

UNIVERSITY OF ZAMBIA

A CLINICAL AND PATHOLOGICAL STUDY OF CHILDREN WITH PNEUMOCYSTIS CARINII PNEUMONIA

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CHAPTER	CONTENT	PAGE
	<i>DEDICATION</i>	<i>I</i>
	<i>ACKNOWLEDGEMENT</i>	<i>II</i>
	<i>COPYWRIGHT</i>	<i>III</i>
	<i>DECLARATION</i>	<i>IV</i>
	<i>APPROVAL</i>	<i>V</i>
1.	SUMMARY	1
2.	INTRODUCTION	3
3.	LITERATURE REVIEW	7
4.	AIMS AND OBJECTIVES	10
5.	METHODOLOGY	11
6.	RESULTS	13
7.	DISCUSSION	27
8.	CONCLUSIONS AND RECOMMENDATIONS	29
9.	PCP CLINICAL ALGORHYTHM	30
10.	REFERENCES	31

DEDICATION

This project is dedicated to my late father Mr Amos CM Lishimpi,

my mother Mrs Anna Lishimpi, my wife Dr Namwinga Chintu,

and my son Chendwa Lishimpi. Without all these people,

I would not be what I am today.

To my fellow clinicians I wish to say let us rise up

to the challenges of the devastating effects of the HIV/AIDS pandemic.

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DECLARATION

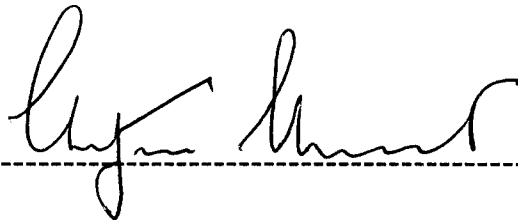
I declare that this dissertation represents my work and has not been presented even in part to any forum or university other than University of Zambia



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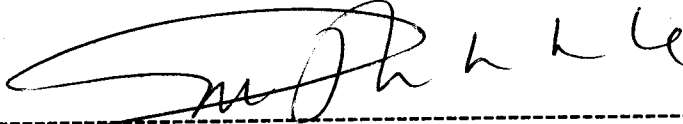
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APPROVAL

The University of Zambia approves this dissertation of Kennedy Lishimpi as partial fulfillment of the requirement for the Master of Medicine Degree in Paediatrics.

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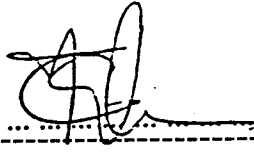
Examiner I

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EXTERNAL EXAMINER

Date of approval 18TH NOVEMBER 2002 -----

1 SUMMARY

The commonest causes of admission to University Teaching Hospital - Children wing are gastroenteritis, respiratory infections, anaemia, malnutrition and malaria. Twenty-eight percent of these admissions are seropositive for HIV-1. Since 1990 respiratory infections have constituted 28% of the total admissions and out of these, about 28% of the respiratory infections are associated with HIV, 11% of whom die during their hospital stay.

A significant number of children infected with HIV develop *Pneumocystis Carinii* Pneumonia (PCP) in their first year of life, which can be fatal. Tuberculosis, cytomegalovirus pneumonitis and PCP are very difficult to distinguish clinically in HIV infected children because of overlapping symptoms. Studies from Zambia and West Africa show that Childhood respiratory diseases are the most common cause of Paediatric hospital admissions. Due to limitations in the current clinical and laboratory criteria used for the diagnosis of respiratory diseases in children accurate information on the specific aetiologies of fatal illnesses are not available. Since Tuberculosis, bacterial pneumonia and PCP have overlapping clinical features, misdiagnosis may occur making management of such patients difficult. Treatment and Prevention of PCP is one of the most important health issues among HIV infected children. The clinical picture of PCP can be severe in HIV infected children such that intensive therapy with intravenous cotrimoxazole in combination with a steroid and inhaled pentamidine may be required. Knowing the actual aetiology of lung diseases in children is important in developing practical diagnostic, therapeutic, prophylactic protocols and for epidemiological surveillance and control. This study was carried out to define the clinical presentation and pathological findings of PCP in Zambian Children, and to try and find an easy method of diagnosis. There has been no study done in Zambia to understand the presentation of PCP in HIV infected Children.

This descriptive study conducted a clinical - pathological study of 264 childhood deaths from pulmonary disease at the University Teaching Hospital. Post mortem lung material were obtained and subjected to histo-pathological examination, and polymerase chain reaction (PCR) for *Pneumocystis Carinii* Deoxyribonucleic Acid (DNA). From the oropharyngeal washings DNA was extracted and PCR performed to detect the presence of *P.carinii*.

The findings were stratified by age and HIV status and correlated with clinical findings, in order to understand how PCP clinically presents in Zambian children, and how other diagnostic procedures compare with histopathological findings of PCP.

Aims and Objectives

- a. To find the prevalence of PCP in Zambian children dying of respiratory diseases.
- b. To collect non invasive samples (oropharyngeal washings) for alternative diagnostic tests such as, Polymerase Chain reaction for identification of *Pneumocystis carinii* deoxyribonucleic Acid (DNA) and find how well this compares with the same test done on DNA extracted from left upper lung, and later correlate with histopathological findings done on lung tissue.
- c. To understand the clinical presentation of PCP, its histological findings as compared to PCR for *P. carinii* DNA done on left upper lung extracts.
- d. To determine the presence of HIV infection in these children by using Enzyme Linked Immunosorbent Assay (ELISA) on all children above 18 months, and PCR for HIV in children less than 18 months.

Results and conclusion

- a. Thirty five percent of children aged 1 month to 14 years dying from pneumonia in UTH have **PCP and 93.2% of these children are missed clinically.**
- b. Out of the children found with PCP, 87.5% were co-infected with HIV.
- c. The sero-prevalence for HIV infection in children who had died of pneumonia was 63%.
- d. PCP is an AIDS defining event / infection even in Zambian children below the age of one year.
- e. At time of death children found with PCP were younger, age less than 8months had long duration of difficulties in breathing, tachypnoea and cyanosis when compared to those with bronchopneumonia. However, children with bronchopneumonia had more significant lymphadenopathy and oropharyngeal Candidiasis.
- f. PCP can be diagnosed from oropharyngeal washings using PCR.

2 INTRODUCTION.

In Sub-Saharan Africa today the Lung is one of the commonest target organ of HIV infection in children. The management of respiratory diseases has a substantial drain on the health resources of all African countries, Zambia inclusive. Infection with HIV is a major risk factor in development of opportunistic infections, such as Tuberculosis, cytomegalovirus and PCP [1,2] to mention but a few.

Pneumocystis carinii pneumonia is the most common serious HIV – associated opportunistic infection among children who are less than twelve months of age. PCP was diagnosed in 1080 (39%) of the 2789 paediatric AIDS patients reported to CDC in 1990. In medical centres caring for large numbers of children with perinatally acquired HIV infection, PCP has been the initial HIV related illness in 8% - 12% of children and for greater than 50% of those children who progress to AIDS within the first year of life [3,4,5,6,7].

Although PCP can occur at any age, in children it is most commonly diagnosed between 3 – 4 months of age. A population based study in Massachusetts found the minimum incidence of PCP during the first year of life to be 2.3% among all infants born to seropositive mothers, or an estimated 7.7% among HIV infected infants [8].

PCP is often the initial clinical sign of HIV infection, particularly among infants. Of the children described in the literature, at least half who developed PCP were not recognised as HIV infected before they were diagnosed as having PCP, although some had had earlier HIV associated symptoms [5,6,7,8,9]. Mortality from PCP among infants and children is high; the median survival time from the first episode is only 1-4 months [4-12]. Among AIDS cases reported to CDC, 35% of children with PCP died within 2 months of diagnosis, compared with 13% of children with other AIDS diagnoses.

