in 37 (15.2%) of BAL or sputum samples were predominantly CMV (table 3.2). Bacteria were cultured from 19 (51.3%) of these samples. Overall, mixed bacterial–viral infections in sputa or BAL occurred in 19 children (7.8%).

3.5.2 Effect of TMP-SMX prophylaxis

Use of TMP-SMX prophylaxis in HIV-positive children did not change the overall incidence of bacteraemia which occurred in 9 of 58 (15.5%) children taking prophylaxis compared with 11 of 86 (12.8%) not on prophylaxis, p=0.64. However, the incidence of *S pneumoniae* bacteraemia was higher in children on prophylaxis [6 of 58 (10.3%) versus 1 of 86 (1.2%), p=0.012]. When the subset of children who had been previously diagnosed with HIV were compared by use of TMP-SMX prophylaxis, the increased rate of pneumococcal bacteraemia was still evident (6 of 58 versus 0 of 29 children, p=0.07). Of the pneumococcal isolates from children taking prophylaxis, 2 were susceptible, 3 were of intermediate resistance and 1 demonstrated high grade resistance to penicillin; the single isolate from a child not on prophylaxis was penicillin sensitive, p=0.248. HIV-infected children on TMP-SMX prophylaxis were not more immunocompromised than those not on prophylaxis as CD4/CD8 ratios ratios [0.3 (0.2-0.4) versus 0.4 (0.2-0.6, p=0.24], CD4 counts [638 (292-1001) versus

758 (404-1276) cells/μl, p=0.196] and percentage CD4 counts [14% (10.9-21.9) versus 15.1% (10.7-20.9), p=0.68] were similar. HIV-positive children with pneumococcal bacteraemia who were on TMP-SMX prophylaxis had similar CD4/CD8 ratios [0.35 (0.3-0.45) versus 0.3 (0.2-0.50, p=0.57] and percentage CD4 counts [19.1% (15.1-23.3) versus 13.7% (8.8-19.7), p=0.15] compared to those without bacteraemia. Other than *S pneumoniae*, there were no differences in the type or antimicrobial susceptibility of isolates from blood by use of TMP-SMX prophylaxis.

Use of TMP-SMX prophylaxis in HIV-infected children was not associated with any significant differences in the type or frequency of bacteria cultured from respiratory secretions except for an increased rate of *S. aureus* from nasopharygneal aspirates and a lower rate of *K pneumoniae* from sputa (Table 3.4). Antimicrobial resistance patterns of bacteria did not significantly differ by use of TMP-SMX prophylaxis except for a lower rate of ampicillin resistance in *K pneumoniae* nasopharyngeal isolates of children on prophylaxis but there were only 2 such isolates (Table 3.5). Although higher rates of resistance to cloxacillin and erythromycin were found in *S. aureus* isolates from sputum/BAL and nasopharygneal aspirates of children taking TMP-SMX prophylaxis, these differences were not statistically significant (Table 3.5).

Table 3.2: Microbial pathogens (number, %) by HIV status of children

Pathogen	HIV positive (n=151)	HIV negative (n=99)	OR (95% CI)
Blood culture (n=244)	n=148	n=96	
Gram positive bacteria	11 (7.4)	8 (8.3)	0.89 (0.31-2.67)
Gram negative bacteria	9 (6.1)	7 (7.3)	0.83 (0.27-2.7)
Sputum (n=210) / BAL (n=32)	n=146	n=96	
P carinii	15 (9.9)	4 (4)	2.62 (0.77-9.77)
Bacteria:			
S aureus	22 (15.0)	3 (3.1)	5.46 (1.56-29.2)*
K pneumoniae	16 (10.9)	14 (14.5)	0.72 (0.31-1.68)
H influenzae	13 (8.8)	12 (12.5)	0.68 (0.27-1.72)
P aeruginosa	12 (8.2)	3 (3.1)	2.76 (0.7-15.6)
*M tuberculosis***	11 (7.4)	8 (8.3)	0.89 (0.31-2.66)
M catarrhalis	4 (2.7)	5 (5.2)	0.51 (0.1-2.44)
S pneumoniae	2 (1.4)	4 (4.2)	0.32 (0.03-2.28)
Viruses:			
CMV	21 (14.3)	10 (10.4)	1.43 (0.61-3.58)
Others (HSV, parainfluenza, RSV)	2 (1.4)	4 (4.2)	0.32 (0.03-2.28)
Nasopharyngeal aspirates	n=135	n=96	
K pneumoniae	11 (8)	6 (6)	1.33 (0.43-4.55)
S aureus	41 (30)	9 (9)	4.22 (1.87-10.4)*
H influenzae	23 (17)	18 (19)	0.89 (0.43-1.88)
S pneumoniae	17(13)	11 (11)	1.11 (0.46-2.77)
M catarrhalis	21 (16)	18 (19)	0.8 (0.38-1.7)

* $p<0.05$
** One additional HIV-positive child cultured *M tuberculosis* on gastric lavage only

Table 3.3: Antimicrobial resistance patterns (no, %) from sputum, BAL and nasopharyngeal aspirates by HIV status

	Sputum/ BAL		NPA	
	HIV pos	**HIV neg**	**HIV pos**	**HIV neg**
K. pneumoniae	n = 16	n = 12	n = 11	n = 6
Ampicillin	4 (25)	4 (33)	10 (91)	5 (83)
TMP-SMX	10 (63)	6 (50)	3 (27)	3 (50)
Cefuroxime	5 (31)	4 (33)	4 (36)	2 (33)
S. aureus	n = 22	n = 3	n = 41	n = 9
Penicillin	19 (86)	3 (100)	36 (88)	9 (100)
Cloxacillin	14 (63)	1 (33)	19 (46)	3 (33)
Erythromycin	17 (77)	1 (33)	24 (59)	4 (44)
H. influenzae	n = 13	n = 13	n = 23	n = 18
Ampicillin	3 (23)	1 (8)	4 (17)	2 (11)
TMP-SMX	7 (54)	4 (31)	12 (52)	6 (33)
Cefuroxime	0 (0)	0 (0)	0 (0)	0 (0)
S. pneumoniae	n = 2	n = 4	n = 17	n = 11
Penicillin	2 (100)	2 (50)	9 (53)	3 (27)
TMP-SMX	2 (100)	3 (75)	11 (65)	6 (55)
Erythromycin	1 (50)	0 (0)	3 (27)	1 (6)
M. catarrhalis *	n = 4	n = 5	n = 21	n = 18
Penicillin	2 (50)	3 (60)	19 (90)	14 (78)
TMP-SMX	3 (75)	0 (0) †	13 (62)	3 (17) ‡
Erythromycin	2 (50)	1 (20)	0 (0)	1 (5)
Cefuroxime	0 (0)	0 (0)	0 (0)	0 (0)

* NCCLS zone diameter criteria applicable for *S. aureus* used

† p= 0.025 compared to sputum/ BAL isolates from HIV-positive children

‡ p=0.005 compared to NPA isolates from HIV-positive children

Table 3.4: Predominant bacterial isolates (no, %) from respiratory secretions in HIV-positive children by use of TMP-SMX prophylaxis

	Sputum / BAL		NPA	
	No TMP-SMX n = 83	TMP-SMX n = 58	No TMP-SMX n = 79	TMP-SMX n = 51
K. pneumoniae	14 (17)	2 (3) *	8 (10)	2 (4)
S. aureus	9 (11)	12 (21)	17 (22)	22 (43) †
H. influenzae	6 (7)	6 (10)	13 (16)	10 (20)
S. pneumoniae	0 (0)	1 (2)	6 (8)	9 (18)
M. catarrhalis	3 (4)	1 (2)	11 (14)	9 (18)

* p = 0.014 compared to sputum/ BAL of children not taking TMP-SMX

† p = 0.009 compared to NPA of children not taking TMP-SMX

Abbreviations: BAL - bronchoalveolar lavage fluid, NPA - nasopharyngeal aspirate, TMP-SMX - trimethoprim-sulphamethoxazole

Table 3.5: Antimicrobial resistance patterns (no, %) from respiratory secretions in HIV-positive children by use of trimethoprim-sulphamethoxazole (TMP-SMX) prophylaxis

	Sputum / BAL		NPA	
	No TMP-SMX n = 83	TMP-SMX n = 58	No TMP-SMX n = 79	TMP-SMX n = 51
K. pneumoniae	n = 14	n = 2	n = 8	n = 2
Ampicillin	13 (93)	1 (50)	8 (100)	1 (50) †
TMP-SMX	8 (57)	1 (50)	2 (25)	0 (0)
Cefuroxime	3 (21)	1 (50)	2 (25)	1 (50)
S. aureus	n = 9	n = 12	n = 17	n = 22
Penicillin	8 (89)	10 (83)	15 (88)	19 (86)
Cloxacillin	5 (55)	9 (75)	6 (35)	13 (59)
Erythromycin	6 (67)	10 (83)	7 (41)	16 (72)
H. influenzae	n = 6	n = 6	n = 13	n = 10
Ampicillin	2 (33)	1 (17)	1 (8)	3 (30)
TMP-SMX	4 (67)	3 (50)	7 (54)	6 (60)
Cefuroxime	0 (0)	0 (0)	0 (0)	0 (0)
S. pneumoniae	n = 0	n = 1	n = 6	n = 9
Penicillin	-	1 (100)	3 (50)	5 (55)
TMP-SMX	-	1 (100)	4 (67)	5 (55)
Erythromycin	-	1 (100)	1 (17)	2 (22)
M. catarrhalis *	n = 3	n = 1	n = 11	n = 9
Penicillin	1 (33)	0 (0)	9 (82)	9 (100)
TMP-SMX	2 (66)	0 (0)	5 (45)	7 (78)
Erythromycin	1 (33)	1 (100)	0 (0)	0 (0)
Cefuroxime	0 (0)	0 (0)	0 (0)	0 (0)

* NCCLS zone diameter criteria applicable for *S. aureus* used
† p= 0.05 compared to isolates from NPAs in children not on TMP-SMX
Abbreviations: BAL - bronchoalveolar lavage fluid, NPA - nasopharyngeal aspirate

3.6 Hospital course and outcome

Most children (78.9%) were treated with supplemental oxygen and 36 (14.4%) with positive pressure ventilation. Thirty-nine children (15.6%, 95% CI 11.5 – 20.5) died in-hospital; the mortality rate was higher in HIV-positive (31 of 151, (20.5%, 95% CI 14.7 – 27.5) compared with seronegative children (8 of 99, 8.1%, 95% CI 3.8 - 14.8, p=0.008) (table 3.6). In addition, HIV-positive children had a longer in-hospital stay (median of 14 days compared to 10.5 days in seronegative patients, p<0.005) (table 3.6). Children who died were younger than those who survived (median age 3 (2-7) months compared to 6 (3-18) months, p=0.016). The nutritional status of those who died was not significantly worse than those who survived as evidenced by a similar median weight for age [73.3% (57.7-91.3) versus 74.3% (61.6-88.5), p=0.768] and median height for age [87.3% (78.8-97.4) versus 89.8% (83.7-95.7), p=0.411]. When the effect of nutrition on outcome was analysed by HIV status, no association between mortality and poor nutrition was found in HIV-positive children. Amongst HIV-positive children, the median weight for age [76.3% (59.5-91.6)] and height for age [89.4% (82.3-97.4)] in those who died was similar to that in survivors [71.6% (60.1-84.2), p=0.411 and 89.4% (82.6-93.1), p=0.974, respectively]. However, amongst HIV-negative children, those who died had a lower median weight for age [65.8% (50.6-78.1) compared with 81.7% (65.5-92.8), p=0.09] and height for age [81.6% (78.8-91.4) compared with 91.6% (84.3-98.2), p=0.181] although these differences were not statistically significant.

Eight (7 HIV-infected) of 19 (42%, 95% CI = 20.3 - 66.5) children with PCP died compared to 31 of 231 (13.4%, 95% CI = 9.3 - 18.5) without PCP [RR=1.5 (1.02-

2.2), p<0.005]. When children with PCP were excluded, the mortality rate for HIV-infected patients was still higher [17.6% compared to 7.4%, RR=1.12 (1.02-1.24), p=0.02]. The mortality rate of 15% for children with tuberculosis was similar to those without TB (15.6%, p=0.9). All HIV-negative children died in ICU in contrast to 6 of 31 (19.4%) HIV-positive patients (p<0.001). A detailed analysis of children admitted to ICU is contained in chapter 4. Risk factors for mortality in HIV-positive children by univariate analysis included younger age, a more severe clinical presentation (as evidenced by a higher presenting respiratory rate or the presence of cyanosis), lower platelet count, serum globulin or CD4 count, higher serum protein, LDH or CD8 count and a diagnosis of PCP (table 3.7). Using multiple logistic regression, only PCP was a significant risk factor for mortality (p=0.03).

Table 3.6: Hospital course and outcome of children by HIV status

	HIV positive (n=151)	HIV negative (n=99)	p
Hospital days	14 (9-20)	10.5 (7-16)	<0.005
ICU days	6 (5-8)	5 (3-10)	0.521
IPPV, n (% of ICU)	8 (38.1)	28 (51)	0.32
Hospital death, n (%)	31 (20.5)	8 (8.1)	0.008

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)

ICU- intensive care unit, IPPV-intermittent positive pressure ventilation

Table 3.7: Risk factors for mortality in HIV positive children with pneumonia

Risk factor	Alive (n=120)	Died (n=31)	p
Age (months)	12 (5-25)	3 (3-9)	<0.001
Presenting signs			
Respiratory rate	50 (40-60)	60 (48-68)	0.002
Cyanosis, n (%)	27 (22.5)	16 (51.6)	0.003
Clubbing, n (%)	28 (23.3)	2 (6.5)	0.04
Lab findings:			
Platelets (10^9/l)	389 (246-446)	229 (41-367)	0.002
LDH (U/l)	307 (243-439)	683 (401-909)	<0.001
Protein (g/l)	79.5 (65.5-91)	65 (55-75)	<0.001
Globulin (g/l)	51 (41-62)	34 (28-45)	<0.001
CD4 (cells/μl)	760 (385-1276)	431 (108-766)	0.002
CD8 (cells/μl)	2486 (1281-3773)	1077 (495-1519)	<0.001
PCP, n (%)	8 (6.7)	7 (22.6)	0.02

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)

LDH – lactate dehydrogenase, PCP- *P carinii* pneumonia

3.7 Discussion

3.7.1 Aetiology of lung disease

PCP was found to be an important AIDS defining infection, occurring in 20% of those newly diagnosed as HIV positive. This PCP incidence is probably an underestimate as the diagnosis was made using induced sputum in most children. Due to resource constraints, BAL could not be performed in sputum negative children nor could post mortem examinations be done, which probably would have increased the incidence of PCP. Our results confirm the need for early diagnosis of HIV infection and institution of TMP-SMX prophylaxis in this geographical area. A detailed discussion of the importance of PCP in this population of children is provided in chapter 5.

