Clinical presentation and outcome of Pneumocystis carinii pneumonia in Malawian children

Graham, Stephen M; Mtitimila, Edward I; Kamanga, Henry S; Walsh, Amanda L; Hart, C Anthony; Molyneux, Malcolm E

Department of Paediatrics (S M Graham FRACP, E I Mtitimila MB, H S Kamanga Dip Lab) and Wellcome Trust Research Laboratories (A L Walsh MIBiol, Prof M E Molyneux FRCP), College of Medicine, University of Malawi, Blantyre, Malawi; School of Tropical Medicine, Liverpool, UK (A L Walsh, Prof M E Molyneux); and Department of Medical Microbiology, University of Liverpool, Liverpool, UK (Prof C A Hart FRCPath)

Correspondence to: Dr Stephen M Graham, Department of Medical Microbiology, University of Liverpool, 8th Floor, Duncan Building, Liverpool L69 3GA, UK
(e-mail: smgraham@liverpool.ac.uk)
Summary

Background: Necropsy studies from Africa have shown that Pneumocystis carinii pneumonia (PCP) is common in infants with HIV infection. We aimed to describe the rate, clinical presentation, and outcome of PCP in young Malawian children with acute severe pneumonia.

Methods: Children aged between 2 months and 5 years who were in hospital with a diagnosis of severe pneumonia were admitted to a study ward for clinical monitoring. We carried out blood culture, immunofluorescence on nasopharyngeal aspirate samples to test for PCP, polymerase chain reaction to detect HIV, and chest radiography.

Findings: 16 cases of PCP were identified among 150 children with radiologically confirmed severe pneumonia. All were HIV-positive and younger than 6 months. 21 children had bacterial pneumonia (including one who was also PCP positive) and 114 were not confirmed. The most common bacterial pathogens among children without PCP were Streptococcus pneumoniae (eight) and non-typhoidal salmonellae (seven). On admission, children with confirmed PCP had a lower mean age, body temperature, and oxygen saturation than children with bacterial pneumonia and were less likely to have a focal abnormality on auscultation. Oxygen requirements were much greater in children with PCP than those with bacterial pneumonias (96 of 105 hospital days vs 15 of 94, p<0.0001). Ten of 16 children with PCP and six of 21 with bacterial pneumonia died (relative risk 2.19 [95% CI 1.0-4.7]). The overall case-fatality rate of severe pneumonia was 22%. In addition to a strong association with PCP, a fatal outcome was...
significantly and independently associated with HIV infection (2.98 [1.1-7.9]) and with age under 6 months (2.76 [1.0-5.2]).

Interpretation: PCP is common and contributes to the high mortality from pneumonia in Malawian infants. Clinical features are helpful in diagnosis. The study highlights the impact of HIV infection and difficult issues of management in countries with few resources.

Introduction

Studies of vertical HIV transmission in many regions of Africa, including Malawi, show that childhood HIV infection is common. Necropsy studies from the region have consistently shown that Pneumocystis carinii is common in children with HIV infection dying of pneumonia. Though P carinii pneumonia (PCP) is commonly recognised clinically, a lack of diagnostic tests has meant that the number of cases of PCP among children presenting with acute pneumonia is unknown. A pilot study in Malawi diagnosed five cases of PCP by means of an immunofluorescence method that identified cysts on nasopharyngeal aspirate samples. We did a prospective study of the causes and outcome of acute severe pneumonia in Malawian children.

Methods

Patients

We did a prospective study at Queen Elizabeth Central Hospital, Blantyre, Malawi, during a 6-month period (July to December, 1996) when the rate of severe malaria is low. Clinical data were collected on all children aged between 2 months and 5 years with an admission diagnosis of severe pneumonia. This hospital serves Malawi’s largest city with a population of about 1 million. Children admitted with acute pneumonia have either presented directly to the hospital's under-5 clinic or have been referred to the clinic from an urban or periurban health centre. Severe pneumonia was defined according to WHO criteria—cough or a difficulty breathing with chest indrawing. Assessment on admission included a thick blood film to detect malaria parasites and measurement of packed-cell volume.