As HIV disease progresses, T- helper lymphocyte (CD4+ cell) counts decline. Among adults with AIDS a CD4+ cell count of less than 200/mm³ is highly predictive of increased risk for PCP [9,10]. In children however, the absolute CD4 count are much higher at which PCP develops [11,12]. In ninety percent of HIV infected infants with PCP the CD4+ cell count ranged from 1500 – 2000 /mm³ [5,9,10,13,14,15,16,17,18,19].

It is recognised that due to the overlap in symptoms and signs of several common respiratory illnesses, misdiagnosis is common especially in HIV-infected children where opportunistic infections such as PCP may mimic Tuberculosis. Several diagnostic, therapeutic and epidemiological dilemmas are created by the inadequacy of the current clinical criteria for the specific diagnosis of respiratory infections in children. It is important to be able to allocate meagre health care funds to areas where they can have the most impact. Diagnosis, treatment and prevention of PCP being such an area, in this era of HIV/AIDS epidemic.

As is the case with Tuberculosis the main problem facing the clinical management of PCP in children in Zambia is that of accurate diagnosis. Saline gargles or mouthwash for amplification by PCR of *Pneumocystis carinii* DNA have been shown to be as sensitive and specific as using BAL fluid [20,21] for diagnosis of PCP. This technique has the potential for practical use as a differentiating diagnostic test in children with respiratory illness. It is relatively expensive and may seem not to be cost effective for routine use especially if frequency of PCP is low. However, if PCP is found to be a significant problem the use of PCR for *Pneumocystis carinii* DNA will be beneficial over time as management will be more specific. This study will try and understand the clinical aspects of PCP, its pathological findings on post mortem lung tissue as it compares with PCR for identification of *pneumocystis carinii* DNA done on left upper lung and oropharyngeal washings.

Community acquired pneumonia constitutes a major cause of morbidity for patients infected with HIV [22,23,24]. The three most common pneumonia syndromes in HIV infected patients are PCP, bacterial pneumonia and tuberculosis [23,25-32]. Clinicians are frequently faced with the challenges of differential diagnosis for these three syndromes, which require prompt treatment decisions often before microbiological diagnosis is made [33]. In most of the Paediatric clinics in Zambia, children present with recurrent respiratory illnesses and are forever breathless despite being treated in the past with different antibiotics and some with anti-tuberculosis drugs with slight or no improvement at all. Since the treatments of these syndromes differ it is important to be able to predict accurately the type of pneumonia based on the patient's clinical presentation.

This could facilitate the timely choice of appropriate therapy, decrease the need for costly invasive procedures, reduce hospital stay, and promote appropriate isolation for suspected tuberculosis cases [34]. In an attempt to improve clinical understanding of these syndromes, several reports have sought to characterise bacterial pneumonia, PCP, and Tuberculosis in the setting of HIV infection. While certain clinical features typical of each syndrome have been identified [35,36], several reports have suggested that clinical and radiological features of these three pulmonary diseases may be indistinguishable [37-40].

In Zambia the commonest causes of admission to the University Teaching Hospital, Paediatric wing are gastro-enteritis, respiratory infections, anaemia malnutrition and malaria. These may or may not be associated with HIV infection. Twenty eight percent (28%) of these admissions are seropositive for HIV-1. Since 1990 respiratory infections, have constituted about 28% of admissions. Out of these respiratory problems 28% are associated with HIV and 11% of whom die during their hospital stay [41]. In Zambia today the commonest organ to be infected in children with HIV is the lung. Infection with HIV is a major risk factor in the development of PCP and other opportunistic infections [1,2]. Studies of vertical HIV transmission in many regions of Africa show that childhood HIV infection is common [42]. *Pneumocystis carinii* pneumonia was initially thought to have been rare in Sub-Saharan children [43] but this is no longer the case at the present moment. Autopsy studies from Africa have shown that PCP is common in children with HIV infection dying of pneumonia [44,45,46]. PCP is closely related to immunosuppression. Preliminary data emerging from clinical, radiological and pathological studies show that PCP may not be an uncommon infection in Zambian children infected with HIV [Tshibwabwa - Tumba et al 1996; Lucas et al 1996]. It is just possible that PCP is being underdiagnosed due to the inadequate diagnostic facility in most of the hospitals in the developing countries.

Currently the most effective method of confirming PCP is by microscopic examination of Bronchoalveolar lavage (BAL) aspirates, lung aspirates, and open lung biopsy and of course after autopsy. All of these procedures are invasive and labour intensive and probably not feasible yet in Zambia as a routine procedure. Methods of amplification of *P.carinii* DNA by the use of PCR have been developed. This method can be applied not only on BAL specimens but also on non-invasive samples such as sputum and mouth / oropharyngeal washings which seem to be better alternatives to the invasive procedures [20,21]. To my knowledge no study

has been done in Zambia to determine the clinical and pathological features of PCP in HIV infected children.

3 LITERATURE REVIEW

Prior to 1991, *Pneumocystis Carinii* (*P. carinii*) was seen infrequently and only in-patients with immunosuppression. The organism was first recognised as the cause of an outbreak in a human population in 1955 in a group of malnourished children [47]. All children are believed to have had been infected with *Pneumocystis carinii* by the age of four years. Transmission is by aerosol and evidence of horizontal transmission exists from community outbreaks [48,49].

It has been shown in the United States of America that *Pneumocystis carinii* is common in homosexual men who are HIV positive with low CD4 cell levels less than 200/mm³. *Pneumocystis Carinii* was first described as both protozoan and fungal organism. Now it has been found to show fungal homology i.e. its 18s ribosomal sequence [50,51], the seven contiguous genes encoded on a 6.8 kilobase pair fragment of mitochondrial DNA [52] - genes encoding dihydrofolate reductase (DHFR); Thymidylate Synthase (TS) [53]; B-Tubulin [54], Transcription factor, Tubulin, cation transporting Adenosine triphosphatase and translation elongation factor (EF#) all show fungal homology. It however, lacks ergosterol in the plasma membrane [55] and does not respond to antifungal chemotherapeutic agents.

Different species of *Pneumocystis Carinii* exist and have antigenic differences. Most antibodies elicited from one species do not cross react with antigens of another species. *Pneumocystis Carinii* is a host specific organism. Transmission across species has not yet been demonstrated. *Pneumocystis Carinii* is transmitted by droplet spread from human to human. The cycle of asexual reproduction is from mature cysts forming eight sporozoites transforming into trophozoite that mature into cysts. This takes place in the alveolar air sacs and surrounding epithelial cells. Infection acquired in infancy represents a primary infection with late childhood disease caused by a resurgence of dormant infection.

PCP was thought to result from activation of latent infection especially in immunocompromised patients. However, this view nowadays has changed because latent infection has not been demonstrated in experimental animals and the persistence of infection

after initial infection has been shown to be cleared by 12 months of age of the child. Most children mount an immune response to *Pneumocystis Carinii* in early childhood. It is the failure of T-Cell function that appears to be of primary importance in the development of PCP [56].

Pneumocystis Carinii causes interstitial plasma cell pneumonia. It causes a mononuclear cell infiltrate of alveolar septae, compressing and collapsing of the alveolar air spaces and ducts with occasional dilatation of alveoli leading to emphysematous changes of lungs and occasionally pneumothorax. A foamy exudate containing organisms fill the alveoli, and appearance similar to that documented in infants dying with PCP and Acquired Immunodeficiency Syndrome (AIDS) [57].