The results of this study indicate an increased rate of *S. aureus* in both upper and lower respiratory secretions of children with pneumonia and HIV infection compared to seronegative patients. Apart from *S. aureus* there were no differences in the type or number of bacteria isolated from respiratory secretions in HIV positive and negative children. Although antimicrobial resistance of bacteria tended to be higher in HIV-positive compared with seronegative children, the only significant difference was a higher rate of TMP-SMX resistance in *M. catarrhalis* isolates. Small sample size may possibly contribute to the lack of statistical significance for many isolates. The importance of increased resistance in *M. catarrhalis* isolates is unclear as this bacteria was never cultured from blood and was rarely isolated from lower respiratory secretions and thus appears to be an uncommon cause of pneumonia. However, as this bacteria frequently causes upper respiratory infections particularly otitis media and sinusitis (Wald 1995), an increased incidence of TMP-SMX resistance may occur in

such infections. In any event, as TMP-SMX resistance was present in a substantial number of *S. pneumoniae* and *H. influenzae* isolates, this antibiotic cannot be recommended for treatment of respiratory tract infections in this population of children. Besides *S aureus*, the similarity in prevalence, organism type and resistance patterns of bacteria in HIV-positive and negative children is consistent with results of a Zambian study that reported that most acute lower respiratory tract infections in HIV-infected children responded to simple antibiotic and supportive treatment (Smyth et al 1997).

The increased rate of *S. aureus* culture in HIV positive children most probably indicates respiratory tract colonisation as this pattern was found in sputum or BAL as well as NPAs. An increased rate of carriage of *S. aureus* in HIV positive adults has been previously described in male homosexuals (Ganesh et al 1989, Battan et al 1991) and intravenous drug users (Styrt et al 1997) in developed countries and in urban Kenyan patients (Amir et al 1995). Reasons for an increased colonisation rate with *S. aureus* in HIV positive individuals are unclear. Acquisition of *S. aureus* may occur in hospital, thus frequent hospital admissions may enhance carriage of this organism in HIV-positive patients (Weinke et al 1992). In support of this, the majority of children with *S. aureus* colonisation (in sputum, BAL or NPAs) had been previously admitted to hospital. However, colonisation with *S. aureus* may also occur at a more advanced stage of respiratory illness resulting in more frequent hospitalisations. Another possibility is that infection with *S. aureus* may be associated with a specific immune defect in HIV infection (Amir et al 1995, Weinke et al 1992). Alternatively, chronic use of antibiotics especially TMP-SMX prophylaxis by HIV positive patients may result in selective colonisation by *S. aureus*. This hypothesis is supported by the results

of the current study as a significantly higher rate of *S. aureus* colonisation was found in NPAs of HIV-positive children who were using TMP-SMX prophylaxis.

Although use of TMP-SMX prophylaxis was associated with a higher rate of *S. aureus* colonisation, no significant change in resistance patterns was found. *S. aureus* isolates from HIV-infected patients have been reported to develop increased erythromycin, TMP-SMX or tetracycline-resistance compared to seronegative patients (Amir et al 1995, Martin et al 1999). Although susceptibility of staphylococci to either tetracycline or TMP-SMX was not assessed, higher rates of erythromycin and cloxacillin-resistance occurred in respiratory isolates from HIV-positive compared with seronegative children although these differences were not significant. Moreover, increased resistance to these antibiotics was found in *S. aureus* respiratory isolates of HIV-positive children taking TMP-SMX prophylaxis, but these differences also did not reach statistical significance. As the number of *S. aureus* isolates is relatively small, it is possible that a type II error may have occurred. The current study population comprised predominantly infants and young children. Although we were unable to determine when *S. aureus* colonisation had occurred or the exact duration of TMP-SMX prophylaxis, it is probable that HIV-infected children were put on prophylaxis at the time of HIV diagnosis in accordance with hospital policy. As the median age of children with *S. aureus* in lower respiratory secretions was 16 months but the median age of HIV diagnosis 7 months, it is likely that these patients were taking prophylaxis for about 9 months. With longer follow-up and more prolonged use of antibiotics, increasing development of drug resistant strains may emerge in HIV-positive children.

Although the incidence and type of bacteraemia did not differ by HIV status, HIV-positive children on TMP-SMX prophylaxis had a higher rate of *S pneumoniae* bacteraemia than those not using prophylaxis. Prior studies have reported an increased incidence of invasive pneumococcal disease in HIV-infected children (Krasinski 1988, Andiman et al 1994, Mao et al 1996), however, the association between use of prophylaxis and occurrence of disease is not clear. No difference in the rate of invasive pneumococcal infection by use of TMP-SMX prophylaxis was found in a study of 82 HIV-infected children (Mao et al 1996). However, an increased risk of pneumonia was reported in HIV-infected children on TMP-SMX prophylaxis compared with those off prophylaxis, but this was attributed to the more advanced disease stage in the former (Mofenson et al 1998). In the current study, HIV-positive children with pneumococcal bacteraemia who were on TMP-SMX prophylaxis did not demonstrate a greater degree of immunosuppression than those without bacteraemia. A higher rate of pneumococcal nasopharyngeal colonisation has been found in children with respiratory illness born to HIV-positive mothers compared with controls (Rusen et al 1997). Moreover increased rates of colonisation with penicillin-resistant pneumococci have been reported in children on TMP-SMX prophylaxis compared to age-matched healthy controls (Abdel-Haq et al 1999). Although the rate of nasopharyngeal colonisation with *S pneumoniae* and of penicillin-resistance were higher in HIV-positive than negative children in the present study, this was not statistically significant. However, this may be a due to the relatively small sample of children. Moreover, there was a trend towards an increased rate of colonisation in HIV-infected children on TMP-SMX prophylaxis. As nasopharyngeal colonisation is a risk factor for disease (Kellner et al 1998), it is possible that use of TMP-SMX may result in increased rates

of colonisation with *S pneumoniae* and penicillin-resistance so predisposing to invasive disease.

For all other bacteria, TMP-SMX prophylaxis was not associated with an increased rate of culture from blood, sputum, BAL or NPA, nor was the antimicrobial susceptibility altered by use of TMP-SMX prophylaxis. Given the high incidence of PCP found in the study, use of TMP-SMX prophylaxis should be an important intervention to reduce mortality and morbidity in South African HIV-infected infants. Moreover, studies of HIV-infected adults have found that TMP-SMX prophylaxis reduced the incidence of other HIV-associated infections and resulted in significantly improved survival, but there is no comparable data for children (Edge and Rimland 1996, Wiktor et al 1999). The impact of TMP-SMX prophylaxis on the epidemiology of other HIV-associated infections and long term survival of children needs further study.

The incidence of culture proven pulmonary tuberculosis was similar in HIV-positive and negative children. In contrast to the data from HIV-positive adults in whom the risk of tuberculosis is increased, studies of the association between HIV infection and TB in children have provided conflicting data (Coovadia et al 1998). Cross sectional studies have reported co-infection in 11-64% of African children but longitudinal and mortality data have not demonstrated an increased incidence of TB in HIV-positive children (Mukadi et al 1997, Luo et al 1994, Harries et al 1997, Lallemant et al 1994). Differences in study design, sampling methods and difficulties in diagnosing both infections, but particularly tuberculosis may account for these discrepancies. In the present study, a culture confirmed diagnosis of tuberculosis was required. However,

the results may be influenced by the use of an HIV-negative control group who were either severely ill or had clinical signs suggestive of AIDS. Finally, as the sample of TB-infected children in the present study is small, a type II error cannot be excluded. The tuberculin skin test was positive in only 33% of HIV-infected children with culture proven TB, demonstrating the high incidence of anergy that is associated with HIV infection (Nachmann and Navaie-Waliser 1996, Graham et al 1992). This rate of tuberculin skin reactivity is consistent with that found in a similar paediatric population in Johannesburg, South Africa in which 21% of HIV-infected children with a clinical diagnosis of TB had a positive skin reaction (Madhi et al 1999). Inability to develop reactions to tuberculin due to impaired cell mediated immunity in HIV-infected children is also evidenced by a significantly lower rate of scar formation at the site of BCG immunisation compared with seronegative patients. The tuberculin skin reaction results for seronegative children are similar to those previously reported in which approximately ten to 25% of HIV-uninfected patients with TB did not demonstrate a positive reaction (Nachman and Navaie-Waliser 1996, Sbarbaro 1986).

LIP occurred in approximately 7% of HIV-infected children and was associated with the presence of digital clubbing. HIV-infected children with clubbing were older than those without this sign suggesting that clubbing had developed in association with chronic lung disease. Older age associated with clubbing might reflect the more favourable prognosis associated with LIP compared with other HIV-related pulmonary illnesses (Schneider 1996, Spira et al 1999). However, as the typical radiological changes of reticulonodular infiltrates and adenopathy may not be present and as non-specific changes such as increased bronchovascular markings may occur in milder cases of LIP (Berdon et al 1993, Marquis et al 1993), it is possible that LIP was under

diagnosed in our study. Due to resource constraints and ethical considerations, we were unable to perform lung biopsies to histologically confirm LIP. Nevertheless, the study suggests that in geographical areas with high HIV seroprevalence rates, the presence of clubbing in a child hospitalised for respiratory disease should raise a suspicion of HIV infection and the possibility of underlying LIP. Diagnosis of LIP is important as symptomatic disease may be amenable to treatment with oral corticosteroids (Bye 1995).

3.7.2 Outcome

Despite the similarities in presenting respiratory signs and in bacterial and viral pathogens, HIV-infected children had a higher mortality rate than HIV-negative patients even when the presence of PCP was excluded. The increased mortality rate found in HIV-positive children is consistent with studies in both developed and developing countries that have reported that HIV infection increases infant and child mortality (Blanche et al 1989, Lallemant et al 1989, European Collaborative Study 1991, Taha et al 1995, Vetter et al 1996). A number of African studies have reported increased mortality rates for HIV-positive compared to seronegative children; these studies have also highlighted the importance of pneumonia as a cause of death. A prospective study of 1385 children in Malawi found that the independent risk of mortality was 5 times higher for infants born to HIV seropositive mothers than for those born to seronegative women (Taha et al 1995). A longitudinal cohort study of infants with vertically transmitted HIV infection in Durban, South Africa found that 17 of 48 (35%) HIV-infected compared to 8 of 93 (8.6%) HIV-uninfected children died; 83% of HIV-related deaths occurred before 10 months of age (Bobat et al 1999).

The mortality rate of 20% for HIV-positive children found in the present study is similar to that reported in a few African studies. A prospective study of 4480 children hospitalised in Cote d'Ivoire reported that 20.8% of HIV positive children died compared to 8.7% seronegative patients [RR 2.4 (1.9-3.1)]. Although this study was not restricted to children with pneumonia, acute respiratory infection accounted for 24% of deaths in HIV positive and 18% in seronegative children respectively (Vetter et al 1996). A study of 150 Malawian children (93 HIV-infected) hospitalised with pneumonia, reported the case fatality rate for pneumonia to be 22%; death was independently and significantly associated with HIV infection [RR 2.98 (1.1-7.9)] (Graham et al 2000). A retrospective study of paediatric hospital admissions at an urban South African hospital Chris Hani Baragwanath from 1992 to 1997, reported a mortality of 13.2% in HIV-infected children compared to 5.1% in uninfected patients; pneumonia was the commonest cause of mortality, accounting for 24.6% of deaths (Zwi et al 1999).

Several factors may account for a higher mortality rate in HIV-infected children. More rapid progression of pneumonia, inability to mount an effective immune response and less aggressive medical management such as reluctance to ventilate HIV-infected children. Although nutritional status has been reported to be associated with mortality from pneumonia (Smyth et al 1997), in the current study, children who died did not have worse nutrition than those who survived. Although there was a tendency for malnutrition to be associated with higher mortality in HIV-negative children this was not evident in HIV-infected children. The high mortality associated with PCP, which occurred in young, well nourished HIV-infected infants may partly explain these

findings (chapter 5, table 5.1). Lack of an association between malnutrition and outcome is consistent with the findings from a Zimbabwean study in which HIV-infected children were found to have a threefold risk of dying from pneumonia compared to seronegative patients (mortality rate of 28% compared to 9%) even after controlling for nutritional status, age and birthweight (Nathoo et al 1993).

Access to care may be an additional factor impacting on the higher mortality rate in HIV-infected children. When resources are limited, it is likely that care is prioritised for immunocompetent children in preference to those who are HIV-positive. As evidence of this, all HIV-negative children who died had received ICU care whereas 80% of HIV-positive children died in a general ward. A detailed discussion of the outcome of children admitted to ICU and those with PCP is contained in chapters 4 and 5.

3.7.3 Limitations

A criticism of this study may be the use of a control group of HIV-negative children who were selected because of the severity of their clinical presentation. Although the results may not be generalisable to HIV negative children with less severe disease, this control group was selected as they more closely resemble the clinical illness of HIV-infected children with pneumonia and because they constitute the sickest children who are most likely to be hospitalised in developing countries (Shann 1986). It is likely that the differences in outcome between HIV-positive and negative children would have been even greater if less severely ill HIV negative patients had been included. The

results therefore reflect differences between HIV positive and negative children with a similar severity of illness.

The number of patients in some subgroups may be too small to detect significant differences or associations. However, the main aim of this study was to investigate differences in aetiology and outcome of pneumonia in HIV-infected children and not differences within subcategories as stratified by aetiological agent. Nevertheless, the findings of differences in some sub-groups may raise interesting considerations for further studies.

Induced sputum was used in the majority of children for the identification of pulmonary pathogens. Induced sputum has not been routinely used in young children and infants; BAL is more widely performed in developed countries when microbiologic confirmation of the aetiology of pneumonia is required (Bye et al 1987). Alternatively, lung biopsy has been regarded as the gold standard for identifying pathogens (Chaudhary et al 1977). However, due to resource and ethical considerations, BAL or lung biopsy could not be performed in most children. In non-intubated children, bronchoscopy and BAL would be done under general anaesthetic in the operating room at Red Cross Childrens Hospital as there is currently no dedicated bronchoscopy facility. Due to limitations in resources both for doing the procedure and for supporting the child in ICU afterwards, bronchoscopy and BAL could not be undertaken in non-intubated children who were sputum negative for pathogens. Dramatic circulatory collapse thought to result from HIV-associated autonomic neuropathy has been reported in HIV-infected adults with suspected PCP who underwent percutaneous transthoracic fine-needle aspiration (Craddock et al 1987);

thus this procedure could not be safely performed in children in the present study. Nevertheless, the results obtained from induced sputum suggest that lower respiratory tract secretions can be successfully obtained even in very young infants (chapter 6). However, BAL may have produced a higher yield for specific pathogens. The yield from induced sputum should therefore be regarded as a conservative measure of the true number of children with specific pulmonary pathogens.

3.8 Conclusion

A quarter of children hospitalised for pneumonia were diagnosed with HIV infection at the time of admission. Amongst these children, PCP occurring in 20%, was an important AIDS indicator disease and was associated with a high mortality. Other than PCP, the aetiology and presentation of acute pneumonia was similar in HIV-positive and negative children however, HIV-positive children had a longer hospitalisation and higher mortality rate. Even when children with PCP were excluded, the mortality rate amongst HIV-infected children was significantly higher than that for seronegative patients. However, allocation of resources may partly account for this difference as almost 80% of HIV-positive children who died did not have the opportunity of ICU care.