Children from this group with severe pneumonia were admitted to the study ward, if beds were available, by one of the clinicians (SG, EM, MM). Children with a diagnosis on admission of severe pneumonia, who were not admitted to the study ward, were followed up to assess clinical course and outcome. Care was taken to avoid misdiagnoses due to other disorders such as severe malaria that also present with cough and respiratory distress. Children with measles were followed up but not included in the study because they must be cared for in isolation. Children with severe malnutrition (eg, marasmus or kwashiorkor) were not included in our study because they must be transferred to the nutritional rehabilitation unit.

Investigation
The clinician enrolling patients collected clinical and demographic data. Investigations included blood culture, immunofluorescence of nasopharyngeal aspirate samples, and chest radiography. Arterial oxygen saturation (SaO2) was measured with pulse oximetry (Ohmeda, Louisville, KT, USA) on admission first with the child breathing air and then in oxygen if the child was hypoxic (SaO2 <90%). Because most patients were infants, HIV infection was sought by nested PCR on DNA extracted from blood clots (with proteinase K and phenol-chloroform). Part of the HIV LTR gene was amplified with a primer (Neil Berry, University College London, London, UK) designed for detection of African HIV strains. The outer primer pair was Tar-3 (5’-ACCAGRTYTGAGCTGGGAGCT) and N2607 (5’-CCTGTTCGGGCGCCACTGCTAGAGCTTTT) and the inner primer pair was prime Tar-2 (5’-TGAGCCTGGGAGCTCTCTGGCT) and N2609 (5’-CTGAGGGATCTCTAGDYACCAGAGT).

In the laboratory of the paediatric department, nasopharyngeal aspirate samples were analysed for presence of P carinii with an indirect immunofluorescent assay technique (DETECT immunofluorescence P carinii test kit, Shield Diagnostics, Dundee, UK). A child was diagnosed as having PCP when three or more typical fluorescent cysts were identified in the nasopharyngeal aspirate sample. Blood was cultured by a conventional method, with brain heart infusion broth and SPS (Equint and Ovine Laboratory, Bonnybridge, Scotland, UK). Isolates were identified by standard methods. Antibiotic sensitivities were assessed by the modified disc diffusion method of Kirby Bauer.

A diagnosis of bacterial pneumonia was made when a child had an abnormal chest radiograph and a positive bacterial isolate on blood culture. Chest radiographs were done on the study ward with the patient in a supine position. Radiographs of children with a final diagnosis of PCP or bacterial pneumonia were reported together by paediatric radiologists at the Alder Hey Liverpool Children's Hospital. The paediatric radiologist was aware that all cases had severe pneumonia but was not aware of the cause. The chest radiography was classified as showing hyperinflation when the posterior end of more than nine ribs were visible through the aerated lung, there was abnormality in the contour of the diaphragm, and evidence of inflated lung projecting over the mediastinum.

The patients were cared for in a designated project ward with no more than four patients per nurse. Chloramphenicol (100 mg/kg daily) was given intramuscularly. Children who were hypoxic on admission were given humidified oxygen from an oxygen concentrator at a rate of 1-2 L/min through an 8-French gauge nasopharyngeal catheter. If immunofluorescence of nasopharyngeal aspirate samples was positive for PCP, or if PCP was suspected clinically but not confirmed, high-dose co-trimoxazole was given orally (120 mg trimethoprim/600 mg sulphamethoxazole three times daily for children weighing less than 5 kg; or 240 mg/1200 mg three times daily if 5 kg or more). Intravenous and oral liquid preparations of co-trimoxazole are not usually available at public hospitals in Malawi, so this treatment was given in a crushed tablet form. Prednisolone (2 mg/kg daily) was added if hypoxia was severe (SaO2 <70%). Chloramphenicol was given to severely hypoxic children for at least 7 days. Clinical monitoring included clinical observations every 4 h and daily measurement of oxygen saturation when the child was breathing air, and if hypoxic, when breathing oxygen.
Oxygen was given to children with hypoxia until no longer indicated, until the child died, or until discharge was requested because the treatment response was poor. Oxygen was not stopped while it was still clinically indicated for any child remaining in the ward. The decision to discharge a child in whom treatment response was poor was taken only after a request for discharge was made by the family. The decision was discussed with the family by nursing and medical staff and the child was still taking oral co-trimoxazole on discharge. Follow-up was arranged. The family were invited to bring these children to the research ward for review whenever they wanted. For those that did not return, no attempt was made to visit the child at home.