PCP is one of the commonest AIDS - defining diagnoses in about 40 -50% children reported [58]. PCP is common in children less than one year, with a mean of age four months at onset [59]. The clinical tetrad of clinical feature of PCP is tachypnoea with or without dyspnoea, cyanosis, cough and fever. It has insidious onset of fast breathing. Cough comes in when the full clinical picture develops. Fever is low grade. Physical findings are limited from a clear chest to fine crepitations with or without stigmata of underlying HIV infection. Hypoxia is common. This progresses rapidly to respiratory failure and death if not treated. It is estimated that of the HIV positive new-borns, 8% - 12% are at risk of developing PCP. However, PCP can develop in children with CD4 cell counts of even greater than 1,500/mm³ [4,5,6,7].

Diagnosis of PCP is essential since it's an AIDS defining infection. In adults induced sputum has been used for diagnosis. BAL fluid or open lung biopsy has been used in adults. In children who are intubated, BAL fluid can be obtained. Tracheal or nasopharyngeal washings are increasingly common samples for diagnosis of *Pneumocystis Carinii* using polymerase chain reaction (PCR) [20]. Detection of *Pneumocystis Carinii* by DNA - amplification using PCR has been developed. Methods have been designed to a number of different loci of *Pneumocystis Carinii* genome including genes encoding - mitochondrial RNA [60,61] 5s rRNA [62], the 18s rRNA and thymidylate synthase [63].

Chest X-rays initially may be normal but complete opacification with air bronchograms are common. The alveolar infiltrate progress peripherally with late apical sparing and small pleural effusions. Occasionally, bullae, cysts and pneumothoraces have been seen.

Cotrimoxazole (Trimethoprim: Sulphamethoxazole) 20mg:100mg / kg / day in four divided doses infused over 1 hour for one week, then orally the next two weeks is the standard treatment of PCP. If side effects to cotrimoxazole occur, Pentamidine intravenously or Atovaquine can be used. Corticosteroids are of benefit in the initial treatment phase but this has not yet been demonstrated in Zambian children. Prophylaxis with Cotrimoxazole two to three times per week can be used in all children born to HIV positive mothers and to all children who have had an episode of PCP. Dapsone, inhaled Pentamidine or Atovaquine can be used in those who develop side effects to cotrimoxazole.

4 AIMS AND OBJECTIVES

AIMS

1. To define the clinical features of childhood PCP in Zambia.
2. To define the histopathological features of childhood PCP in Zambia.
3. Determine the prevalence of PCP in HIV positive Children dying from respiratory illness and determine whether PCP is an AIDS defining infection.
4. To elucidate the Clinical and Pathological features of PCP in Zambian children paying particular attention to the salient features that may help distinguish PCP from other opportunistic infection of Lungs in HIV infected children.

OBJECTIVES

1. To compare histopathological diagnosis of PCP on Lung tissue with polymerase chain reaction amplification of *P. carinii* DNA extracted from left upper lung and oropharyngeal washings.
2. To devise an easy and method of diagnosing PCP using oropharyngeal washings.
3. To try and develop a clinical and management algorithm to help in diagnosis of PCP.

5 METHODOLOGY

The Study was a build up on the already ongoing Post mortem (PM) study in Zambian children dying of respiratory illnesses. It is a descriptive prospective as well as a retrospective clinical study of children found to have had PCP at post-mortem. Both the PM and this study were approved by the Ethics Committee of the University of Zambia and the University Teaching Hospital.

Setting: study done at UTH Department of Paediatrics, Microbiology, virology and Pathology, Lusaka, Zambia.

Eligibility: informed consent, child died from respiratory infection, clinical files and CXR's available, clinical data form complete.

Subjects: Children aged between 2 months and 15 years, dying from a respiratory infection in UTH.

Sample size: The total number of admissions to the paediatric wing over six month is about 10 000. Using the formula

$$(Za) NP (1-P) \\ (N-1) E P + Za P (1-P)$$

Where $Za = 1.96$, $a = 0.05@95\%$ level of significance, P estimated prevalence of PCP 30%(0.3)[pilot study], N target population (10000) E deviation (margin of error) 0.18 allows confidence level 12% - 48%. The sample size was calculated at **269.127**.

Procedure: Using 10 mls of normal saline the oropharynx was washed to obtain oropharyngeal washings (sample I). Post mortem was restricted to the chest. Two separate left upper lung tissue samples (sample II a & b) were obtained. Blood was collected from one of the heart chambers (sample III). Samples so obtained were sent to respective laboratories at UTH for analysis i.e. sample (sample I) molecular biology laboratory for DNA extraction, sample (sample II a) histopathology laboratory and (sample II b) molecular biology laboratory for DNA extraction, and sample (sample III) to virology laboratory for ELISA and molecular biology laboratory for DNA extraction for HIV testing.

Using sample III HIV was determined by (1) ELISA on all subjects irrespective of age and (2) amplification of the POL and GAG regions of the HIV genome using PCR after DNA extraction done on all subjects regardless of age using another aliquot of this same sample.

Using sample II a, the presence of *P. carinii* was demonstrated histologically by staining prepared slides with silver-grocott stain. The left upper Lung tissue so obtained was sent for histopathological examination where the tissue was processed onto slides and stained with Silver-grocott impregnation. On high power microscopic examination PCP reveals polygonal cysts, which stain darkly, and some have tiny black dots inside which are trophozoites of *Pneumocystis Carinii*.

To demonstrate the presence of *P. carinii*, the left upper lung tissue (sample IIb) and oropharyngeal washings (sample I) were used for DNA extraction using the Proteinase K digestion and phenol chloroform extraction method to obtain DNA, which was then, amplified using the primers

pAZ 102E 5' GATGGCTGTTTCCAAGCCCA 3' and

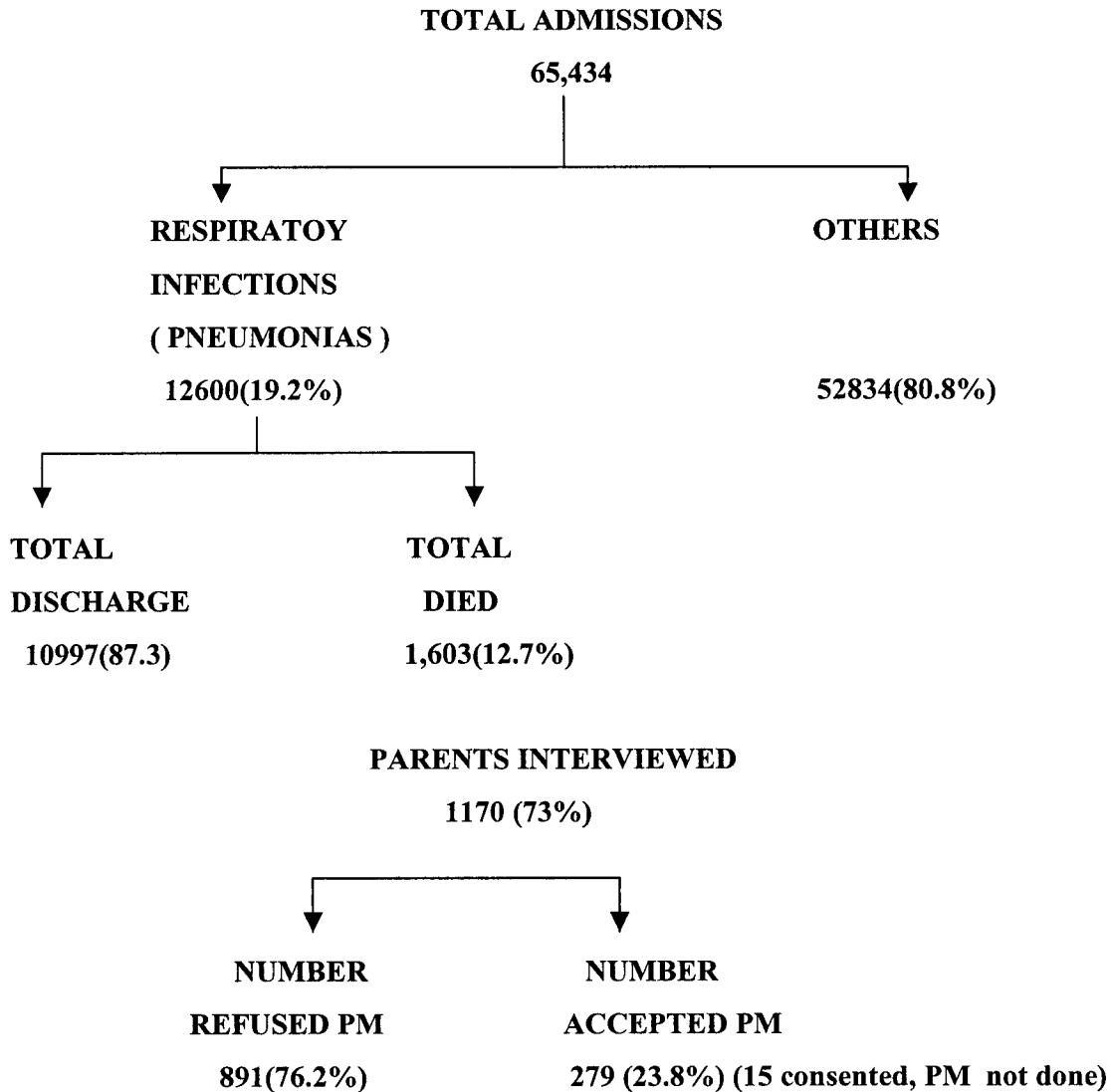
pAZ 102H 5' GTGTACGTTGCAAAGTACTC 3' to obtain a 340 base pair fragment.