The microbiological results suggest that the risk of invasive pneumococcal disease is increased in HIV-infected children on TMP-SMX prophylaxis and that *S. aureus* may become an increasingly important potential pathogen in HIV-positive children and in the community. Although the number of children with pneumococcal bacteraemia was small, the increased incidence associated with TMP-SMX prophylaxis use is of

concern. The findings are of importance in the management of infections not only in HIV-infected children but also in the general population as the type and antimicrobial susceptibility of bacteria carried in the nasopharynx reflect pathogens circulating and causing infection in the community (Gehanno 1998, Kellner 1998). Long term monitoring of bacterial pathogens and their resistance patterns is needed particularly in the light of the rising HIV epidemic in sub-Saharan Africa. Additional, larger paediatric studies are needed to clarify the impact of TMP-SMX prophylaxis on bacterial respiratory tract colonisation, invasive infection and on antimicrobial susceptibility.

CHAPTER 4: RESULTS II

Aetiology and outcome of pneumonia in children admitted to an intensive care unit

4.1 Patients enrolled

One hundred and three children were admitted to an intensive care unit (ICU) for pneumonia of whom 76 (74%) were enrolled in the study. Twenty-seven children were not enrolled as they died before consent for the study could be obtained or more than 48 hours since ICU admission had elapsed or a parent refused permission for participation. Of the 27 children not enrolled, 9 (1 HIV-positive, 4 HIV-negative, 4 not tested) died. The median age of children enrolled was 3 (1-4) months. Twenty-one (27.6%, 95% CI =18 - 39.1) were HIV-infected; of these 15 (71.4%, 95% CI = 47.8 - 88.7) were diagnosed as HIV-positive at the time of admission. None of the children diagnosed with HIV on admission had been previously hospitalised while all those known to be HIV-infected had a prior hospitalisation. HIV-positive and negative children were of a similar age but females predominated in the former (table 4.1).

4.2 Clinical signs

HIV-positive children had a higher respiratory rate at presentation but the incidence of lung findings and chest indrawing was similar in the 2 groups (table 4.1). Clinical signs of HIV infection (hepatomegaly, splenomegaly and generalised lymphadenopathy) were more common in HIV-infected children and occurred in 19 of 21 (90.5%, 95% CI = 69.9 - 98.8) HIV-infected children. Clubbing occurred exclusively in 3 HIV-positive older children with a median age of 28 (25-38) months.

4.3 Radiology

The most common radiological findings were alveolar infiltrates and consolidation (table 4.1). Chest radiology findings did not differ according to HIV status (table 4.1).

4.4 Laboratory findings

HIV-infected children had lower platelets and percentage CD4 lymphocytes but higher serum protein and lactate dehydrogenase (LDH) on presentation (table 4.1). Ten of 21 (47.6%) HIV-positive children were severely immunosuppressed [CD4/ T cells (%) less than 15%] compared to none of those who were HIV-negative, $p<0.005$.

4.5 Microbiology

The incidence of bacteraemia [11 of 72 blood cultures (15.3%, 95% CI =7.9 - 25.7)] was similar in HIV-infected (15.1%) and uninfected children (15.8%), p=0.94. *S pneumoniae* [in 5 patients (6.8%)] and *S aureus* [in 3 children (4.1%)] were the predominant isolates (table 4.2). Gram negative bacteraemia (*S typhi*, *C jejuni* and *S marcescens*) occurred in single patients each and exclusively in HIV-negative children. One HIV-negative child cultured *S pneumoniae* and *H influenzae* from a single blood culture.

PCP occurred in 9 (11.8%, 95% CI = 5.6 – 21.3) children (median age 3(2-3) months), 8 (89%) of whom were HIV-infected (table 4.2). However, HIV infection was diagnosed in 7 of the eight only at the time of admission despite the presence of clinical features of AIDS in six. One HIV positive child developed PCP while on TMP-SMX prophylaxis but adherence to medication could not be confirmed. PCP therefore occurred in 8 of 21 (38.1%, 95% CI = 19.5 - 59.8) HIV-infected children and was the AIDS defining illness in 7 of 15 (47%, 95% CI = 23.2 - 71.3). A single HIV-negative malnourished child (median weight for age 64%) developed PCP and *S pneumoniae*

bacteraemia; HIV status was confirmed with a negative PCR in the child and a negative HIV ELISA in the mother. In 7 children PCP was diagnosed on BAL fluid while 2 had a positive IF on induced sputum. Children with PCP had a lower CD4 count [880 (520-1140) versus 1780 (1100-2600) cells/µl, p=0.014], percentage CD4/T lymphocytes [15 13.4-27.5) versus 38.2 (24.5-45.7), p=0.003] and CD4/CD8 ratio [0.7 (0.4-1) versus 2.1 (1.3-3), p=0.04] but higher serum LDH (552 (388-1214) versus 369 (294-575) U/l, p=0.044] than those without PCP.

Bacteria, cultured in 17 of 32 (53%) BAL and 26 of 41 (63%) induced sputa specimens did not differ by HIV status (table 4.2). *M tuberculosis* was cultured from 6 (8%) children (table 4.2). Infection with TB occurred in 3 of 21 HIV positive patients (14.3%) which was similar to the rate of 3 of 52 (5.8%) in HIV negative children; p=0.23. Viruses, cultured in 8 children (11%), grew predominantly CMV (table 4.2). The prevalence of viruses did not differ by HIV status. Mixed bacterial–viral infections occurred in 4 children (5.5%).

Table 4.1: Presenting features of children admitted to ICU by HIV status

	HIV positive (n=21)	HIV negative (n=55)	P
Age (months)	3 (2-9)	2 (1-4)	0.14
Male:female	0.6	2.7	0.005
African race, n (%)	19 (90.5)	35 (63.6)	<0.001
Clinical signs			
Heart rate	160 (150-180)	154 (140-164)	0.07
Respiratory rate	60 (60-70)	56 (50-65)	0.04
Temp (° C)	37 (36.5-38)	37.1 (36.6-37.6)	0.7
Weight for age (%)	89 (68.5-100.2)	76.2 (60.2-92.6)	0.18
Height for age (%)	95.5 (88-106)	89.5 (83-96)	0.04
Weight for height	90 (83-100)	95 (89-106)	0.1
Clubbing, n (%)	3 (20)	0 (0)	0.004
Cyanosis, n (%)	14 (66.6)	26 (47.3)	0.13
Adenopathy, n (%)	11 (52.3)	8 (14.5)	<0.001
Hepatomegaly, n (%)	19 (96)	34 (72.7)	0.02
Splenomegaly, n (%)	13 (64.9)	8 (25.2)	<0.001
Lower chest indrawing	12 (54.3)	25 (53.5)	0.37
Lung findings**,n (%)	19 (92.7)	53 (92.9)	0.31
Blood tests			
Haemoglobin (g/dl)	9.8 (8.1-11.5)	10.1 (9.5-11.9)	0.202
Platelets (10^9/l)	252 (56-380)	447 (296-621)	0.02
Protein (g/l)	76 (56-93)	55 (48-63)	<0.001
Albumin (g/l)	26 (23-34)	33 (26-38)	0.075
WBC (10^9/l)	10.5 (8.1-17.1)	14.0 (9.5-22.3)	0.118
CD4 (% lymphocytes)	15.0 (10.8-21.9)	39.7 (31.3-45.6)	<0.001
CD4/CD8	0.4 (0.3-1.0)	2.3 (1.7-3.6)	<0.001
LDH (U/l)	552 (372-825)	360 (291-529)	0.017
Radiology, n (%)			
Normal Xray	2 (9.5)	3 (5.4)	0.522
Hyperinflation	11 (52.3)	32 (58.2)	0.650
Alveolar infiltrate	19 (90.5)	49 (89.1)	0.861
Consolidation	19 (90.5)	53 (96.4)	0.307
Adenopathy	1 (4.8)	3 (5.4)	0.904
Cavity	0 (0)	2 (3.6)	0.379
Pleural effusion	3 (14.3)	4 (7.3)	0.348

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)

** presence of adventitious sounds including wheezes, bronchial breathing or crackles

Table 4.2: Microbiological isolates (n) of children admitted to ICU by HIV status

	HIV positive	HIV negative	OR (95% CI)	p
Blood culture (n)	19	53		
S pneumoniae	2	3	1.96 (0.2-18.5)	0.48
S aureus	1	2	1.42 (0 – 28.6)	0.78
Gram negative bacteria	0	4	0 (0-4.3)	0.22
BAL / sputum (n)	21	52		
*P carinii**	8	1	31.4 (3.5-1418.5)	<0.001
K pneumoniae	3	8	0.9 (0.1-4.5)	0.93
H influenzae	3	6	1.28 (0.2-6.8)	0.75
M tuberculosis	3	3	2.7 (0.3-21.9)	0.23
A baumanii	1	5	0.5 (0 – 4.6)	0.5
P aeruginosa	3	2	4.17 (0.4-52.4)	0.11
S aureus	2	2	2.63 (0.2-38.1)	0.34
M catarrhalis	2	2	2.63 (0.2-38.1)	0.34
S pneumoniae	0	2	0 (0-13.3)	0.36
CMV	2	2	2.63 (0.2-38.1)	0.34
Other viruses	1	3	0.82 (0-10.9)	0.86

Footnotes: **P carinii* was detected either by silver stain or immunofluorescence

4.6 Hospital course and outcome

Intermittent positive pressure ventilation (IPPV) was used in 36 children (47.4%, 95% CI =35.8 - 59.1) for a median of 3 (2-6) days. Use and duration of IPPV and ICU and hospital stay were similar for HIV-positive and negative children but HIV-infected patients experienced more severe hypoxia with a lower median PaO_2 and higher oxygen requirement (table 4.3). Median stay in ICU of 5 (3-9) days was longer for those who received IPPV [7 (5-10) versus 5 (3-9) days; $p<0.05$]. Eight of 9 children (88.9%, 95% CI = 51.8 - 99.7) with PCP received IPPV compared to 26 of 67 (38.8%, 95% CI = 27.1 - 51.5) without PCP [OR 12.62 (1.5-573.1), p=0.005]. HIV-infected children with PCP had a higher rate of IPPV compared to HIV-positive patients without PCP but among those selected for IPPV there was no significant difference in ventilation requirements (table 4.4).

Fourteen of 76 (18.4%, 95% CI = 10.5 - 29) children died in-hospital. Although the in-hospital mortality rate for HIV-positive children was double that for negative patients, this did not reach statistical significance [6 of 21 (28.6%, 95% CI = 11.3 - 52.2) compared to 8 of 55 (14.5%, 95% CI = 6.5 - 26.7); RR 1.33 (0.83-2.13), p=0.16]. Mortality in those found to be HIV-infected on admission compared to those previously diagnosed with HIV was also higher but this difference was not statistically significant [5 of 15 (33%, 95% CI = 11.8 - 61.6) versus 1 of 6 (16.7%, 95% CI = 0.4 - 64); RR 2 (0.29-13.74), p=0.46]. All children who died received IPPV except for 2 HIV-infected patients. Four of 9 children (44.4%, 95% CI = 13.7 - 78.8) with PCP died compared to 10 of 67 (15%, 95% CI = 7.4 - 25.7) without PCP [RR 1.29(0.92-1.8), p=0.03]. Excluding children with PCP, 23% of HIV-positive compared to 13%

of HIV-negative children died [RR 1.18 (0.77-1.80), p=0.36]. Risk factors for mortality in all children included PCP, a high presenting heart rate, an enlarged liver or spleen, low platelets or serum albumin and high LDH; ventilation requirements were lower in survivors (table 4.5). However, multivariate analysis did not identify any significant risk factors for mortality. On univariate analysis, no variables were predictive of mortality in the subgroup of HIV-infected children, but the sample size was small.

Table 4.3: Ventilation requirements and outcome of children by HIV status

Outcome	HIV positive (n=21)	HIV negative (n=55)	p
IPPV, n (%)	8 (38.1)	28 (51)	0.31
IPPV (days)	4 (2-6)	3 (2-7)	0.73
ICU (days)	6 (5-8)	5 (3-10)	0.52
Hospital (days)	16 (9-19)	11 (7-19)	0.19
Highest PIP (cm H_2O)	28 (25-29)	25.5 (24-30)	0.75
Highest PEEP (cm H_2O)	8 (6-12)	6 (5-9)	0.11
Highest FIO_2 (%)	0.7 (0.6-0.95)	0.5 (0.4-0.8)	0.06
Highest $PaCO_2$ (mmHg)	52 (47-62)	66 (47-74)	0.30
Lowest PaO_2 (mmHg)	27 (26-28)	42 (32-55)	0.01
Deaths, n (%)	6 (28.6)	8 (14.3)	0.16

Footnotes : Continuous variables are median (25^{th}-75^{th} percentile)

: PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; FIO_2, fraction of inspired oxygen; $PaCO_2$, partial pressure arterial carbon-dioxide; PaO_2, partial pressure arterial oxygen

Table 4.4: Intermittent positive pressure ventilation (IPPV) requirements and outcome of HIV-positive children admitted to ICU by presence of PCP

Outcome	PCP (n=8)	No PCP (n=13)
IPPV, n (%)	7 (87.5)	1 (7.7)*
IPPV (days)	4 (2-6)	2 (2-2)
ICU (days)	7 (6-9)	6 (4-8)
Hospital (days)	10.5 (7-16.5)	18 (10-21)
Highest PIP (cm H_2O)	27 (25-28)	29 (29-29)
Highest PEEP (cm H_2O)	8 (7-12)	6 (6-6)
Highest FIO_2 (%)	0.7 (0.6-1.0)	0.9 (0.9-0.9)
Deaths, n (%)	3 (37.5)	3 (23.1)

Footnotes : Continuous variables are median (25^{th}-75^{th} percentile)

: PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; FIO_2, fraction of inspired oxygen; $PaCO_2$, partial pressure arterial carbon-dioxide; PaO_2, partial pressure arterial oxygen

Table 4.5: Factors associated with mortality in children admitted to ICU for pneumonia

Risk factor	Alive (n=62)	Died (n=14)	p
PCP, n (%)	5 (8)	4 (29)	0.033
Presenting signs			
Heart rate	155 (140-162)	175 (155-190)	0.015
Hepatomegaly, n (%)	39 (63)	14 (100)	0.007
Splenomegaly, n (%)	13 (21)	8 (57)	0.007
Lab findings			
Platelets (10^9/l)	438 (273-632)	55 (31-367)	0.002
Albumin (g/l)	33 (26-37)	24.5 (18-32)	0.023
LDH (U/l)	369 (294-560)	561 (427-1184)	0.043
IPPV requirements			
Highest PIP (cm H_2O)	25 (22-27)	35 (32.5-36)	<0.001
Highest PEEP (cm H_2O)	6 (5-7)	9 (8-11)	0.017
Highest FIO_2 (%)	0.5 (0.4-0.6)	0.8 (0.7-0.9)	0.003

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)

IPPV, intermittent positive pressure ventilation; PIP, peak inspiratory pressure; PEEP, positive end-expiratory pressure; FIO_2, fraction of inspired oxygen

4.7 Discussion

4.7.1 HIV infection in the ICU

More than a quarter of children admitted to ICU for pneumonia were found to be HIV positive. However, HIV infection was diagnosed in over 70% of cases only at the time of admission despite the presence of clinical features of AIDS in most of these children. The high prevalence of HIV infection amongst children admitted to ICU for pneumonia reflects the rising epidemic of this disease in South Africa and the increased burden that this has placed on health care resources (UNAIDS/WHO report 2000, Jeena et al 1999, Zwi et al 1999).