Parents of guardians were informed of all procedures before admission to the project ward. One mother refused enrolment of her child in the study. Results of investigations done during the admission period were given to parents. Clinicians explained to parents that blood would be taken for anonymous HIV testing on completion of the study and that the testing would not be done in Malawi. Parents were, however, told that they could be informed of the child's HIV status if they wanted. If results were requested, the usual hospital procedure with pre-test and post-test counselling would have been followed, but no parent requested HIV information.

Data analysis

Coded data were entered and analysed with a statistical programme (Epi-info 6). Associations were assessed with the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. Mantel-Haenszel analysis was used to find the independence of individual risk factors for mortality. The study protocol was approved by the National Health Sciences Research Committee of Malawi.

Results

During the 6-month study period, a final diagnosis of severe pneumonia was made in 333 children; 67 (20.1%) died. Of the 333 children, 150 were fully investigated in the study ward and had their pneumonia radiographically confirmed. Only this group were included in further analyses. The median age was 5.0 months (range 2-59) and there were 80 boys and 71 girls. Many children had had previous treatment: 79 (53%) had received an antibiotic, penicillin or co-trimoxazole in most cases; 66 (43%) had received aspirin, and 29 (19.5%) had received traditional medicine. 135 (90.2%) had a complete immunisation record.

P. carinii was identified in 16 (10.7%) children and all were HIV-positive (table 1). The proportion of confirmed cases of PCP who had received co-trimoxazole before admission was the same as for the rest of the study group (18%). 21 (14%) children had positive blood cultures; the bacteria most commonly detected were Streptococcus pneumoniae, non-typhoidal salmonellae, and Haemophilus influenzae type b. Among the children with positive blood cultures, previous antibiotic usage was reported in 11 (52%). All bacterial isolates, including salmonellae were sensitive in vitro to chloramphenicol.
The cause of illness was not found in 114 children. PCP was suspected clinically in 24 of them, of whom 13 (54%) died in hospital and 11 were still hypoxic on discharge from hospital despite treatment for PCP. 21 of 24 children with clinically suspected PCP were subsequently diagnosed as HIV positive. Nine children were diagnosed and treated for tuberculosis because of a history of contact and a chest radiograph showing consolidation. No cases of tuberculosis were confirmed microbiologically and eight of nine were HIV positive.

Table 1 compares the clinical presentations of children with PCP and those with bacterial pneumonia. On admission to hospital, children with PCP had a significantly lower mean age, mean body temperature, and mean oxygen saturation and higher proportions had no abnormality or diffuse abnormalities on auscultation.

Treatment response and outcome for PCP were poor (table 3). Hypoxia was more persistent in children with PCP than in those with bacterial pneumonia. Children with PCP received oxygen for 96 of a cumulative 105 inpatient days compared with 15 of 94 inpatient days for patients with bacterial pneumonia (p<0.0001). The case-fatality rate was significantly higher for patients with PCP than for those with bacterial pneumonia (relative risk 2.19 [95% CI 1.0-4.7], p=0.04). Of five children with PCP who were discharged from hospital between day 6 and day 12 with persistent hypoxia, three died within a month after discharge. Because all children with PCP were less than 6 months of age, the effect on overall outcome was confined to this group. Mortality for PCP was higher than for other cases of this age-group (3.13 [1.7-5.7], p=0.001).

Table 3 compares the treatment response for PCP and bacterial pneumonia. 33 patients died in hospital and 21 were discharged with persistent hypoxia after at least 5 days of inpatient care (table 1). 93 (62%) children were HIV positive. 77 of 134 (57%) patients without PCP were HIV positive. 63 of 93 HIV-positive children were
less than 6 months of age and the proportion of children who were HIV infected was significantly higher in this age group \( (p=0.002) \) than in other age-groups. HIV-infected children had lower oxygen saturation, required more oxygen therapy, and had a poorer outcome than children without HIV (table 4). On single table analysis, mortality was significantly higher for HIV-positive children (relative risk 3.43 \( [95\% \text{ CI } 1.4-8.4] \), \( p=0.004 \)) and for infants younger than 6 months \( (2.76 [1.2-5.9], p=0.008) \). The effect of HIV infection on mortality remained significant \( (2.98 [1.1-7.9], p=0.01) \) when stratified analysis was done with age as a confounder, and also for analysis of children younger than 6 months \( (2.76 [1.0-5.2], p=0.03) \) with HIV status as a confounder. The effects of HIV status on outcome was independent of the association between HIV and PCP.