PCR was performed on 1microlitre-extracted DNA, 0.35ul each of pAZ102E and pAZ102H, MgCl₂ 3ul, DNTPs 0.4ul, Tris-buffer 5ul, distilled water 39.5ul and Taq-polymerase 0.4 ul. Amplification was performed in a thermocycler. For a hot start, the PCR mixture was preheated to 94°C. Thirty-five cycles were carried out consisting of (1) denaturation for one minute at 94°C, (2) annealing for one minute at 55° C, (3) extension for 90 seconds at 72 ° C. A final extension was performed for 10 minutes at 72 ° C after the last cycle . PCR samples were stored at -20 ° C until further analysis was performed. All PCR products were investigated by agarose gel electrophoresis, stained with ethidium bromide, and visualised under UV light.

For all those cases that revealed PCP, the files and CXR's were reviewed and clinical data from the patient's files was recorded in order to understand the symptoms and sings of PCP. This data was correlated with HIV seropositivity in these children. A clinical diagnostic and management algorithm was then developed.

RESULTS

264 post mortems were done from September 1997 to July 2000. The total number of admissions during this period was 65,434. Of these 12,600 (19.2%) were diagnosed as Pneumonia. The case fatality was 1,603 (12.2%).



Only 220 cases were completely analyzed. 44 not analyzed because 30 had no complete clinical forms and 14 had either aCXR or clinical form missing. For some others reagents ran out.

CLINICAL CHARACTERISTICS OF THE 220 SUBJECTS

Male ; female ratio (150 : 100) 3:2.

SYMPTOM / SIGN	DURATION (averages given)		
	PCP	BRONCHO- PNEUMONIA	TUBERCULOSIS
Total numbers	80	116	54
Age at time of death	7.4months	8.9 months	22months
Duration of admission	3.8 days	4 days	5.4 days
Cough	6.2 days	6.4 days	12.9 days
Difficulty breathing	6 days	3.9 days	10.3 days
Fever	5.6 days	5 days	15.7 days
Temperature	35.6° c	38.4° c	37.5° c
Respiratory rate	49/min	57/min	69/min
Tachypnoea	18 subjects	0	0
Lymphadenopathy	4	9	5
Cyanosis	11	2	1
Oral thrush	14	25	9
Weight at time of death	5.1Kg	5.7Kg	5.7Kg
Auscultation :			
clear chest	50%	very few < 10%	40%
crepitations : bilateral	fine crepitations	bilateral crepitations	nil
Localised	nil	10% bronchial breathing	

Children with bacterial pneumonia and PCP are younger when compared to those with tuberculosis. The duration of symptoms is the same in the PCP and bacterial pneumonia group but longer in the tuberculosis group. In the PCP group tachypnoea was more significant than in those children with pneumonia. Lymphadenopathy and oral thrush were a more common finding in children with bacterial pneumonia as compared to those with PCP. Cyanosis was very often found in children with PCP. At time of death it appears as if malnutrition is more significant in children with tuberculosis.

1. CLINICAL SUSPICION OF PCP VERSUS HISTOPATHOLOGICAL DIAGNOSIS OF PCP

	HISTOPATHOLOGICAL PCP	
	POSITIVE	NEGATIVE
CLINICALLY PCP POSITIVE	3	1
CLINICALLY PCP NEGATIVE	41	211
	44	212
		256

Ninety-three point two percent of children found with PCP were clinically missed and only 6.8% of the cases with PCP were clinically suspected.

2. PCP HISTOPATHOLOGY VERSUS PCR FOR *P. CARINII* DNA

(Using histopathology as a gold standard)
PCP HISTOPATHOLOGY

	POSITIVE	NEGATIVE
PCP PCR POSITIVE	40	30
PCP PCR NEGATIVE	4	146
	44	176
		220

SENSITIVITY 90.9% SPECIFICITY 82.9%

PCR for PCP picks up more PCP as opposed silver-groccott stain of slides for PCP. On PCR 30 more, negative on histopathology, are pcked up. Histopathology only manages 4 more that are negative on PCR.

3. HIV POSITIVITY VERSUS HISTOLOGICALLY PROVEN PCP

PCP HISTOPATHOLOGY

POSITIVE NEGATIVE

HIV POSITIVE ON PCR
HIV NEGATIVE ON PCR

34	102	136
10	74	84
44	176	220

Point estimate [95% Conf. interval]

Odds ratio **2.466667** 1.159815 5.232835 (Cornfield)
Attr. frac. ex. .5945946 .1377935 .808899 (Cornfield)
Attr. frac. pop .1486486
chi2(1) = 5.57 **Pr>chi2 = 0.0183**

77.35% of those with PCP on histopathology were co infected with HIV

4. HIV SEROPOSITIVITY VERSUS PCR FOR P.CARINII DNA

HIV POSITIVITY ON PCR

POSITIVE NEGATIVE

PCP PCR POSITIVE
PCP PCR NEGATIVE

70	10	80
66	74	140
136	84	220

Point estimate [95% Conf. Interval]

Odds ratio **7.848485** 3.777974 16.26398 (Cornfield)
Attr. frac. ex. .8725869 .7353079 .9385144 (Cornfield)
Attr. frac. pop .7635135
chi2(1) = 35.13 **Pr>chi2 = 0.0000**

51.5% of those found to be HIV positive were found with PCP. However, **13%** of the HIV negative subjects were found with PCP, giving an overall rate for PCP at **35%** . Of those with PCP **87.5%** were coinfectd with HIV infection.