4.7.2 Aetiology of lung disease

The incidence and type of bacterial and viral pathogens were similar in HIV positive and negative children including *M tuberculosis*. However, PCP, occurring in almost half of those newly diagnosed with HIV infection, was the most important AIDS indicator disease and was associated with a mortality rate of 44%. The PCP incidence found in the study is probably an underestimate of the number of children with the infection due to limited diagnostic resources. *P carinii* was identified from lower respiratory tract secretions using induced sputa in non-intubated patients and BAL in intubated children; BAL was performed in only 33 (43%) children. Nevertheless, sputum induction yielded a diagnosis of *P carinii* in 2 children. Transbronchial biopsy or percutaneous lung aspiration would probably have provided a higher yield of *P carinii*, however, these techniques are associated with an increased risk of air leaks, bleeding or circulatory collapse (Chaudhary et al 1977, Broaddus et al 1985, Craddock et al 1987). The yield of *P carinii* may also have been increased if the more sensitive

technique of DNA amplification of *P carinii* PCR had been used (Lipshik et al 1992, Khan et al 1999); however, this was not available in the laboratory. The high incidence of PCP is similar to that reported from developed countries in the early stages of their HIV pandemic (Notterman et al 1990, Marolda et al 1989, Vernon et al 1988, Wong and Chundu 1994, Bye et al 1990). Although PCP has been considered to occur relatively rarely in Africa, the present study indicates that this infection is a major cause of severe pneumonia and mortality in HIV-infected infants in South Africa.

4.7.3 Outcome

Despite the similarity in bacterial and viral pathogens, HIV-infected children tended to have a higher mortality rate than HIV negative patients even when children with PCP were excluded. Although HIV positive children had double the mortality rate of HIV-negative patients, the difference in mortality rate (14.1%) was not statistically significant. This is probably due to the small sample size. A sample size of 144 children would have been needed for this difference to reach statistical significance at a power of 90% and alpha of 5%. However, the mortality rate for HIV positive children (29%) was much lower than that reported in 2 earlier South African studies in Johannesburg and Durban despite the fact that most (90%) had established AIDS (Mathivha et al 1998, Jeena et al 1996). In the Johannesburg study of 110 children admitted to ICU for pneumonia, the mortality rate for HIV-positive children without signs of AIDS was 88% (Mathivha et al 1998). In the Durban study, children admitted to ICU who had AIDS had a mortality of 100% with a rate of 55% in their controls; however, HIV-positive patients without AIDS had a similar mortality rate (38%) to HIV-negative controls (46%) (Jeena et al 1996). The present study differs from these

two in that an ante-mortem diagnosis of PCP was vigorously sought and treatment with intravenous TMP-SMX and corticosteroids was instituted in suspected cases.

The mortality rate in the present study was also lower than that reported from developed countries in the initial stages of their HIV epidemics (Notterman et al 1990, Vernon et al 1988). Timely and effective use of anti-pneumocystis therapy including corticosteroids may have improved outcome, as suggested by 5 children who survived PCP. The improved mortality rate is consistent with more recent data for children who had survival rates of 100% for PCP when treated with corticosteroids (Bye et al 1994, Sleasman et al 1993). Improved IPPV techniques may have also impacted on survival of ventilated children. The results may also reflect a global trend of improved outcome for respiratory failure in HIV-infected patients after the first years of the epidemic (Curtis 1996). However, the overall mortality rate would have been higher (27%) if we had included deaths of the 9 children who were not enrolled in the study; as the HIV status of 4 of these children was not known while one was confirmed to be HIV-positive, the HIV-associated mortality could have ranged from 7 of 22 (31.8%) to 11 of 26 (42.3%). Furthermore, as access to ICU is limited due to the relatively small number of available beds, the mortality rate only reflects deaths of children selected for ICU admission. It is likely that a selection bias operates in the hospitals in which the study was conducted such that immunocompetent children are more likely to be admitted to ICU than patients with advanced AIDS. This is borne out by the mortality results for all hospitalised children enrolled in the study of the aetiology and outcome of pneumonia in which all deaths amongst HIV negative but only 20% of deaths in HIV-positive children occurred in the ICU (chapter 3). It is probable that this selection bias may also affect IPPV use as evidenced by two HIV-infected children

who died in ICU without receiving IPPV. Nevertheless, amongst children selected for ICU admission, the mortality rate for HIV-positive patients was relatively good.

The mortality rate for children with PCP (44%) was high. This poor outcome for children with PCP is consistent with that reported from developed countries in the early stages of their HIV pandemic (Notterman et al 1990, Marolda et al 1989, Vernon et al 1988, Wong and Chundu 1994, Bye et al 1990). In these studies, acute survival rates of HIV-infected children with PCP ranged from 13% - 60% with lower rates associated with respiratory failure (Notterman et al 1990, Vernon et al 1988). The present study lacks the statistical power to compare outcome in children with PCP by use of IPPV as 8 of 9 children with PCP were mechanically ventilated. The study confirms the need for early identification of HIV-infected children and use of chemoprophylaxis in this population in order to prevent the severe pneumonia resulting from primary infection with *P carinii*.

No reliable predictors of death in HIV-positive children could be identified. Furthermore, multivariate regression analysis did not identify any significant risk factors for mortality for all patients. This is probably a result of the small sample size. Additional studies of the acute and long-term survival of both HIV-positive and negative children following severe pneumonia in developing countries are needed in order to develop rational guidelines for utilisation of scarce ICU resources.

4.8 Conclusion

An increased demand for ICU beds for HIV-positive infants and children in South

Africa has occurred following the sharp rise in HIV infection rates in both high and low HIV-prevalence areas over the past 4 years (Jeena et al 1999, UNAIDS/WHO 2000). The present study was performed in an area of comparative low HIV prevalence, nevertheless almost a quarter of the children admitted to ICU for pneumonia were HIV-infected. Furthermore, in more than 70% of HIV-positive patients, HIV infection was diagnosed only at the time of admission. From the experience of other parts of the country, the proportion of patients who can be accommodated in ICU cannot keep pace with the demand (Zwi et al 1999, Jeena et al 1999, Mathivha et al 1998, Jeena et al 1996). Furthermore, because of cutbacks in tertiary health expenditure and the increased emphasis on primary care, ICU resources have become increasingly scarce within the academic hospitals. Some hospitals such as Chris Hani Baragwanath in Johannesburg have implemented a policy that excludes children known to be infected with HIV from ICU (Zwi et al 1999). Set in this context, the observations in the present study that with effective ICU management, a satisfactory outcome from pneumonia can be achieved in many HIV-positive children poses yet another dilemma to health care providers in developing countries experiencing the HIV epidemic.

For children admitted to ICU with pneumonia some general recommendations for management can be made for this geographic area. Infants with pneumonia whose HIV status is unknown but who have signs of AIDS should be investigated for HIV and PCP and presumptively treated for the latter. Broad-spectrum antibiotic coverage for gram positive and negative bacteria are indicated. Prevention of PCP and of the subsequent need for ICU should be included in management guidelines by prioritising early identification of HIV-infected infants and use of chemoprophylaxis. All children

who have experienced an episode of PCP should be maintained on life long TMP-SMX prophylaxis as subsequent episodes of PCP increase in clinical severity (Masur 1992).

The short-term outcome for pneumonia in HIV-infected children admitted to ICU was worse than that for HIV negative patients but due to the small sample size this was not statistically significant. The mortality rate for children with PCP was higher than that of patients without PCP. Additional, larger studies are needed to identify factors predictive of mortality in both HIV-positive and negative children requiring ICU care in developing countries. Finally studies are needed to investigate the long-term survival of both HIV positive and negative children following severe pneumonia in developing countries in order to develop guidelines for utilisation of scarce ICU resources.

CHAPTER 5: RESULTS III

Pneumocystis carinii pneumonia (PCP) in HIV-infected patients

5.1 PCP in HIV-infected children

Of the 151 HIV-infected children [71 (47%) female, age 9 (3-23) months], 87 had been previously diagnosed with HIV while 64 (42.4%) were found to be HIV-positive at the time of admission. The incidence of PCP, which occurred in 15 children, was 9.9% (95% CI = 5.9-15.5); 13 of these patients had been previously undiagnosed as HIV-infected. PCP was therefore the AIDS indicator disease in 13 of 64 children (20.3%, 95% CI = 11.8-31.5). One child developed PCP while receiving TMP-SMX prophylaxis but adherence to medication could not be established. HIV-infected children with PCP were younger with a male predominance and were less likely to have had a prior hospitalisation compared with those without PCP (table 5.1). Thirteen of 15 children (87%) with PCP were 6 months or younger while 14 (93%) were less than a year old. The incidence of PCP stratified according to age was 13 of 62 (21%) in those 6 months or younger, 14 of 92 (15.2%) under a year, 14 of 115 (12.2%) under 2 years and 15 of 151 (9.9%) under 3 years of age. Use of TMP-SMX prophylaxis was associated with a reduced risk of PCP: 1 of 59 children receiving prophylaxis (1.7%) developed PCP compared with 14 of 92 (15.2%) not taking prophylaxis [RR 0.11 (0.02-0.82), p=0.007].

Cough, fast breathing and fever were the predominant symptoms reported by caregivers; no symptoms distinguished children with PCP from those without PCP except for vomiting which was less common in the former (table 5.1). Only one of the 15 children with PCP had diarrhoea. The median duration of cough [5 (3-14) days] and other symptoms before presentation at hospital was similar in both groups except for

fast breathing which was present for a shorter duration in those with PCP [1.5 (1-4) compared with 3 (2-7) days, p=0.024].

PCP-infected children had more severe pulmonary disease as reflected by a higher presenting respiratory and heart rate and a greater incidence of cyanosis (table 5.1). However, the prevalence of lower chest indrawing and abnormal chest auscultatory findings did not differ. The prevalence of clinical signs of HIV infection (in 96% of children) including hepatomegaly, splenomegaly and parotid enlargement were similar in both groups. PCP infected children had better nutritional status than those without PCP as reflected by higher median weight and height for age. This most likely reflects the younger age of PCP infected infants (table 5.1) in whom PCP was an initial manifestation of AIDS. A BCG scar was absent in 8 children with PCP, present in 5 PCP-infected children and not recorded in two. When children with PCP were stratified by the presence or absence of a BCG scar, no association between the absence of a scar and development of PCP was found, p=0.743. The median presenting baseline arterial oxygen saturation in air was 92.5% (88-95) and 94 % (92-97) for PCP infected and uninfected groups respectively, p=0.071.

5.2 Laboratory results

PCP was diagnosed either by silver stain or IF in 9 induced sputa and 6 BAL specimens; all corresponding NPAs were negative for *P carinii*. The median age of children in whom *P carinii* was identified using induced sputum was 4 (3-5) months. There were no laboratory investigations that distinguished children with PCP from those without PCP except for serum LDH which was higher in the former (table 5.2).

Using a cutoff of greater than or equal to 500 U/l for LDH to predict PCP infection, 50% of PCP-infected children and 18.5% of uninfected children would have been identified, OR 4.39 (1.03-18.25), p=0.001, providing a sensitivity of 50% and specificity of 81%. Similarly using 400U/l LDH as the lower limit, would have identified 50% of PCP-infected and 28% of uninfected children, OR 2.59 (0.63-10.53), p=0.116 which provides a sensitivity of 52% and specificity of 72%. A value of greater than or equal to 300 U/l would have yielded 83% of PCP infected and 48% of uninfected children, OR 5.32 (1.04-51.7), p=0.023, providing a sensitivity of 83% and specificity of 52%. These results indicate that raised LDH levels (above the standard normal value of 153-253 U/l) may be useful for diagnosis of PCP; higher levels are associated with greater specificity but lower sensitivity for PCP.

The incidence of bacteraemia [2 children with PCP (13.3%) compared to 16 without PCP (11.8%), p=0.86] was similar in both groups.

5.3 Radiology

There were no diagnostic radiological features for PCP but patchy alveolar infiltrates and hyperinflation occurred in 94.4% and 72.2% respectively (table 5.2). None of the children with PCP were reported to have hilar or mediastinal lymphadenopathy, pleural effusion or a diffuse nodular pattern (table 5.2).

Table 5.1: Presenting features of HIV-positive children stratified by the presence of PCP

	PCP (n=15)	Without PCP (n=136)	p
Age (months)	3 (3-4)	10 (4-24)	<0.001
Male:female	12:3	68:68	0.028
African race, n (%)	15 (100)	133 (97.8)	0.563
Prior admission, n (%)	2 (13.3)	88 (63.2)	<0.001
Newly diagnosed with HIV	13 (86.7)	51 (37.5)	<0.001
TMP-SMX prophylaxis	1 (6.6)	58 (42.6)	0.007
Symptoms, n (%)			
Cough	15 (100)	129 (94.9)	0.340
Fast breathing	13 (86.7)	105 (77.2)	0.402
Fever	12 (80)	96 (70.6)	0.445
Rhinorrhoea	6 (40)	81 (59.5)	0.147
Vomiting	2 (13.3)	64 (47)	0.013
Diarrhoea	1 (6.7)	38 (27.9)	0.075
Vital signs			
Respiratory rate	63 (60-73)	50 (40-60)	<0.001
Heart rate	160 (136-180)	140 (120-152)	0.025
Axillary temp (^{o} C)	37 (36.5-37.5)	37 (36.5-37.8)	0.748
Weight for age (%)	88.5 (76.3-98.2)	70.7 (58.3-81.3)	0.002
Height for age (%)*	97.4 (91.3-102.1)	88.8 (81.2-92.6)	0.004
Physical exam, n (%)			
Cyanosis	8 (53.3)	35 (25.7)	0.025
Clubbing	1 (6.7)	29 (21.3)	0.178
Lower chest indrawing	6 (40)	76 (55.9)	0.243
Lung findings**	13 (86.7)	123 (90.4)	0.643

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)
: * height available for 132 children
: ** presence of adventitious sounds (wheezes, bronchial breathing or crackles)

Table 5.2: Laboratory results and chest radiology of HIV-positive children with PCP

	PCP (n=15)	Without PCP (n=136)	p
Blood tests			
Haemoglobin (g/dl)	9.8 (8.5-11.4)	8.8 (7.8-9.9)	0.019
Platelets (10^9/l)	367 (173-446)	337 (219-444)	0.918
WBC (10^9/l)	12.4 (10.5-19.4)	12.7 (9.3-17.9)	0.758
Neutrophils (10^9/l)	6.7 (3.8-8.6)	5.6 (3.4-9.4)	0.586
Lymphocytes (10^9/l)	4.6 (3.3-8.2)	4.4 (2.5-6.7)	0.433
CD4 (cells/µl)	871 (290-1150)	667 (360-1120)	0.575
CD4/T cells (%)	16.4 (12.7-27.6)	23.6 (14.5-38.7)	0.058
CD8 (cells/µl)	2660 (900-5500)	2010 (1100-3600)	0.562
CD4/CD8	0.4 (0.2-0.7)	0.3 (0.2-0.4)	0.454
Protein (g/l)	67.5 (56-75)	78.5 (63-88.5)	0.141
LDH (U/l)	626 (450.5-1098.5)	307 (243-465)	<0.001
Bacteraemia, n (%)	2 (13.3)	16 (11.8)	0.859
Radiology, n (%)	n=15	n=118	
Normal Xray	0 (0)	3 (2.5)	0.534
Hyperinflation	11 (73.3)	73 (61.9)	0.387
Diffuse alveolar pattern	11 (73.3)	106 (89.8)	0.065
Diffuse nodular pattern	0 (0)	9 (7.6)	0.270
Consolidation	15 (100)	108 (91.5)	0.243
Adenopathy*	0 (0)	23 (19.5)	0.061
Cavity	0 (0)	3 (2.5)	0.534
Pleural effusion	0 (0)	14 (11.9)	0.160

Footnotes: Continuous variables are median (25^{th}-75^{th} percentile)

* hilar or mediastinal

5.4 Hospital course and outcome

HIV-infected children with PCP, as compared with those without PCP, were more likely to be admitted to ICU [8 of 15 (53%) versus 13 of 136 (10%), p<0.001] and to receive intermittent positive pressure ventilation [7 of 15 (47%) versus 2 of 136 (1%), p<0.001]. Although duration of hospitalisation was shorter for PCP-infected children [10 (7-14) versus 15 (9-20) days, p=0.015] this difference was not significant when those who died were excluded [11.5 (8.5-15) compared to 15 (9-20) days for PCP infected and uninfected surviving children respectively, p=0.317]. Seven of 15 (46.7%, 95% CI = 23.2 - 71.3) children with PCP died while hospitalised compared to 24 of 136 (17.6%, 95% CI = 11.9 - 24.7) without PCP [RR=1.21 (0.99-1.47), p=0.008]. There were no clinical or laboratory findings predictive of mortality in HIV-infected children with PCP compared to those without PCP although LDH tended to be higher in those who died [708 (594-1289) compared to 390 (387-570) U/l, p=0.088]. Ventilation requirements and outcome of HIV-infected patients who received IPPV for PCP compared to those who were admitted to ICU but not ventilated are reported in chapter 4 and table 4.4.