<table>
<thead>
<tr>
<th>Oxygen saturation</th>
<th>HIV-positive (n=45)</th>
<th>HIV-negative (n=62)</th>
<th>( \chi^2 ) ( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desaturation and hypoxia</td>
<td>41 (91.1%)</td>
<td>51 (82.8%)</td>
<td>5.393 ( 0.02 )</td>
</tr>
<tr>
<td>Desaturation and hypoxia</td>
<td>26 (57.4%)</td>
<td>39 (62.9%)</td>
<td>0.897 ( 0.34 )</td>
</tr>
</tbody>
</table>

Table 4: Effect of HIV infection on oxygen requirements and outcome

Discussion

A previous study from Malawi identified P carinii in five of 52 consecutive cases of severe pneumonia, accounting for four of 14 deaths.\(^5\) PCP was recognised as the most common serious opportunistic infection among children with HIV infection in the USA and UK before the use of co-trimoxazole prophylaxis.\(^{11,12}\) In another study 27 cases were identified during a 10-year period (1980-90) with more sensitive techniques, including open lung biopsy.\(^{13}\) 12 cases of PCP were identified during a 1-year period of established active surveillance (1992-93) in the entire British Isles.\(^{12}\) The high proportion of PCP in our study in Malawi is probably a result of high HIV prevalence and vertical-transmission rates in the region.\(^{1}\)

In our study there was probably bias towards selection of the most severe cases for admission to the research ward, and therefore for enrolment in the study. This bias is suggested by the frequency and severity of hypoxia in children on admission to the ward and the high case-fatality rate. Despite the more intensive nursing care and clinical monitoring available, the mortality rate was higher among children admitted to the research ward than among those with severe pneumonia who were not included (22 vs 18%). Our hospital accepts referrals from surrounding clinics, so the study may have included the most severe cases of community-acquired pneumonia. In addition, this study is not representative of all children admitted with severe pneumonia because it did not include children with severe malnutrition or measles.\(^{14}\)

We identified clinical features that might be useful in distinguishing PCP from other community-acquired pneumonia. Confirmatory diagnostic techniques are not commonly available in most African countries where childhood HIV infection is common. Acute pneumonia is usually managed according to clinical signs of severity, because most cases of fatal pneumonia are caused by bacterial infection.\(^{6,15}\) We compared PCP with pneumonia diagnosed by a positive bacterial blood culture because the latter was the only other group in which cause of disease had been confirmed. The children in this group probably represent the most severely ill among all...
children with bacterial pneumonia, and they had a higher mortality rate than children with pneumonia of unknown cause (table 1). Use of lung puncture would have increased the identification rate of bacterial pathogens, but we believe that this procedure is not safe in this group of patients, of whom many may have pulmonary hyperinflation. Chest radiography are commonly used as a diagnostic tool to differentiate causes of pneumonia. Interstitial infiltration and hyperinflation are consistent with a diagnosis of PCP and did contrast in this study with the focal abnormalities seen in children with bacterial pneumonia. However, chest radiographs cannot diagnose bacterial pneumonia or PCP and coinfection of PCP with other pathogens is common. We did not test for respiratory syncytial virus, a recognised cause of hyperinflation.

Immunofluorescence can be done on site if fluorescent microscopy is available and if the test provides a rapid diagnosis. The more sensitive technique of amplification of P carinii DNA by PCR was not available for this study. The sensitivity of testing nasopharyngeal aspirate samples is not known. In our study, 13 children in whom PCP was suspected but not proven died. Pneumonitis syndromes caused by other pathogens may present with similar features in infants. Sputum induction with nebulised hypertonic saline is another technique for PCP diagnosis and has been used in Malawian children as young as 3 years to confirm tuberculosis. We are not certain of the risk of using this technique in severely hypoxic infants without intensive respiratory support. Bronchoalveolar lavage, which was not available for our study, has been used successfully to diagnose PCP in infants as young as 2 months. Lung samples obtained by open lung or transbronchial biopsy provide greater diagnostic sensitivity than respiratory secretions. Percutaneous lung aspiration would have provided a higher yield of both P carinii and bacterial isolates, however, we decided not to use this technique because of the danger of air leaks in PCP.