5. HISTOLOGICALLY PCP POSITIVE TISSUE VERSUS PCR POSITIVE OROPHARYNGEAL WASH

		OROPHARYNGEAL WASH		
		POSITIVE	NEGATIVE	
HISTOPATHOLOGY FOR P.CARINII	POSITIVE	15	7	22
	NEGATIVE	0	48	48
		15	55	70

SENSITIVITY of PCR 15/22=68.2% SPECIFICITY 48/48 = 100%
POSITIVE PREDICTIVE VALUE 15/15 = 100%
NEGATIVE PREDICTIVE VALUE 48/55 = 87%

6.HIV SEROPOSITIVITY

	HIV PCR POSITIVE	HIV PCR NEGATIVE	
ELISA POSITIVE	126	4	130
ELISA NEGATIVE	9	79	88
	135	83	218

Rate of HIV PCR positivity **62%**; ELISA POSITIVE **59%**.

7. HIV POSITIVE VERSUS AGE

HIV PCR POSITIVE

HIV PCR NEGATIVE

17months and below	18months and above	
103	33	136
63	17	80
166	50	216

63% were are HIV positive. $103/166=62\%$ are positive for HIV among those 17 months and below. $33/50=66\%$ are positive for HIV among those above 17 months.

Number of subjects 17 months and below $166/216=77\%$.

Number of subjects above 17 months $50/216=23\%$

8. ELISA POSITIVITY VERSUS AGE

ELISA POSITIVE

ELISA NEGATIVE

17months and below	18months and above	
100	26	126
67	19	86
167	45	212

$126/212=59.4\%$ are HIV positive. $100/167=59.9\%$ are positive for HIV among those below 18 months. $26/45=57.8\%$ are positive for HIV among those above 17 months.

Number of subjects below 18 months $167/212=78.8\%$.

Number of subjects above 17 months $45/212=21.2\%$

9. ELISA VERSUS HIV PCR IN CHILDREN 18 MONTHS AND ABOVE

(using PCR as a gold standard or reference test)

HIV PCR

POSITIVE NEGATIVE

ELISA POSITIVE
ELISA NEGATIVE

31	0	31
0	17	17
31	17	48

SENSITIVITY 100% SPECIFICITY 100%

10. ELISA VERSUS HIV PCR IN CHILDREN 17 MONTHS AND BELOW

HIV PCR

POSITIVE NEGATIVE

ELISA POSITIVE
ELISA NEGATIVE

96	3	99
7	61	68
113	64	167

SENSITIVITY 84.9% SPECIFICITY 95.3%

11. ELISA VERSUS HIV PCR IN CHILDREN 15 MONTHS AND BELOW

HIV PCR

POSITIVE NEGATIVE

ELISA POSITIVE
ELISA NEGATIVE

95	3	98
3	55	58
98	58	156

SENSITIVITY 96.9% SPECIFICITY 94.8%

12. ELISA VERSUS HIV PCR IN CHILDREN 16 MONTHS AND ABOVE

	HIV PCR	
	POSITIVE	NEGATIVE
ELISA POSITIVE	33	1
ELISA NEGATIVE	2	23
	35	24
		59

SENSITIVITY 94.3%

SPECIFICITY 95.8%

13. ELISA VERSUS HIV PCR IN CHILDREN 12 MONTHS AND BELOW

	HIV PCR	
	POSITIVE	NEGATIVE
ELISA POSITIVE	84	3
ELISA NEGATIVE	2	50
	86	53
		139

SENSITIVITY 97.7%

SPECIFICITY 94.3%

14. ELISA VERSUS HIV PCR IN CHILDREN 13 MONTHS AND ABOVE

	HIV PCR	
	POSITIVE	NEGATIVE
ELISA POSITIVE	44	1
ELISA NEGATIVE	3	28
	47	29
		76

SENSITIVITY 93.6%

SPECIFICITY 96.5%

14. ANTIBIOTIC THERAPY ON ADMISSION VERSUS FINAL DIAGNOSIS

FINAL DIAGNOSIS

BACTERIAL PCP
PNEUMONIA

COTRIMOXAZOLE

OTHER

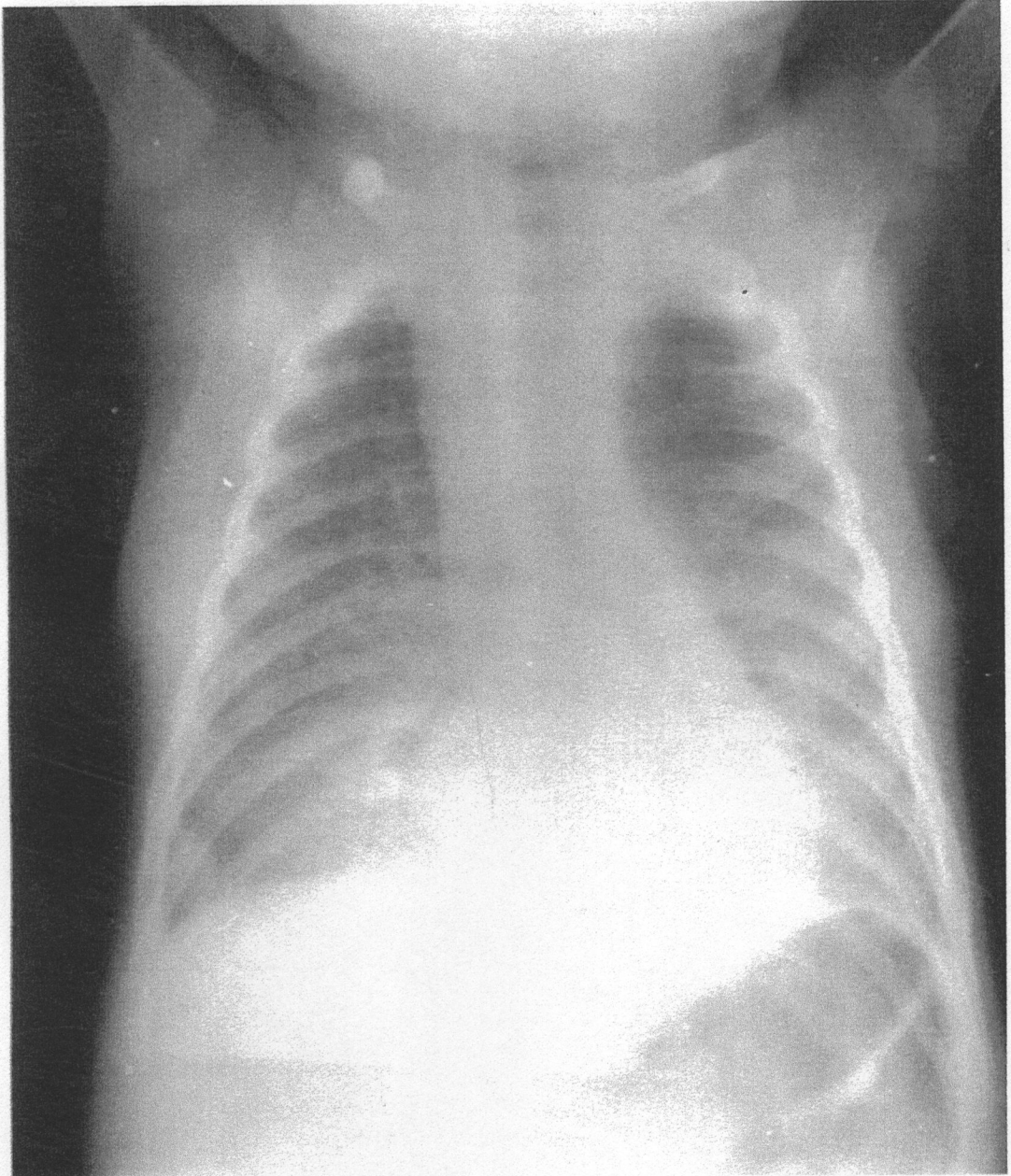
6	5	11
96	70	166
102	75	177

Other in the table above means PENICILLIN and/or GENTAMYCIN OR CHLORAMPHENICOL.