5.5 Discussion

PCP accounted for 10% of acute pneumonia episodes in hospitalised HIV-infected children and was the AIDS defining infection in 20% of those newly diagnosed as HIV-positive. HIV-infected children with PCP had a high in-hospital mortality (47%) which is consistent with the paediatric PCP-associated mortality (40-87%) reported in developed countries during the early stages of the HIV pandemic (Notterman et al 1990, Marolda et al 1989, Vernon et al 1988, Wong and Chundu 1994, Bye et al

1990). Furthermore, the results indicate that PCP is potentially preventable as the majority of those with PCP were infants in whom HIV infection had not yet been diagnosed and who were therefore not using chemoprophylaxis. However, use of TMP-SMX prophylaxis in children known to be HIV-positive effectively prevented PCP. These results highlight the need for early diagnosis of HIV infection so that chemoprophylaxis can be instituted and PCP prevented.

This is one of the first large, prospective studies of the incidence of PCP in children hospitalised with pneumonia in Africa. Although this PCP incidence is probably an underestimate because the diagnosis was made using induced sputa specimens in most children, the results indicate that PCP is an important cause of pneumonia in HIV-infected infants in South Africa. PCP has been considered to occur uncommonly in Africa (Lucas et al 1989, Elvin et al 1989, Carme et al 1991, Abouya et al 1992, Machiels et al 1992, Atzori et al 1993, Kamanfu et al 1993, Batungwanayo et al 1994, McLeod 1989, Malin et al 1995) but technical difficulties in diagnosing the infection and geographical variation may account for differences in reported prevalence (Russian and Kovacs 1995). Although there is little data on PCP incidence in HIV-infected children in Africa, the incidence in the present study is similar to the rate of 17% recently reported in Malawian HIV-infected children hospitalised with severe pneumonia in whom PCP was diagnosed using IF on NPAs (Graham et al 2000). Amongst the subgroup of HIV-infected children who died in the present study, PCP occurred in 7 of 31 (23%) of children. This is also similar to the incidence found in 2 post mortem studies of African HIV-positive children in Zimbabwe and Cote d'Ivoire (Ikeogu et al 1997, Lucas et al 1996). In the Zimbabwean study in which autopsies were performed on children who died at home, a PCP incidence of 16% was reported

(Ikeogu et al 1997). In Cote d'Ivoire, a post mortem study of consecutive HIV-positive children who died found PCP in 11 of 78 cases (14%); however, all PCP cases occurred in children under 15 months increasing the PCP incidence in this age group to 11 of 36 (31%) (Lucas et al 1996). The importance of PCP in HIV-infected children is further supported by results of a study from Gambia that reported serological evidence of *P carinii* infection in 70% of healthy children by 8 years of age (Wakefield et al 1990).

Differences in reported PCP incidence between developed countries and Africa may be accounted for by a number of factors. Technical difficulties in diagnosing PCP are one factor as *P carinii* must be identified from lower respiratory tract secretions and requires specialised laboratory staining. Bronchalveolar lavage (BAL), lung biopsy or sputum induction have been successfully used in adults but such invasive procedures are frequently not feasible in developing countries with limited resources and facilities. Moreover, these diagnostic procedures are especially difficult in young children and infants in whom the peak incidence of PCP is at 3-6 months of age (Hughes 1991, Masur 1992). Sputum induction which compared with BAL has a reported sensitivity of 55-95% for diagnosis of PCP in immunocompromised adults, has rarely been used in children and infants (Leigh et al 1989, Ognibene et al 1989). In the present study, sputum induction was successfully used to diagnose 9 of 15 (60%) PCP cases in HIV-infected children, seven of who were younger than 6 months of age. *P carinii* may be isolated from nasopharyngeal aspirates (Kamiya et al 1997, Hague et al 1990, Graham et al 2000); however, the sensitivity of this technique compared with BAL or induced sputum has not been tested. In the present study, upper respiratory tract secretions in the form of NPAs were not found to be useful for identifying *P carinii*. A

discussion of the diagnostic utility of induced sputum and NPAs for *P carinii* can be found in chapter 6.

The identification of *P carinii* in respiratory secretions poses another technical difficulty as this requires special stains and a pathologist skilled in the recognition of the pathogen. A variety of stains have been used – silver methenamine and toludine-blue for the cyst forms and Giemsa for the trophozoites (Hughes 1991). The development of immunofluorescence (IF) techniques using monoclonal antibodies has resulted in a more sensitive method for detecting *P carinii* (Orhholm et al 1990). However, use of IF in developing countries is further limited by the relatively high cost of this test. Polymerase chain reaction techniques (PCR) have demonstrated a high sensitivity and specificity and may also improve diagnostic accuracy, but require specialised skills and well equiped laboratories (Lipshik et al 1992, Roux et al 1994).

Differences in patient selection criteria are another factor contributing to reported discrepancies in PCP incidence (Russian and Kovacs 1995). The method used in some studies of selecting patients who are unresponsive to standard antibiotics or smear negative for acid fast bacilli will undoubtedly identify a higher rate of PCP (McLeod et al 1989, Malin et al 1995, Mahomed et al 1999). Similarly, selecting patients who are admitted to an intensive care unit (ICU) will also identify a higher incidence of PCP as the infection is usually clinically severe.

Environmental factors and geographic variability are additional factors that may affect the PCP incidence (Russian and Kovacs 1995). Seasonal variation in the incidence of PCP has been described which suggests that factors such as temperature and humidity

may be important (Miller et al 1992). Geographic variability in the prevalence of *P carinii* may also play a role (Morris et al 2000), but this is not supported by the results of serologic studies. Reduced exposure to *P carinii* may be another factor if adult disease results from reinfection rather than reactivation; however, little is known about the ecological niche and lifecycle of *P carinii* outside a host. Host genetic susceptibility to PCP may play a role as evidenced by studies reporting a lower risk of PCP in black Africans residing in different geographical areas. In support of this, in a retrospective study of attendees at HIV clinics in Cape Town (table 1.3) and in a retrospective chart review of HIV-positive patients hospitalised in Johannesburg (Mohamed et al 1999), adult HIV-infected black Africans have been reported to have a lower risk of developing PCP compared with Caucasians. A similar racial difference in susceptibility to PCP has been described in black African HIV-infected people living in London, UK compared with their white counterparts (Del Almo et al 1996). It has been suggested that differences in molecular genotypes of *P carinii* may be additional factors contributing to pathogenesis and virulence (Huang et al 2000).

An alternative explanation for the apparent lower incidence of PCP in HIV-infected people in Africa is the high prevalence of other infections such as tuberculosis or bacterial sepsis, which may result in death at an earlier stage of HIV infection when CD4 lymphocyte counts are relatively high and the risk of PCP low. In support of this, the major pathogen identified in almost all African studies is *M tuberculosis* (table 1.2). Even in a study which excluded patients who were sputum smear-positive for acid fast bacilli, *M tuberculosis* was the commonest pathogen identified, occurring in 39% (Malin et al 1995). Furthermore, the existing adult data from Africa rarely defines the immunological stage of HIV infection. As the risk of PCP is largely restricted to

adults with CD4 counts lower than 200 cells/μl (Phair et al 1990, Kaplan et al 1998), PCP will be under-represented if patients with pulmonary infiltrates and relatively high CD4 counts are studied as the commonest causes in this region are bacterial pneumonia and tuberculosis. Compounding this under-representation is the fact that in Africa survival after the onset of AIDS is short (Morgan et al 1997). However, even though survival in Cape Town is similar to that reported in industrialised countries in the era prior to highly active anti-retroviral therapy (HAART), the risk of PCP still appears lower in Africans than Caucasians (Maartens et al 1997).

Distinguishing HIV-infected children with PCP from those without this infection by clinical methods is difficult. The clinical presentation is characterised by a tetrad of signs including tachypnoea, dyspnoea, cough and fever (Hughes 1991). However, these signs are non-specific, occurring in children with lower respiratory tract infections (LRTIs) of differing aetiologies or in acute asthma (Bye 1995). The chest Xray changes, usually progressive air-space disease with air bronchograms, are also non-specific and may occur in LRTIs due to other pathogens (Berdon et al 1993). Moreover, the only biochemical abnormality found to be useful in distinguishing PCP-infected children from those without PCP was an elevated LDH level. Higher LDH values also tended to be associated with mortality. LDH has previously been described as a marker of the severity of lung disease in PCP with higher values reported for children who developed respiratory failure (Bye et al 1990). However, LDH may be a non-specific marker of lung injury as indicated by data in HIV-infected adults in which LDH concentrations correlated with the extent of radiological abnormality regardless of the underlying aetiology (Opravil et al 1994).

5.6 Conclusion

PCP is a common AIDS defining infection in infants in South Africa. The study suggests that in this geographic area, patients less than a year of age who are hospitalised with pneumonia and have signs of AIDS should be treated presumptively for PCP. The high mortality associated with PCP highlights the need for early identification of HIV-positive children and timely use of chemoprophylaxis. Diagnostic and management algorithms should include guidelines for diagnosing and treating PCP in HIV-infected patients with severe pneumonia and for use of chemoprophylaxis.

CHAPTER 6: RESULTS IV

Evaluation of diagnostic methods: induced sputum and nasopharyngeal aspirates

6.1 Diagnostic procedures

Sputum induction was performed on non-intubated children for bacterial, viral and mycobacterial culture and *P carinii* identification while nasopharyngeal aspirates were done on all children for bacterial culture and *P carinii* identification, as described in chapter 2. Sputum induction has not been used in infants or young children before for the identification of pulmonary pathogens. A few small studies in older children have utilised sputum induction as a diagnostic procedure. A study of 18 immunosuppressed paediatric patients (mean age 7.3 years) detected 9 episodes of PCP in 8 children using sputum induction with immunofluoresence for *P carinii* detection (Ognibene et al 1989). Sputum induction done in 14 immunocompromised children with respiratory symptoms identified CMV in one and *H influenzae* in two (Foot et al 1992). In a Malawian study of the efficacy of sputum induction in children aged 3 to 15 years, sputum was successfully obtained in 29 of 30 patients undergoing investigation for pulmonary tuberculosis and the diagnosis was confirmed in 8 (28%) (Shata et al 1996). The present study is therefore the first to investigate the utility of induced sputum as a diagnostic test in infants and young children. In order to do this, the rate of culture for *M tuberculosis* from induced sputa and gastric lavage specimens was compared and the bacterial yield from induced sputa was compared with that from nasopharyngeal aspirates.

Nasopharyngeal aspirates have been reported to be useful for the diagnosis of PCP in a few case reports (Hague et al 1990, Kamiya et al 1997) and in a recent study of children hospitalised with pneumonia (Graham et al 2000). However, the yield of *P carinii* from NPAs has not been compared with that from sputum or BAL. To

investigate this, the yield for *P carinii* from NPAs using immunofluorescence was compared with that obtained from lower respiratory tract secretions.

6.2 Sputum induction compared to gastric lavage for the diagnosis of pulmonary tuberculosis

6.2.1 Efficacy and safety

Gastric lavages were done in 149 children as described in chapter 2. Corresponding sputum specimens were obtained in 142 of 149 (95%) of children with a median (25^{th}-75^{th} percentile) age of 9 (3-20) months (table 6.1). The youngest child in whom sputum was successfully obtained was 1 month old. Seven children (5%) were either considered too ill to tolerate sputum induction or developed increasing tachypnoea or cough during nebulisation or suctioning necessitating termination of the procedure. Only a minority of children 14 (10%) could expectorate and sputum was obtained by suctioning in the remainder. No serious adverse reactions occurred during sputum induction but minor events including mild epistaxis in 6 (4.2%), marked increase in coughing in 8 (5.6%) and wheezing that was responsive to an inhaled bronchodilator in 3 (2.1%) occurred. The baseline median respiratory rate of children was 50 (40-60) and arterial oxygen saturation in air was 94% (92-97). Amongst this group of children, 100 (70%) were HIV-infected.

6.2.2 Yield of M tuberculosis

M tuberculosis was cultured from sputum or GL in 16 children (11.3%, 95% CI = 6.8 – 17.3). Sputum cultures grew *M tuberculosis* in 15 of 142 children (10.6%, 95% CI = 6.3 – 16.4) but GL was positive in only 9 (6.3%, 95% CI = 3.1 – 11.3) (table 6.2). A

single GL was obtained in 39 children [of which 2 (5.1%) were positive], two pooled lavages in 77 [4 (5.2%) positive] and three pooled specimens in 26 [3 (11.5%) positive]. In only one case was the GL culture positive for mycobacteria while the corresponding sputum was negative. The difference (95% confidence interval) between yields for *M tuberculosis* from culture of induced sputum compared to GL was 4.3% (95% CI 0% to 5.6%), p=0.08. Of the specimens culture positive for *M tuberculosis*, 3 of 15 induced sputum (20%) and 3 of 9 GLs (33%) were positive by microscopy for acid-fast bacilli.

The median age of children with bacteriologic confirmation of tuberculosis was 12 (7-25) months which was similar to those without tuberculosis [9 (3-21.5) months]; p=0.19. The youngest child in whom *M tuberculosis* was cultured from induced sputum was 3 months of age while seven of 15 (47%) were less than 1 year. No child had cavitatory tuberculosis. Ten children culture positive for *M tuberculosis* (62.5%) were HIV-infected. This represents 10% of all HIV-infected children which was similar to the percentage of HIV negative children with tuberculosis [six of 42 (14.3%); OR 0.67 (0.2-2.41), p=0.46].