PCP in infants without HIV has been described. All 16 children with PCP in our study were HIV-positive and younger than 6 months. This finding is consistent with evidence from previous necropsy studies in African children and suggests that PCP is a primary infection. Although most common in infants, PCP has been described in older children with vertically acquired HIV infection. In healthy African children the prevalence of antibody to P carinii shows a steady increase with age, similar to that of healthy British children, with 70% of children with evidence of infection by the age of 8 years. Epidemiological data are important if a PCP prophylaxis strategy is to be considered.

The outcome for infants with PCP in our study was poor. Children were provided with the best available care under the circumstances. Such a low ratio of patients to nurses in a separate ward is not usual. Pulse oximetry was used to assist in identifying hypoxia and humidified oxygen was delivered via a carefully placed nasopharyngeal catheter, estimated to deliver at least 45% oxygen. Co-trimoxazole was not available as an intravenous or oral liquid preparation so we used crushed portions of tablets. The poor outcome may have been related to decreased drug bioavailability because of the mode of administration, although patients receiving high-dose co-trimoxazole have similar serum concentrations whether treated orally or intravenously.

The early initiation of corticosteroid therapy reduced morbidity in adults with PCP and is recommended for those with moderate or severe respiratory failure. Controlled trials have not been done in children, although adjunctive corticosteroid use has been associated with a
significant improvement in survival, or at least with successful withdrawal of assisted ventilation and discharge from intensive care.\textsuperscript{29,30} We took a cautious approach to steroid use because of the immunosuppressive properties of corticosteroids, the possibility of mixed infection, and the high rate of primary infection with Mycobacterium tuberculosis in the district.\textsuperscript{31}

In a previous study \textsuperscript{4} of 16 cases of PCP identified at necropsy, cytomegaloviral infection was diagnosed in 11 children and adenovirus infection in two. There have been reports in both HIV-infected children \textsuperscript{30} and adults \textsuperscript{32} of overwhelming cytomegaloviral infection within weeks of the start of corticosteroid therapy. There are no available data on the epidemiology of cytomegalovirus in Malawi. Ganciclovir, the drug of choice for treatment of cytomegalovirus infection, is not available. Malin and colleagues \textsuperscript{33} reported that six of 21 Zimbabwean adults with PCP (identified by bronchoalveolar lavage) were coinfected with M tuberculosis.

PCP is associated with a poor prognosis, even when more intensive treatment and greater therapeutic options are available.\textsuperscript{12,13} The increased and extended oxygen requirement of a patient with PCP commonly denies access to the limited supply of oxygen concentrators to children with other causes of severe pneumonia for whom oxygen could be life saving.\textsuperscript{34} We do not know whether more vigorous steroid administration to patients with PCP in this study would have resulted in clinical improvement and less oxygen dependency.

The rate of HIV infection in this study was unexpectedly high (62\%) and the effect on outcome was severe. The hospital setting and methods of selection of patients may have skewed our study patients towards those with the most severe disease. Compared with our hospital, HIV prevalence was lower (28-32\%) in children with pneumonia admitted to large urban hospitals serving populations with a similar background HIV prevalence than in Malawi, and where less specific diagnostic methods were used.\textsuperscript{35} Higher pneumonia-related mortality has been reported among children with supportive evidence of HIV infection than among those without.\textsuperscript{35,36} A Zambian study \textsuperscript{37} used PCR to diagnose HIV infection, and found a case-fatality rate in children with severe pneumonia of 36\% for HIV-infected children compared with 14\% for children not infected with HIV (p=0.08).

Our study has provided prospective evidence of the effect of PCP among infants admitted to hospital with severe pneumonia in an HIV-endemic region, and highlights common difficulties of clinical management in a setting with a few resources.

\textbf{Contributors}

All investigators contributed to study design. Steve Graham, Malcolm Molyneux, and Edward Mtitimila provided clinical care and supervision. Henry Kamanga, Mandy Walsh, and Tony Hart did the laboratory work. Steve Graham analysed the data and wrote the paper, with major contributions from Malcolm Molyneux and Tony Hart.

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