Only **5(0.07%)** subjects who had PCP confirmed after PM, received Cotrimoxazole. The rest of the **70(93.3%)** that had PCP received other antibiotics on admission.

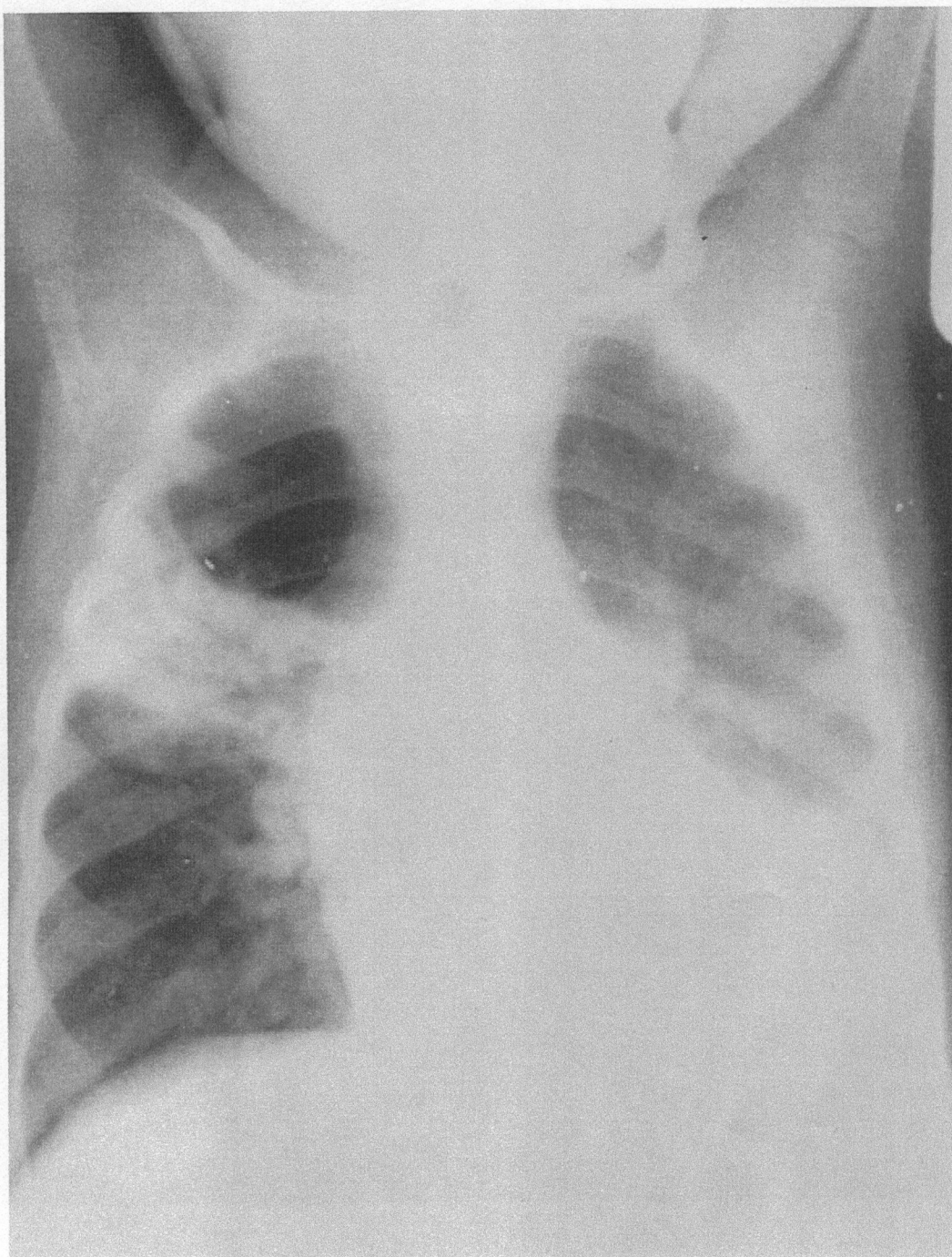
CHEST X RAYS FOR SOME OF THE PATIENTS WITH PCP

The CXR shows a ground glass appearance in a child found with PCP at postmortem.

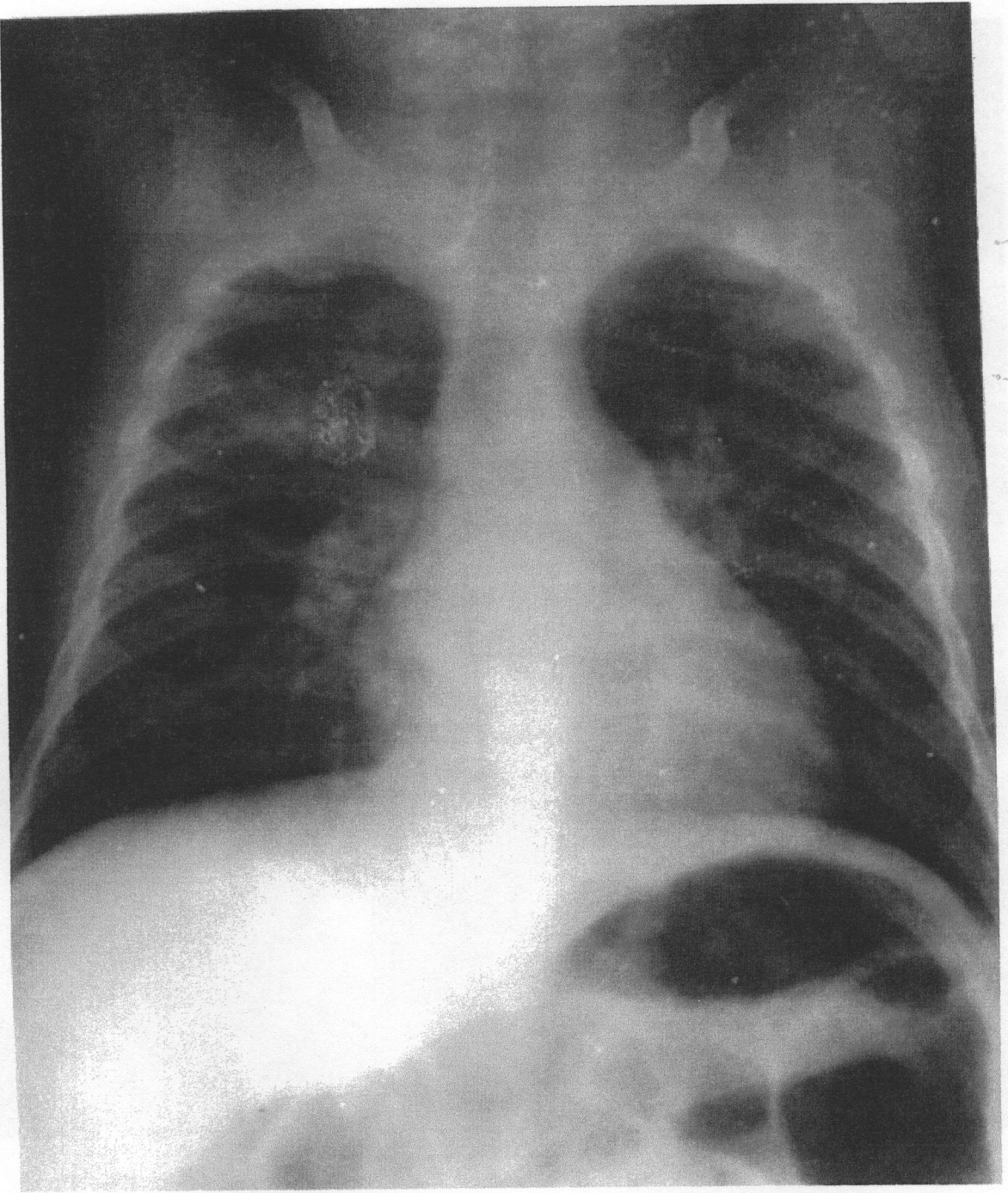


This CXR has the following abnormalities ;