Table 6.1: Characteristics of children in whom sputum induction and gastric lavage were performed

Characteristic	Children without TB (n=126)	Children with TB (n=16)
Age (months)	9 (3-21.5)	12 (7-25)
M:F	1.2	2.2
ICU admission, n (%)	18 (14)	2 (12)
HIV positive, n (%)	90 (71)	10 (63)
TB contact, n (%)	24 (19)	7 (44)*
Use of supplemental O_2, n (%)	83 (66)	8 (50)
Baseline O_2 saturation in air	94 (90.5-97)	93.5 (88-98)
Baseline RR	50 (40-60)	53 (40-63.5)

*p=0.04
Footnote: Continuous variables are median (25th-75th percentile)

Table 6.2: Diagnosis of children with tuberculosis by sputum induction or gastric lavage

	Gastric lavage	
	Culture positive (n)	Culture negative (n)
Induced sputa **Culture positive (n)**	8	7
Culture negative (n)	1	126

6.3 Bacterial isolates from induced sputa compared with nasopharyngeal aspirates

Bacterial culture results for paired specimens of induced sputa and NPAs were available in 200 children. In these children, a significantly higher rate of *S aureus, H influenzae, M catarrhalis* and *S pneumoniae* was found in NPAs compared to induced sputa (table 6.3). This pattern was particularly evident in HIV-infected children who had a significantly higher rate of these bacteria cultured from NPAs compared to induced sputa (table 6.4). In seronegative children, the yield for *M catarrhalis* from NPAs was also significantly higher than that from sputa; although the rate of culture from NPAs compared to sputum for *S aureus, H influenzae* and *S pneumoniae* was higher this did not reach statistical significance (table 6.4). In HIV-positive and negative patients, *K pneumoniae* was more frequently isolated from induced sputa although this difference was not significant. A detailed analysis of the bacterial isolates obtained from sputa and NPAs and the effect of TMP-SMX prophylaxis can be found in section 3.5.1 and tables 3.3, 3.4 and 3.5.

Table 6.3: Comparison of bacterial isolates (n, %) from 200 paired specimens of nasopharyngeal aspirates and induced sputa

Bacteria	Nasopharyngeal aspirates	Induced sputa	p
S aureus	49 (24.5)	25 (12.5)	0.002
H influenzae	38 (19)	21 (10.5)	0.017
M catarrhalis	38 (24.5)	8 (4.0)	<0.001
S pneumoniae	26 (13)	5 (2.5)	<0.001
K pneumoniae	13 (6.5)	22 (11)	0.112
P aeruginosa	13 (6.5)	11 (5.5)	0.674

Table 6.4: Comparison of bacterial isolates (n,%) from paired specimens of nasopharyngeal aspirates (NPA) and induced sputa (IS) by HIV status

Bacteria	HIV positive			HIV negative		
	NPA (n=129)	IS (n=129)	p	NPA (n=71)	IS (n=71)	p
S aureus	41 (31.8)	22 (17.1)	0.006	8 (11.3)	3 (4.2)	0.118
H influenzae	22 (17.1)	11 (8.5)	0.041	16 (22.5)	10 (14.1)	0.194
M catarrhalis	21 (16.3)	3 (2.3)	<0.001	17 (23.9)	5 (7.0)	0.006
S pneumoniae	17 (13.2)	2 (1.5)	<0.001	9 (12.7)	3 (4.2)	0.071
K pneumoniae	9 (7.0)	13 (10.1)	0.373	4 (5.6)	9 (12.7)	0.146
P aeruginosa	10 (7.8)	9 (7.0)	0.812	3 (4.2)	3 (4.2)	1.000

6.4 Identification of *P carinii* – comparison of lower respiratory tract secretions with nasopharyngeal aspirates

P carinii was identified in 19 children (15 HIV-infected) in lower respiratory tract secretions; of these 7 (37%) were identified in BAL and 12 (63%) in induced sputa specimens. Of the 12 children diagnosed with PCP using induced sputum, 9 were HIV-positive. Six of seven children with PCP diagnosed on BAL were HIV-infected. A detailed analysis and discussion of HIV-infected children with PCP can be found in chapter 5. Immunofluorescence for *P carinii* on the corresponding NPAs of these 19 children were all negative.

6.5 Discussion

The present study demonstrated that sputum induction can be effectively and safely performed in infants and young children including those with HIV infection. The procedure can be successfully performed in infants as young as a month of age. Sputum induction was well tolerated even in children who were hypoxic or who had AIDS. Although continuous monitoring of arterial oxygen saturation during sputum induction could not be performed, very few children were unable to complete the procedure and no child appeared to clinically deteriorate following it. The efficacy of sputum induction in infants and young children in the study is evident in that sputum induction was a more sensitive method than GL for culture of *M tuberculosis*, detecting almost twice the number of children with pulmonary tuberculosis. Furthermore, the type and proportion of bacteria cultured on NPAs compared with induced sputa differed significantly, providing further evidence that sputa represent lower respiratory tract secretions. Finally, *P carinii* was identified in over 60% of the cases of PCP by induced sputum examination but not in NPAs.

Childhood tuberculosis is an increasingly important public health problem particularly in developing countries. The diagnosis of tuberculosis is notoriously difficult in children especially those who are HIV-infected due to the development of anergy which limits the use and importance of the skin tuberculin test. In addition, other HIV-associated lung diseases may mimic the clinical and radiological picture of pulmonary tuberculosis. Gastric aspirates have been reported to give isolation rates of *M tuberculosis* ranging from 28-40% in children with suspected tuberculosis (Lloyd 1968, Starke and Yaylor-Watts 1989) but higher rates of up to 75% have been

described in infants (Vallejo et al 1994). Although 7 children had TB diagnosed on induced sputum while GLs were negative and only a single child had a positive GL and corresponding negative sputum, the difference between the yield from induced sputum compared to GL tended towards statistical significance using a two-tailed McNemar test ($p=0.08$). The lack of statistical significance for this difference is most likely due to the small number of children with tuberculosis. The small number of pulmonary tuberculosis cases reflects the relatively low risk of infection amongst the study group as they were hospitalised for acute pneumonia. Nevertheless, pulmonary tuberculosis was a common diagnosis in this group of children from a high TB prevalence area, who had clinical features of acute respiratory infection.

This is the first paediatric study to compare the yield for *M tuberculosis* from induced sputum with that from GL. Studies comparing GL to induced sputum in adults with suspected tuberculosis reported induced sputum to be more effective (Lillehei 1961, Jones 1966). In a study of 87 adult male patients with suspected pulmonary tuberculosis, induced sputum yielded bacteriologic confirmation of TB in almost twice the number of cases than did GL (Lillehei 1961). In a second study of 155 adults hospitalised with active pulmonary TB, induced sputum and GL yielded a positive culture in 51% and 30% of specimens respectively ($p<0.001$) (Jones 1966). In a large study of 2385 paired specimens obtained to investigate adult patients with pulmonary infiltrates of unknown aetiology, the order in which procedures (gastric lavage and sputum induction) were performed was varied. Induced sputum was found to be superior to GL only when GL preceded sputum induction, yielding *M tuberculosis* in 67 sputa compared to 53 GLs in 1116 paired specimens. When sputum induction was done first, the yield from sputum and GL in 1269 paired specimens were similar, 72

and 74 respectively (Carr et al 1967). A small study of children with suspected pulmonary tuberculosis reported an improved yield from GL when preceded by a nebulisation of superheated isotonic saline; a positive GL culture for *M tuberculosis* was obtained in 12 of 13 children (Giammona and Zelkowitz 1969). Although there are no published studies comparing induced sputum to GL for culture of *M tuberculosis* in children, 2 paediatric studies of GL compared to BAL found that GL provided a higher yield and suggested that lower respiratory tract secretions may be less useful (Abadco and Steiner 1992, Somu et al 1995). The relatively lower yield from BAL was ascribed to the smaller area sampled by BAL compared to GL, the paucibacillary nature of childhood pulmonary TB, dilution of BAL specimens with installation of successive saline aliquots and possible antibacterial activity of lidocaine used in BAL (Abadco and Steiner 1992, Rankin 1991, Schmidt and Rosenkranz 1970). However, a study of 29 Malawian children found that induced sputum yielded a diagnosis of TB in 8 (28%) of patients (Shata et al 1996). The results of the present study confirm that lower respiratory tract secretions derived from sputum induction are useful for bacteriologic confirmation of pulmonary TB in children.

Although GL was done according to a standard protocol (section 2.7.5), different nurses performed this procedure as an early morning pre-prandial specimen was required. The relatively lower culture rate of *M tuberculosis* on GL compared to sputum induction may therefore reflect some variability in GL technique and the lack of well standardised guidelines for the procedure. Whereas GL is time consuming, distressing to the child and caregiver and should be repeated on consecutive days, induced sputum is easier to perform, relatively non-invasive, does not require hospitalisation, and can be repeated if necessary. Although GL can be successfully

performed in an ambulatory setting (Lobato et al 1998), the majority of children require hospitalisation; in contrast sputum induction is easily done as an outpatient procedure.

In addition to *M tuberculosis*, microbiologic confirmation of the aetiology of pneumonia in children is extremely difficult as demonstrated in a study of HIV-infected children with pneumonia in a developed country in which only 16 (12%) of 131 acute pneumonia episodes had an identified aetiology (Mofensen et al 1998). Although lung aspiration is the gold standard for identifying pathogens in pneumonia, this is an invasive procedure and associated with the risk of pneumothorax and clinical decompensation especially in children with severe respiratory illness (Chaudhary et al 1977). Moreover, HIV-infected adults who underwent percutaneous transthoracic fine-needle aspiration for suspected PCP have been reported to develop sudden circulatory collapse probably due to an HIV-associated autonomic neuropathy (Craddock et al 1987) making this procedure unsuitable for children in the present study. The use of BAL in developing countries is limited by the lack of resources and skilled personnel to perform this procedure; furthermore, clinical deterioration may occur following lavage (Bye et al 1987). Thus it is desirable to develop non-invasive, effective diagnostic techniques for children with pneumonia. Sputum induction is such a technique, however, there is little information on its use for microbiologic diagnosis of pneumonia in children. A study of 17 immunocompromised children (mean age 9.3 years) with suspected respiratory infection, reported that in 3 patients, sputum induction successfully identified pathogens (CMV in one, *H influenzae* in two) (Foot et al 1992). A second paediatric study of 18 immunosuppressed children cultured bacterial pathogens from induced sputum in 4 children who were negative for PCP

(Ognibene et al 1989). A larger study of 157 children admitted to hospital with pneumonia reported that after inducing a cough by pharyngeal stimulation, a swab taken from the oropharynx cultured bacteria in 56% of cases (Utsunomiya et al 1998). Distinguishing bacteria colonising the upper airway from lower respiratory pathogens in sputa may be difficult. However, in the present study, differences in the type and proportion of bacteria cultured on NPAs compared to induced sputa suggest that the latter were lower respiratory tract secretions. Furthermore, sputum induction was useful for diagnosing PCP whereas NPAs were not. The efficacy of sputum induction for the diagnosis of PCP has previously been reported in a study of 18 immunosuppressed children (mean age 7.3 years) in which 9 episodes of PCP were documented (Ognibene et al 1989). In a subgroup of 4 children sputum negative for *P carinii*, bronchoscopy and BAL were done; none had evidence of PCP.

Sputum induction mobilizes secretions from the lower respiratory tract. Hypertonic saline, deposited in the lower airways causes interstitial fluid to move into this area by osmosis (O'Byrne and Hargreave 1994). Furthermore, hypertonic saline stimulates the cough reflex causing secretions from the lower respiratory tract to be moved upwards. The efficacy of sputum induction in this study may be due to the application of a standard technique by a few people trained in its use and to rapid processing of the specimen by the laboratory. The diagnostic certainty for induced sputum can be improved by microscopic examination of a gram stained smear of the specimen to document the type and number of cells. The presence of white cells particularly polymorphonuclear cells or high numbers (25 or more) of white cells with low numbers of squamous epithelial cells is likely to represent true lower respiratory tract secretions (Pellegrino et al 1992). Additionally quantitative bacterial culture (greater

than 10^7 CFU/ml) may also help to distinguish pathogenic bacteria from commensals (Matsumoto et al 1978). Due to resource limitations, these techniques were not performed in the present study.

Sputum induction is a relatively simple technique that does not require sophisticated equipment and can be taught to health care workers. Precautions to prevent the spread of *M tuberculosis* or other respiratory pathogens to patients and staff should be taken when performing the procedure. In children, sputum induction is unlikely to result in transmission of tuberculosis due to the paucibacillary nature of their disease as evidenced by a minority of children who were smear positive. Nevertheless, it is recommended that sputum induction be performed in a room with negative pressure ventilation (CDC, MMWR 1990); in the absence of such a facility (as occurs in most developing countries), the procedure should be done in a well-ventilated room and equipment sterilised between patients.

In the present study, NPAs were not useful for diagnosing PCP. This is consistent with studies that have demonstrated that the principal interaction of *P carinii* with human tissue is adherence of the trophozoite to the type I pneumocytes lining the alveoli (Yoneda and Walzer 1980, Yoneda and Walzer 1981). *P carinii* is therefore not usually found in upper airway secretions as it inhabits the lungs exclusively. It is possible that in overwhelming infection, *P carinii* may be detected from the upper airways using methods that are sufficiently sensitive to detect small numbers of organisms (Hague et al 1990, Richards et al 1994). This is supported by the high fatality rate [10 of 16 patients (63%)] reported in Malawian children with PCP in a study in which *P carinii* was identified using NPAs (Graham et al 2000). In a second

small study in which 5 cases of PCP were diagnosed by identification of *P carinii* on NPAs, 4 of 5 children (80%) died (Kamiya et al 1997).

Although NPAs were not useful for identifying *P carinii*, bacterial culture provided information on colonising organisms. Knowledge of the type and antimicrobial susceptibility of bacteria carried in the nasopharynx is important to identify pathogens circulating and causing infection in the community (Gehanno et al 1998, Kellner et al 1998). Colonisation rates and antimicrobial susceptibility may be altered by HIV-infection, antibiotic treatment for intercurrent infections or use of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis (Rusen et al 1997, Arason et al 1996, Slobod and Leggiadro 1994). Monitoring the type and resistance patterns of colonising bacteria is important for rational and optimal use of antibiotic therapy.

6.6 Conclusion

Sputum induction can be safely and effectively performed in infants and young children. Sputum induction was well tolerated even in children who were on supplemental oxygen or who had AIDS. Induced sputum provides a satisfactory and more convenient specimen for bacteriologic confirmation of pulmonary tuberculosis in HIV-infected and uninfected children than GL. Use of induced sputum should be considered as a first line investigation in children suspected of having pulmonary tuberculosis especially in circumstances in which a culture confirmed diagnosis should be vigorously sought such as when the source case is unknown, drug resistance is suspected or cutaneous anergy occurs.

Sputum induction also provided a suitable specimen for diagnosis of PCP. In contrast, nasopharyngeal aspirates did not provide a useful specimen for identification of *P carinii*. Bacterial cultures from induced sputa differed from those obtained from NPAs, suggesting that lower respiratory secretions were obtained during sputum induction. However, knowledge of the type and antimicrobial susceptibility of bacteria colonising the nasopharynx is important to identify circulating pathogens responsible for infection in the community and for monitoring changes in the type or antimicrobial susceptibility patterns of bacteria.