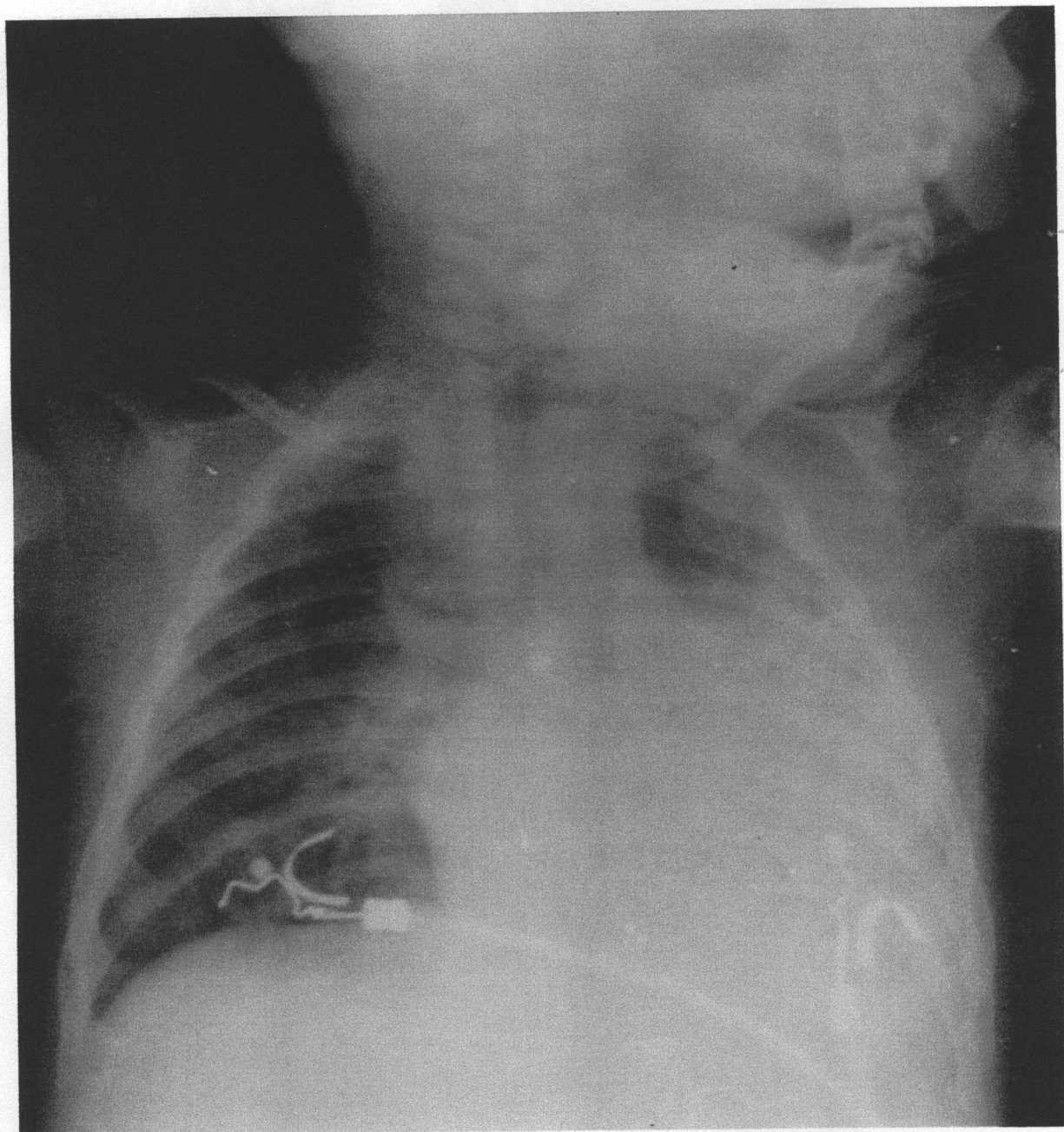
- (1) pneumatocoeles
- (2) areas of consolidation
- (3) milliary shadowing.



This CXR is almost normal except for right hilar infiltrates.
The round like opacity on the right upper zone is an artefact.

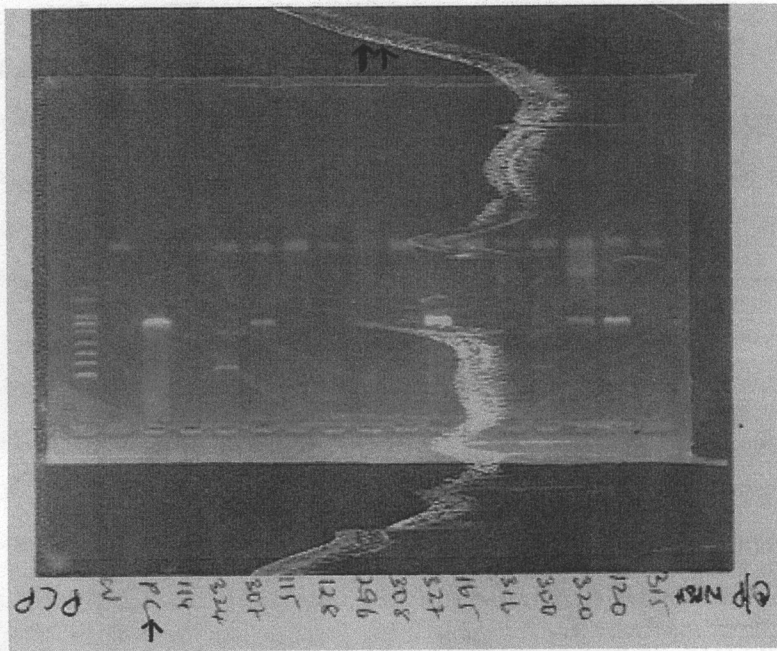


This CXR shows a consolidation on the left lower zone, a widened mediastinum and milliary shadowing on the right side.

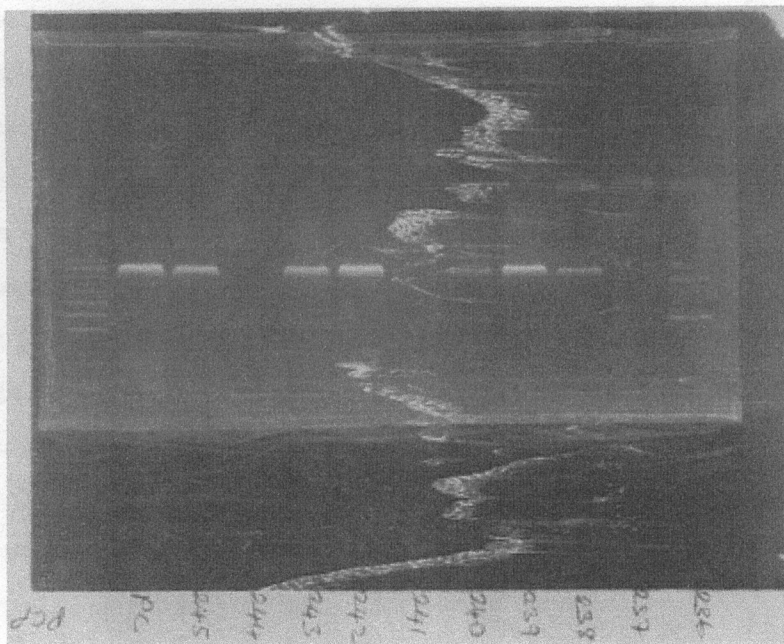


PICTURES OF GEL ANALYSED WITH ULTRAVIOLET CAMERA

The two gel pictures shown below are PCR products after DNA amplification. The one on the top is obtained from the oropharyngeal sample and the one below is from the left upper lung.