Future studies should address the sensitivity of induced sputum compared to BAL for identification of respiratory pathogens in young children. As induced sputum is relatively non-invasive, technically easier and less costly than BAL, this procedure may be considered as an initial investigation in infants and children hospitalised for pneumonia.

CHAPTER 7: SUMMARY AND RECOMMENDATIONS

7.1 Summary

The major findings of the study are summarised as follows:

7.1.1 Diagnosis of HIV infection

The study indicates that a substantial number of HIV-positive children (more than 40%) admitted to hospital with pneumonia are only diagnosed with HIV infection at the time of admission. As the majority of these children had clinical signs of AIDS, it is possible that HIV infection could have been diagnosed earlier. Routine screening of pregnant women for HIV infection would enable early identification of infected infants and effective prevention of PCP by use of chemoprophylaxis. Other opportunities for diagnosis of HIV infection in young infants are routine visits to primary health care well baby clinics for immunisations (currently at 6, 10 and 14 weeks and at 9 months according to national South African guidelines). In the context of the increasing number of HIV-infected children in South Africa, education of primary health care workers on the signs ans symptoms of HIV infection may improve screening for HIV-positive children at these clinics. Early identification of HIV-infected infants has implications for prevention of PCP and for use of other interventions.

7.1.2 Aetiology of pneumonia

PCP was an important AIDS indicator disease occurring in 20% of children diagnosed with HIV infection at the time of admission. PCP was a preventable disease as demonstrated by the efficacy of TMP-SMX prophylaxis in that only a single HIV-infected child on prophylaxis developed PCP. Prevention of PCP requires early

identification of HIV-infected children, reinforcing the need for antenatal HIV screening.

Bacteria were the most common cause of pneumonia. Bacteraemia in association with pneumonia occurred in 14% of children; gram positive and negative organisms occurred with equal frequency but *S pneumoniae* and *S aureus* were the predominant isolates. An increased incidence of *S pneumoniae* bacteraemia was found in HIV-infected children using TMP-SMX prophylaxis compared to those not on prophylaxis; this requires further investigation. The type and antimicrobial susceptibility of bacterial pathogens were similar in HIV-positive and negative children except for *S aureus* which was more common in NPAs and sputa of children who had been previously diagnosed as HIV-infected. Use of TMP-SMX prophylaxis in HIV-positive children was not associated with any significant differences in antimicrobial resistance patterns but this analysis was limited by the small number of isolates.

Although children were admitted for acute pneumonia and were therefore not suspected of having tuberculosis, a culture confirmed diagnosis of pulmonary tuberculosis was made in 8%, reflecting the high incidence of this infection in this geographic area. The incidence of TB did not differ by HIV status. Viruses were cultured from respiratory tract secretions in 15% of children; the incidence did not differ by HIV status. CMV was the commonest virus isolated. Coinfection with bacteria was present in half of those with positive viral cultures.

7.1.3 Diagnostic investigations

Induced sputum

Induced sputum was a safe and effective procedure that was well tolerated even in very young children. The proportion of bacteria cultured on sputa compared with NPAs was significantly different particularly in HIV-infected children, providing evidence that sputa was derived from lower respiratory tract secretions. Induced sputum yielded a culture confirmation of TB in almost twice the number of children than did gastric lavages. Furthermore, *P carinii* was identified on induced sputa in 12 of 19 (63%) of children found to have PCP.

Other investigations

A blood culture was a useful investigation, yielding a pathogen in 14% of children; the incidence of bacteraemia did not differ by HIV status.

Serum LDH was useful in distinguishing HIV-positive children with PCP from those without PCP with higher presenting values associated with PCP (median 626U/l compared with 307 U/l). Moreover, LDH values were higher in the subset of HIV-infected children who died compared to survivors.

A skin test for diagnosis of *M tuberculosis* infection was positive in only 33% of HIV-infected children with culture confirmed pulmonary TB. This reflects the development of anergy in HIV-positive children and is consistent with studies that have reported that most HIV-infected children rather than producing smaller reactions to tuberculin, do not react at all (Madhi et al 1999).

A chest Xray did not identify any specific changes associated with pneumonia in HIV-positive children. Furthermore, there were no distinguishing features on chest radiology that identified children with PCP. This is consistent with prior studies in which a chest Xray has not been found to be predictive for a specific pulmonary pathologic diagnosis in HIV-infected children and in which a wide spectrum of chest radiographic abnormalities in patients with PCP has beeen described (Zimmerman et al 1987, Berdon et al 1993, Sivit et al 1995). Children with culture confirmed pulmonary TB had hilar or mediastinal lymphadenopathy more commonly than those without TB.

Nasopharyngeal aspirates were not useful for diagnosing PCP as they were negative in all cases in which *P carinii* was identified from lower respiratory tract secretions. However, bacterial culture of NPAs is useful for monitoring the type and antimicrobial susceptibility patterns of colonising bacteria, which represent pathogens causing disease in the community. Moreover, culture of nasopharyngeal colonising bacteria is important for monitoring the effect of chronic TMP-SMX use on the type and resistance of microbial flora.

7.1.4 Outcome

HIV-infected children had a longer duration of hospitalisation than seronegative patients (median of 14 days compared to 10.5 days). For children admitted to ICU, use and duration of intermittent positive pressure ventilation and the number of days spent in ICU was similar in HIV-positive and negative children.

The in-hospital mortality of HIV-infected children with pneumonia (20%) was significantly higher than that of seronegative patients (8%). PCP was the major risk factor for mortality. However, even when children with PCP were excluded the mortality for HIV-infected children (18%) was higher than that for seronegative patients (7%). The mortality of those HIV positive children selected for admission to ICU (29%) was almost twice that of seronegative children (15%) but this difference did not reach statistical significance probably due to the small sample size. However, the mortality rate for HIV-infected children admitted to ICU was better than that reported in prior South African studies. An ICU selection bias based on HIV status may have affected the overall mortality rate for HIV-positive patients as 80% of these children died in a general ward; in contrast 100% of seronegative children died in ICU.

7.2 Recommendations

Timely identification of HIV-infected infants is important to implement TMP-SMX prophylaxis and provide optimal supportive care for such children. Antenatal screening for HIV-infected mothers is important for early detection of HIV-positive infants. Opportunities for diagnosing HIV infection or referring infants suspected of being HIV positive at well- baby visits to primary care clinics should be optimised so as to improve early case detection of such children.

Wider use of sputum induction as a diagnostic investigation should be attempted particularly in children who are hospitalised for pneumonia or those in whom pulmonary tuberculosis is suspected. Although sputum induction was more effective than gastric lavage for culture confirmation of *M tuberculosis*, larger studies are

needed to confirm this. In addition, training of personnel in the performance of sputum induction is essential to optimise the yield from this procedure. The efficacy and feasibility of sputum induction for diagnosis of pulmonary pathogens needs further study in primary care settings.

Nasopharyngeal aspirates are not useful for diagnosis of PCP but bacterial culture may provide useful information about the type and antimicrobial susceptibility of prevalent bacterial pathogens. Ongoing surveillance of the type and susceptibility patterns of bacteria colonising the respiratory tract is important to monitor changes in type or resistance, particularly in HIV-infected children on chronic TMP-SMX therapy.

Specific management recommendations for HIV-infected children admitted to hospital with acute pneumonia in this geographical include:

- provision of effective PCP treatment for any child under 12 months of age who has signs of AIDS but who has not been diagnosed with HIV infection
- provision of effective PCP treatment for an HIV-positive infant who has not been taking TMP-SMX prophylaxis
- provision of effective PCP treatment for any HIV-positive child who has a previous diagnosis of PCP and who is not on TMP-SMX prophylaxis
- use of broad spectrum antibiotics effective against gram positive and negative bacteria
- use of antibiotics effective against *S aureus* in HIV-infected children with chronic disease
- use of oxygen therapy for accompanying hypoxia.

The high incidence of PCP and associated mortality as well as the efficacy of TMP-SMX prophylaxis for prevention of PCP, provide clear evidence for the use of prophylaxis in all HIV-infected infants. However, long term monitoring of the effect of such therapy on the development of other infections and outcome of HIV-infected children is important.

Development of clear policy guidelines regarding the admission of HIV-infected children to health facilities including intensive care units is needed. The study found that HIV-positive children with pneumonia have a higher in-hospital mortality compared with seronegative children. However, the majority (80%) of HIV-infected children hospitalised with pneumonia survived, given appropriate therapy. Even amongst the HIV-positive children selected for admission to ICU, 71% survived compared to 85% of seronegative children. In developing countries, reliable prognostic indicators for survival in children with pneumonia need to be identified in order to develop policies for hospital and ICU admission. Moreover, additional, larger studies in developing countries are needed to investigate the short and long-term outcome of children who are hospitalised for pneumonia so as to inform hospital admission and management policies.

7.3 Other issues

Prevention of pneumonia and HIV infection are important issues that should be included in management and policy guidelines, particularly in developing countries where these interventions may provide the greatest benefit given the limited resources

available. A detailed discussion of preventative strategies is beyond the scope of this thesis, however a brief summary of the possibilities follows.

7.3.1 Prevention of pneumonia

Besides prevention of PCP with chemoprophylaxis, protection against other potential pathogens causing pneumonia may be provided by immunisation. Immunisation of HIV-infected children against measles virus, *C diptheria, B pertussis* and *H influenzae type b* infection is important. These vaccines currently form part of the routine schedule recommended by the Department of Health in South Africa. BCG is also currently given at birth for all children in South Africa but the protective efficacy of this immunisation in HIV-infected infants is unknown. Increasing evidence suggests that the benefits of immunisation are greater than protection against the specific disease; vaccine-induced immuno-stimulation may produce non-targeted effects with considerable impact on child survival (Kristensen et al 2000). Whether such effects will occur in HIV-infected children needs further study. The efficacy of *S pneumoniae* immunisation for prevention of pneumonia in HIV-infected people is questionable, although this has been recommended as the standard of care in developed countries (CDC 1989). Of concern is a recent randomised placebo-controlled trial of 23-valent polysaccharide pneumococcal vaccine in HIV-infected adults in Uganda in which immunisation was found to be ineffective and associated with an increased rate of invasive disease and pneumonia (French et al 2000). However, a 7-valent conjugate pneumococcal vaccine has recently been approved for use in all children aged 23 months and younger in the USA (American Academy of Pediatrics, 2000). Studies on

the efficacy of pneumococcal vaccine in HIV-infected children are necessary before routine immunisation can be considered in developing countries.

Attention to adequate nutrition is also important in prevention and management of pneumonia. Early and aggressive nutritional intervention in HIV-infected children may help prevent malnutrition and disease progression (Periquet 1995). Micronutrient deficiency may play a role in the development and severity of pneumonia in HIV-infected children. Vitamin A supplementation in children admitted to hospital with pneumonia resulted in a 49% reduction in mortality and a 63% reduction amongst HIV-infected children. (Fawzi et al 1999). However, a meta-analysis of the impact of vitamin A supplementation as an intervention to prevent childhood pneumonia in developing countries found no effect on pneumonia incidence or pneumonia-specific mortality (The Vitamin A and Pneumonia Working Group 1995). A pooled analysis of randomised controlled trials of zinc supplementation reported a substantial reduction in the rate of pneumonia in children in developing countries, but these studies were not of HIV-infected children specifically (Bhutta et al 1999). Selenium deficiency has been reported to be associated with disease progression and mortality in a study of 24 HIV-infected children but the causes of death were not stated (Campa et al 1999).

7.3.2 Prevention of HIV infection

Prevention of HIV infection *per se* through educational campaigns, behavioural interventions and promotion of safe sexual practices should be part of an overall strategy to contain the HIV epidemic. Implementation of well-designed and adequately resourced behavioural interventions has resulted in a decline in HIV

incidence in targeted communities (Donovan and Ross 2000). Increased condom use, treatment of sexually transmitted diseases and reduction in the number of sexual partners have all been shown to be effective in preventing HIV transmission in developing countries (Ainsworth and Teokul 2000). Uganda has reduced its estimated prevalence from 14% in the early 1990's to approximately 8% using strong prevention campaigns (UNAIDS 2000).

Urgent measures to prevent mother to child transmission of HIV in developing countries are needed. The use of antiretroviral therapy in pregnant women has resulted in a dramatic decrease in the number of HIV-infected children in developed countries, with HIV transmission rates declining by 50 to 66% within the first 3 years of use of zidovudine (ZDV) in pregnancy (Simonds et al 1998, Mayaux et al 1997). Short course ZDV produced a 50% reduction in maternal to child transmission in Thailand (Shaffer et al 1999). A 37% reduction at 3 months of age occurred in children in Cote d'Ivoire who were mostly breastfed (Wiktor et al 1999). A trial of nevirapine, a rapidly acting anti-viral with a long half life that is orally administered reported a substantial reduction in transmission with an HIV transmission rate of 13% at 14 to 16 weeks amongst breastfed Ugandan infants (Guay et al 1999). Use of this drug may be a possibility in developing countries as it is much cheaper and easier to administer than other anti-retrovirals. An economic analysis of short course antiretroviral regimens in South Africa reported that vertical transmission of HIV could be reduced by 60% and would be cost-effective (Söderlund et al 1999). A single-dose nevirapine regimen for mothers and babies has been reported to be highly cost effective for prevention of perinatal transmission in areas of high HIV seroprevalence, particularly sub-Saharan Africa (Marseille et al 1999).

The choice of infant feeding method may affect HIV transmission. Epidemiological data suggest that breastfeeding is associated with an approximate doubling of HIV transmission risk (Mofenson and McIntyre 2000). In a study of Kenyan infants, formula feeding reduced postnatal transmission by 44% (Nduati et al 2000), implying that provision of formula to uninfected infants born to HIV positive mothers protects against HIV acquisition. However, recent data has reported mixed feeding (breastmilk and any additional liquid or food) to be associated with the highest HIV transmission rate, while the infection rate in exclusively breast fed infants was similar to that in children taking formula alone (Coutsoudis et al 1999). Moreover, the cost and impact of formula feeding on overall childhood mortality rates in developing country settings needs further study.

The development of a safe, affordable and effective vaccine against HIV-1would offer the best long term hope for control of the epidemic, particularly in developing countries (Esparza and Bhamarapravati 2000). Small clinical trials of HIV-1 vaccine have been running since 1987 but phase III efficacy trials have only recently commenced (Girard et al 1999, Nitayaphan and Brown 1998). However, most HIV-1 candidate vaccines have been based on subtype B strains, which predominate in the USA and western Europe or subtype E which is prevalent in Thailand (Brown and McNeil 1998, Esparza and Bhamarapravati 2000). The situation in Africa where subtypes C, A and D are prevalent, is complex and may require a cocktail-vaccine approach. Recently greater emphasis has been placed on vaccine designs applicable for developing countries especially subtype C, the dominant subtype in sub-Saharan Africa (Esparza and Bhamarapravati 2000). As a result a number of different vaccines are

now in pre-clinical development. The International AIDS Vaccine Initiative, an international scientific organisation committed to ensuring the development of safe, effective and accessible HIV vaccines for use throughout the world, has spearheaded many of these initiatives (Berkley and Koff 2000). The South African Aids Vaccine Initiative (SAAVI) has been established to develop an effective vaccine for local use. Phase I trials of a candidate vaccine are due to begin in the latter part of 2000 (Galloway 2000). Support for vaccine development has been increasingly provided by a number of national and international agencies, non-governmental institutions and the private sector. The global challenge is not only to produce a safe and effective vaccine but also to make it available in a timely way to all those who need it.

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APPENDICES

APPENDIX 1: DATA SHEET FOR HIV-PNEUMONIA STUDY

DEMOGRAPHIC
NAME:_____
STUDY NO:
FN:
R/S:
DOB:<DD/MM/YY>
MONTHAGE:
SEX: M/F

ADMISSION INFORMATION
ADM.WARD (1=B1,2=B2,3=E1,4=E2,5=NSH, 6=NSHICU, 7=CON, 8=GSH)
DOA:<DD/MM/YY>
DOD:<DD/MM/YY>
NO.OF DAYS IN HOSP:
ADM.ICU:Y/N NO.OF ICU DAYS:
DOAICU:<DD/MM/YY>
DODICU:<DD/MM/YY>

SOCIOECONOMIC
POSTAL CODE:
MOTHER'S AGE:
NO.OF SIBS:
MOTHER KNOWN HIV Y/N
MOTHER'S EDUCATION (0=NONE,1-10=STD.PASSED,11=TERTIARY):
MOTHER CURRENTLY AT SCHOOL Y / N
HOUSING (1=SHACK, 2=HOUSE,3=FLAT,
4=OTHER/SPECIFY):_____

ENVIRONMENTAL
MATERNAL SMOKING Y/N
SMOKER IN HOUSEHOLD Y/N_
INDOOR COOKING FUEL (1=WOOD, 2=COAL, 3=PARAFFIN, 4=GAS,
5=ELECTRICITY, 6=OTHER)
PETS AT HOME (0=NONE, 1=CAT, 2=DOG, 3=BIRD, 4=OTHER)

HISTORY
SYMPTOMS AND DURATION
COUGH(Y,N, DK/NO.OF DAYS):_
FEVER(Y,N, DK/ DAYS):_
RHINORRHEOA(Y,N, DK/DAYS):_
LOSS OF WT.(Y,N, DK/WEEKS):_
STRIDOR(Y,N, DK/NO.DAYS): _
WHEEZE(Y,N, DK/NO.DAYS): _
FAST BREATHING (Y,N, DK/NO. DAYS) _
VOMITING(Y,N, DK/DAYS): _
DIARRHOEA(Y,N, DK/DAYS): _
POOR FEEDING(Y,N, DK/D): _

CURRENT MEDICATION
COTRIMOXAZOLE PROPHYLAXIS(Y,N,DK):__
BR'DILATOR (Y,N,DK/SPECIFY):
STEROIDS (0=NONE, 1=INHALED, 2=ORAL/SPECIFY, 3=DK)
DURATION OF ORAL STEROID THERAPY (DAYS)
DURATION OF INHALED STEROID THERAPY (DAYS)
ANTIBIOTICS(0=NONE, 1=AMOXICILIN, 2=COTRIMOXAZOLE,
3=ERYTHROMYCIN, 4=PEN V, 5=CEPHALOSPORIN, 6=NALIDIXIC ACID,
7=AUGMENTIN, 8=OTHER (specify), 9=DK) _____
DURATION OF ANTIBIOTIC THERAPY (DAYS)

HIV STATUS
KNOWN HIV(Y,N,DK):_
MONTHAGE @ DIAGNOSIS:
SUSPECTED HIV(0=NO, 1=LYMPH, 2=HEPAT, 3=SPLENO, 4=CANDIDA,
5=PAROTITS, 6=DIARRHOEA, 7=LOWWT):

BIRTH HISTORY
BW(GMS/DK=DON'T KNOW: __
GESTATION (1=FT, 2=PREM NOT VENTILATED, 3=PREM VENTILATED,
4=DK):
BREASTFED (Y,N,DK):__
DURATION (MONTHS):
ROAD TO HEALTH CARD(Y,N,DK):__
IMM.UP TO DATE (0=DK, 1=Y, 2=NOT FOR DWT, 3=NOT FOR MEASLES,
4=NOT FOR BCG, 5=OTHER):

PAST HISTORY
PREVIOUS ADM (0=NONE,1,2,3,ETC)
REASONS FOR ADMISSIONS (1=RESP, 2=GIT, 3=CNS, 4=SKIN, 5=HAEM)
No OF PREVIOUS ADMISSIONS FOR RESP
PRIOR ICU ADMISSION (0=NONE, 1,2,3 ETC)
PRIOR PCP DIAGNOSIS Y/N/DK __
DATE OF LAST ADM:<DD/MM/YY>
ASTHMA(Y,N,DK):__
ALLERGY (Y,N,DK) __
PRIOR TB TREATMENT (Y,N,DK) __ if Y TREATMENT CARD SEEN (Y/N) _
CURRENT TB TREATMENT (Y, N, DK) if Y DURATION OF TREATMENT
(MONTHS)

FAMILY HISTORY
ASTHMA (Y,N,DK):__
ALLERGY (Y,N,DK):__
TB HOUSEHOLD CONTACT Y/N/DK: __

CLINICAL EXAMINATION

WT(KG):
HT(CM):
BCG SCAR(Y,N):_
CYANOSIS IN ROOM AIR Y/N
DEHYDRATED (Y,N) _
CLUBBED (Y/N) _
PALE (Y/N) _
JAUNDICE (Y,N):_
ORAL CANDIDA (Y,N):_
ENLARGED PAROTIDS (Y,N):_
ORAL HERPES (Y,N):_
RHINORRHOEA (Y,N) _
HEPATOMEGALY (Y,N):_ CM:
SPLENOMEALY (Y,N):_ CM:
LYMPHADENOPATHY (O=NONE, 1=CERVICAL, 2=AXILLARY, 3=INGUINAL, 4=SUPRACLAVICULAR, 5=GENERALISED) # # # #
ECZEMA(Y,N):_
CHEST: *complete daily illness chart on admission day*

DISCHARGE
STATUS(1=ALIVE,2=DEAD):#

APPENDIX 2: DAILY ILLNESS CHART

DAY	*Adm*	2	3	4	5	6	7
Date (dd/mm)							
Care (1=gen, 2=high care, 3=icu)							
Temp (**highest** in 24 hrs)							
HR (at rest)							
RR (at rest)							
Hyperinflated Y/N							
Chest indrawing (0=none,1=subcos, 2=intercostal, 3=supraster)							
Air entry equal bilateral Y/N							
Wheeze (0=none, 1= local, 2=diffuse, 3=audible)							
Bronchial breathing (0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL)							
Creps (0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL)							
Feed(0=NPO, 1=ng, 2=poor po,3=well)							
O2 sat in room air							
CLINICALLY IMPROVED Y/N	------						
TREATMENT							
O_2 (0=none, 1= facemask, 2=CPAP 3=cannulae, 4=hbox, 5=ippv)							
% O_2 or flow rate							
Ab (0=none, 1= cefurox, 2=amp, 3=pen G, 4=genta, 5=cotrimox iv, 6=ceftriax, 7=cotrimox po, 8=augmentin, 9=amox, 10=erythro, 11= clox, 12=cefotax, 13-amik, 14=pip, 15=metro 16=INH, 17=Rif, 18=PZA, 19=cefip, 20= amp, 21=chloro, 22=other							
S/E Ab (0=none, 1=diarr, 2=vomit, 3=rash, 4=Steven John, 5=hepatitis, 6=aplastic anaem, 7=other (specify)							
Antibiotic added/ changed Y/N							
Antifungal (0=none, 1=nystatin, 2=flucon, 3=ampho, 4=griseof, 5=ketoc, 6=micon, 7=other)							
Antiviral (0=none, 1=acyclovir)							
Steroid (0=none, 1=oral, 2=iv)							
Bdilator (0=none, 1=MDI, 2=nebs, 3=po B2, 4=theoph, 5=other)							
Cardiac meds (0=none, 1=digoxin, 2=iv inotrope).							
Skin test PPD (0=neg, 1=pos, 2=ND) ---mm							

APPENDIX 2: DAILY ILLNESS CHART

DAY	8	9	10	11	12	13	14
Date (dd/mm)							
Care (1=gen, 2=high care, 3=icu)							
Temp (**highest** in 24 hrs)							
HR (at rest)							
RR (at rest)							
Hyperinflated Y/N							
Chest indrawing (0=none,1=subcos, 2=intercostal, 3=supraster)							
Air entry equal bilateral Y/N							
Wheeze (0=none, 1= local, 2=diffuse, 3=audible)							
Bronchial breathing (0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL)							
Creps (0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL)							
Feed(0=NPO, 1=ng, 2=poor po,3=well)							
O2 sat in room air							
CLINICALLY IMPROVED Y/N	------						
TREATMENT							
O_2 (0=none, 1= facemask, 2=CPAP 3=cannulae, 4=hbox, 5=ippv)							
% O_2 or flow rate							
Ab (0=none, 1= cefurox, 2=amp, 3=pen G, 4=genta, 5=cotrimox iv, 6=ceftriax, 7=cotrimox po, 8=augmentin, 9=amox, 10=erythro, 11= clox, 12=cefotax, 13-amik, 14=pip, 15=metro 16=INH, 17=Rif, 18=PZA, 19=cefip, 20=amp, 21=chloro, 22=other							
S/E Ab (0=none, 1=diarr, 2=vomit, 3=rash, 4=Steven John, 5=hepatitis, 6=aplastic anaem, 7=other (specify)							
Antibiotic added/ changed Y/N							
Antifungal (0=none, 1=nystatin, 2=flucon, 3=ampho, 4=griseof, 5=ketoc, 6=micon, 7=other)							
Antiviral (0=none, 1=acyclovir)							
Steroid (0=none, 1=oral, 2=iv)							
Bdilator (0=none, 1=MDI, 2=nebs, 3=po B2, 4=theoph, 5=other)							
Cardiac meds (0=none, 1=digoxin, 2=iv inotrope)							

APPENDIX 3: RADIOLOGY DATA SHEET HIV-PNEUMONIA STUDY

Patient Sticker:

CHEST XRAY

DATE READ	/ /98	/ /98
Radiological sign	Initial CXR	F/U CXR
DATE TAKEN	/ /1998	/ /98
Adequate inspiratory film	Y / N	Y / N
Adequate technique	Y / N	Y / N
General disease (0=normal, 1=hyperinflated, 2=diffuse, 3=focal, 4=patchy)		
Pattern (0=normal, 1=interstitial, 2=alveolar, 3=nodular, 4=mixed, 5=air bronchograms)		
Consolidation (0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL, 7=axillary seg, 8=diffuse, 9=atelectasis)		
Cavity (0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL, 7=R pneumatocoele, 8=L pneumatocoele)		
Granuloma(0=none, 1=RUL, 2=RML, 3=RLL, 4=LUL, 5=lingula, 6=LLL)		
Calcification (0=none, 1=pleural, 2=mediastinal, 3=hilar, 4=diffuse lung, 5=RUL, 6=RML, 7=RLL, 8=LUL, 9=lingula, 10=LLL,)		
Cyst (0=none, 1=diffuse, 2=perihilar, 3=RUL, 4=RML, 5=RLL, 6=LUL, 7=lingula, 8=LLL, 9=pleural)		
Pleura (0=normal, 1=L pneumo, 2=L effusion, 3=thickening, 4=R pneum, 5=R effusion)		
Adenopathy (0=none, 1=hilar, 2=mediastinal)		
Mediastin(0=normal, 1=wide, 2=pneumo, 3=R shift, 4=L shift)		
Trachea (1=central, 2=displaced, 3=indented)		
Bronchi (1=normal, 2=R narrow, 3=R displaced, 4=L narrow, 5=L displaced)		
Cardiomegaly Y / N	Y / N	Y / N
Radiological diagnosis (0=normal, 1=diffuse pneumonia, 2=LIP, 3=TB, 4=ARDS, 5=pulm oedema, 6=abscess, 7=bronchiolitis, 8=bronchiectasis, 9=PCP, 10=cyst, 11=non-specific, 12=pneumothorax, 13=pl effusion, 14=R pneumonia, 15=L pneumonia, 16=round pneumonia, 17=atelectasis, 18=pneumatocoele, 19=other (specify))		
F/U CXR (0=same, 1=worse, 2=improved)	----------	

APPENDIX 4: CONSENT FORM

You and your child are requested to participate in a study. The aim of this study is to find out the cause of your child's chest problem. In order to do this, a number of tests will be done. Firstly we will examine your child and measure the amount of oxygen in his / her blood using a sticker placed on the finger. Thereafter we will do some blood tests, a chest Xray, a skin test for TB and a swab of the nose. To do the nose swab we will put a few drops of salt water in the nostrils and then gently suction it out We will also need to get some phlegm from the chest. To do this we will give your child a breathing treatment after which a physiotherapist will either help your child to cough the phlegm out or will suction it out. These tests will be sent to the laboratory to try and find the exact germ causing the chest problem. There are no major side effects from these tests.

If these tests do not help to find the cause of the chest problem and if your child is not getting better within 72 hours then we will need to do an additional test called bronchoalveolar lavage (BAL). In this test a pipe is put into the airways to look into the lungs and to obtain some of the phlegm in the lungs to send to the laboratory. Your child will need to be sedated for this test. This test is one of the best ways of finding out what the chest problem is. However, there can be side effects such as difficulty with breathing afterwards, sleepiness or worsening of the chest problem. Nevertheless this test is very important to do if your child is not improving or getting worse, so that effective treatment can be started.

All the above tests are necessary in order to provide the best treatment for your child so that he/ she may recover from his / her illness.

I, _____, the parent/ legal guardian of _____ agree to allow her/him to participate in this study. I understand that participation is voluntary and I will not hold any of the investigators or hospital liable for any adverse effect that may occur as a result of participation in this study.

Signed: _____

Date: _____

Witness: _____

Patient sticker:

APPENDIX 4: CONSENT FORM FOR HIV TESTING ON CHILDREN ADMITTED TO HOSPITAL

I, ... the mother/father/guardian of

.....(sticker)..... have been told by doctor .....

that it is the hospital's policy to ask ALL PARENTS/GUARDIANS of children admitted to this hospital to give their permission for an HIV blood test. This may be necessary to find the cause of the child's sickness so that the correct treatment can be given.

MARK THE OPTION BELOW WHICH IS APPLICABLE

A. I give my consent for an HIV test to be done on my son/daughter. I understand that if this test is done, I will be told the result and its meaning explained to me by a doctor before my child is discharged from hospital. The result will not be given to any other family member or friend.

B. I do not want an HIV test to be done on my child

C. A test has already been done on my child

at ..... on ...../...../19.....

The test may/MAY NOT be repeated if the doctor considers it necessary.

Parent's Signature: .....

Doctor's Signature: ..... Date: ...../...../1998

INFORMATION FOR PARENT / GUARDIAN

The doctor taking the test should ensure that the parent /guardian has received the following couselling at the time of obtaining consent for HIV testing

1. HIV infection is common in the Western Cape
2. Children with HIV infection may get sick from many different causes.
3. A child's illness can be better treated if it is known whether that illness is related to HIV infection.

NB: If both parents are present then consent should be obtained from both.

COMPLETE AT THE TIME OF HIV TESTING

I. Dr. _____performed an HIV test on the above patient on / / 199 and undertake to ensure that the results of this test will be relayed to the parent/guardian of this child.