The white wavy lines are artefacts(↑↑). PC positive control and all white bars that correspond to the PC are positive for PCP(↑).



DISCUSSION

Out of 264 Post mortems done only 220 were completely analysed. 63% were HIV positive. The proportion of children who are HIV positive in the groups 18 months and above and 17 months and below is the same 62% and 67% respectively. The number of subjects in the group 17 months and below is more than those in the group 18 months and above. When PCR for HIV(pol and gag regions) was compared to ELISA in subjects at 15 months and above, it was found that doing an Elisa was as sensitive and specific as HIV PCR. This needs to be re-evaluated using one cohort which can then be followed up to the age of twenty four months.

Out of 220 subjects 80(35%) were found to have had PCP. Of the 136 HIV positive subjects, 70(51.5%) had PCP. Out of 84 HIV negative subjects 10(13%) had PCP at time of death. PCR for PCP DNA, picked up 30 more PCP subjects as compared to histology which identified 4 more who were negative on PCR. The results show that PCR used for diagnosis of PCP can be used as a gold standard. **The rate of HIV positivity in those children with PCP is 77.3% - 87.5%, showing that PCP in Zambian children is also an AIDS defining infection.**

This data also reveal that there are more children 17 months and below being admitted, than those who are 18 months and above. Thirty five percent of these were found with PCP, a further 30% had bacterial pneumonia and 15% had Tuberculosis. In this necropsy study, after analysis of the clinical files the **average age of children with PCP was the lowest** as compared to those with Pneumonia and Tuberculosis, children with PCP were younger. The duration of symptoms in those with PCP and Bacterial pneumonia were same but longer in those found with Tuberculosis. Those with PCP however, had significantly longer durations of **difficulties in breathing** reported, than those with bacterial pneumonia. There were more children with **cyanosis** in PCP group than in the bacterial pneumonia group. More children in the bacterial pneumonia group were found with **candida and lymphadenopathy** as compared to those in the PCP group. The children in the PCP group had a low-grade fever and respiratory rates average 49/minute, while those in the Bacterial pneumonia group had very high fever, tachypnoeic (respiratory rates of 60/minute and above).

In previous studies from Zambia PCP was found to be very rare in children with HIV infection [42]. In this study it has been demonstrated that PCP accounts for 35% of respiratory mortality in UTH especially in those children below 18 months and co-infected with HIV. The high prevalence of PCP found was expected as perinatal transmission of HIV-1 in Zambia has been reported to be 39%. The mortality of HIV infected children at 2 years is 44% and that by the age of 12 months most HIV infected children have had pneumonia, thereafter, they develop diarrhoea, recurrent fevers, chest infections, candidiasis, and lymphadenopathy [64], which this study has reconfirmed.

Despite the high frequency of PCP found in these children with HIV infection **most clinicians did not suspect PCP** and their initial response to the treatment was targeted at bacterial pneumonia. Clearly the possibility of PCP in these children was overlooked. This is exemplified by the fact that **only 6%** of children found with PCP received cotrimoxazole as compared to 94% found with PCP and did not receive cotrimoxazole. Ninety four percent of children found with Bacterial pneumonia received Penicillin and/or Chloramphenicol and only 6% received cotrimoxazole as an initial antibiotic on admission.

The pick up rates of *Pneumocystis carinii* from oropharyngeal washings using PCR was compared to histologically proven tissue with PCP and it has been shown that the method has 68.2% sensitivity and 100% specificity, with a 100% positive predictive value and 87% negative predictive value. Oropharyngeal washings can be used as an alternative less invasive specimen to aid in diagnosis of PCP. Other studies have also shown that it is possible to diagnose PCP from oropharyngeal mouthwashes using PCR [65].

Thirteen percent (Ten subjects) of the HIV negative subjects were found with PCP. Two of these were female subjects and the rest were male. Two were found to be above 7 months of age (thus eight and nine months respectively). The 8 month old subject was the only one who was malnourished. The rest of the subjects had normal weight for age. Every child is exposed to *P.carinii* infection by the age of two months [66]. Some children who are HIV negative carry *P.carinii* transiently in the respiratory tract without any symptoms [66]. For these particular children they might have had though rare hereditary immunodeficiency states which were not looked for.

CONCLUSIONS

This study has shown that PCP is common in children dying of respiratory illness and coinfecting with HIV. 35% of the children dying of respiratory infections were found with PCP and that 88% of these are co-infected with HIV virus showing that PCP is an AIDS defining infection even in Zambia. Further more 94% of these children are missed when they present to hospital. Majority of these children are not treated with cotrimoxazole, on admission but instead, get penicillin, gentamycin and/or chloramphenicol. The majority of admissions are below the age of 18 months. 63% of the children dying of respiratory infections are coinfecting with HIV. Diagnosis of PCP from oropharyngeal washings using PCR looking for *P.carinii* DNA is just as specific as histopathology, but this method needs further evaluation in living subjects. There is also need to improve on the extraction of DNA from these washings, so that pick up rates can be improved.

RECOMMENDATIONS

1. PCP should be suspected in all children with pneumonia more so those below the age of 12 months see the suggested algorithm for management of PCP below.
2. Attending physician should be aware of the symptoms and signs which can distinguish PCP from bacterial pneumonia *i.e.* for PCP low grade fever, difficulties breathing, cyanosis, respiratory rates between 40 and 60 /minute and age below 7 months one should consider PCP in the differential diagnosis. As for bacterial pneumonia age is not critical, they tend to have more lymphadenopathy, oral thrush and respiratory rates of more than 60/minute.
3. Voluntary counseling and testing of all expectant mothers should be encouraged.
4. Encourage the use of cotrimoxazole in all children suspected to have PCP early than late.
5. PCP prophylaxis should be given to all children born to HIV positive mothers. If the HIV status of the mothers can be established then selection of those requiring PCP prophylaxis can be done. Although other criteria for prophylaxis need to be in place.
6. Elisa test can be done on all children aged 12 months and above.

PCP ALGORITHM

Fever, cough, tachypnoea, dyspnoea, cyanosis,

No lymphadenopathy.

AGE

< 12 months of age

> 12 months of age

HIV EXPOSURE

HIV EXPOSURE

STATUS

STATUS

-VE

+VE

-VE

+VE

PCP UNLIKELY

PCP UNLIKELY

PCP UNLIKELY

CXR

Localised

bilateral diffuse

bilateral diffuse

normal

Infiltrates

miliary infiltrates

infiltrates

Unlikely to be
PCP

TREAT ALL THESE AS *P. CARINII* PNEUMONIA
HIGH DOSE COTRIMOXAZOLE IV PLUS
PREDNISOLONE

CONFIRM DIAGNOSIS BY COLLECTING
OROPHARYNGEAL OR NASOPHARYNGEAL WASHINGS AND DO TOLUIDINE BLUE STAIN OR
PCR FOR *P. CARINII*

KEY: +ve positive for HIV, -ve negative for HIV, CXR chest x ray, HIV exposure status include any of the following ;
maternal HIV status, Epidemiology consistent with retroviral infection, clinical presentation of the child.

This algorithm needs to be tested in a clinical setting(future study).

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